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In vitro evaluation of twelve fungicides against three major fungal pathogens of tropical tuber crops

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Abstract

The productivity of tropical tuber crops is significantly threatened by a variety of diseases caused by fungi and viruses. These pathogens not only reduce yield but also affect the quality of the tubers, leading to substantial economic losses. Fungal pathogens, such as *Sclerotium rolfsii, Colletotrichum gloeosporioides* and oomycete, *Phytophthora colocasiae* are among the most destructive, causing diseases like collar rot in elephant foot yam, anthracnose in yam and taro leaf blight. Mancozeb is a broadly used fungicide for the control of plant diseases, including taro leaf blight and it has been banned for agricultural use by few countries due to its hazardous effects to humans and the environment. In a search for replacement of fungicides, twelve fungicides at different concentrations (100, 200, 400, 800 and 1600 ppm) were tested for their ability to arrest the major fungal pathogens of tropical tuber crops viz., *Colletotrichum gloeosporioides, P.colocasiae* and *Sclerotium rolfsii.* The chemicals recommended for the management of the diseases, mancozeb and carbendazim alone and their combinations with other chemicals were included to get the comparative efficiency of alternative options. The results suggest use of fungicides, difenoconazole, hexaconazole and metalaxyl-M+ chlorothalonil to manage diseases caused by *Colletotrichum gloeosporioides.* Metalaxyl-M+ chlorothalonil (Folio Gold) and cymoxanil+ famoxadone (Equation Pro) can provide better protection from *Phytophthora colocasiae.* Hexaconazole and difenoconazole showed highest mycelial growth inhibition of *S.rolfsii* even at the lowest concentration tested. The lead obtained from the study will be a stepping stone to revisit the chemical management strategy followed to combat the pathogens.

Keywords : Fungicide, *Sclerotium rolfsii, Colletotrichum gloeosporioides*, *Phytophthora colocasiae,* Tuber crops

Introduction

Tuber crops constitute a considerable part of the world's food supply and are a valuable source of animal feed. Second to cereals, tuber crops are important cultivated staple energy source. Greater yam (*Dioscorea alata*) is a staple food in the regions of West Africa, Southeast Asia and the Caribbean and is part of the monocotyledonous Dioscoreaceae family (Liu et al., 2007). One of the major constraints in yam production is the fungal infestation which causes a great loss on the production of yam worldwide. Anthracnose disease of yam is caused by the fungus, *Colletotrichum gloeosporioides*, an asexual facultative parasite. The disease can cause 100% leaf abscission and premature death of up to 76% of inoculated plants under controlled environment conditions (Shahana et al., 2018). Extensive crop damage and lack of high levels of

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host resistance cause difficulty in managing anthracnose of yam (Arya et al., 2021).

Taro is recognized as one of the oldest known crops (Ahmed et al., 2020). It is an important cash crop cultivated worldwide in tropical and subtropical regions. Leaf blight caused by the oomycete *Phytophthora colocasiae* is the most devastating disease of taro. It has become a significant problem worldwide in taro cultivation including India. *P. colocasiae* affects leaves, petioles and corms of taro plants and reduced corm yield up to 50% when leaves were infected (Singh et al., 2012). The symptoms appear as small, water-soaked spots, which increase in size and number. The lesions then become enlarge and irregular in shape and dark brown in color with yellow margins (Nath et al., 2016; Abdulai et al., 2020). Currently, resistant cultivars and chemicals applications are the main strategies to control the disease. Since only a few resistant cultivars are available in taro production, chemical control becomes the main effective strategy for management of taro leaf blight (Tchameni et al., 2018; Zhang et al., 2021). Collar rot disease caused by *Sclerotium rolfsii* is a serious threat to cultivation of elephant foot yam. The loss due to this pathogen is calculated as 10–100% (Aswathy et al., 2019). *S. rolfsii* is one of the most destructive soil borne fungi initially described by Rolfs (1982) on tomato. It is a fast spreading and destructive disease (Shirsole et al., 2019).

Among the different management strategies for plant diseases, the use of resistant cultivars is the most viable option, but the non-availability of enough quantity of quality seed materials of resistant cultivars forces the farmers to grow susceptible cultivars with proper fungicide scheduling. In agriculture, chemical control remains the most effective, easiest and most commonly used means of control and fungicides play a pivotal role around the world for plant disease management in many crops (Kongcharoen et al., 2020; Aneetta et al., 2022). However, the high frequency of applications poses a real threat to consumers and the environment. Fungicide-resistant strains can develop due to excessive use of fungicides with similar actions. It is necessary to use fungicides having novel modes of action to lessen selection pressure, develop resistance, and successfully manage infections over the long term (Thind et al., 2009). Use of new fungicides with unique mechanism of action or combination of new and traditional fungicides is the present-day strategy to treat plant diseases. Most of the new-generation fungicides are produced by various companies and have a highly specific and single-site mode of action. Therefore, it is important to identify and evaluate a new fungicide or a combination of two fungicides with a novel mode of action under field conditions. Keeping these points in view, a study was conducted in laboratory condition to assess the efficacy of various fungicides to arrest the growth of three major pathogens of tropical tuber crops *viz*., *C. gloeosporioides, P. colocasiae* and *S. rolfsii.* Care has been taken to include the chemicals, which are presently being recommended along with various groups of fungicides available in the market.

Materials and Methods Microbial culture

Cultures of *S.rolfsii*, *C. gloeosporioides* and *P. colocasiae* maintained at microbial repository of ICAR-Central Tuber Crops Research Institute (CTCRI), Sreekariyam were sub cultured on Potato Dextrose Agar (PDA-HIMEDIA) and used for the study. The details of the fungicides used in the study are given in Table.1

Table 1. Details of fungicides used for the study

Sl.No.	Fungicide	Composition	Formulation	Registrant
1	Amistar	Azoxystrobin 23%	Suspension Concentrate (SC)	Syngenta India Ltd
2	Folio Gold	Metalaxyl – M 3.3% Chlorothalonil 33.1%	Suspension Concentrate (SC)	Syngenta India Ltd
3	Equation Pro	Cymoxanil 22.1% + Famoxadone 16.6%	Suspension Concentrate (SC)	Corteva agriscience
4	Score	Difenoconazole 25%	Emulsifiable Concentrate (EC)	Syngenta India Ltd

5	Contaf	Hexaconazole 5%	Suspension Concentrate (SC)	Rallis India Limited
6	Ridomil Gold	Metalaxyl – M 4%+ Mancozeb 64% WP	Wettable Powder (WP)	Syngenta India Ltd
7	Indofil M-45	Mancozeb 75%	Wettable Powder (WP)	Indofil
8	Curzate	Cymoxanil 8% + Mancozeb 64%	Wettable Powder (WP)	DuPont
9	Antracol	Propineb 70%	Wettable Powder (WP)	Bayer
10	Tagstin	Carbendazim 50%	Wettable Powder (WP)	Tropical Agro
11	Blitox	Copper Oxychloride 50%	Wettable Powder (WP)	TATA
12	Sprint	Carbendazim 25% + Mancozeb 50 %	Wettable Powder (WP)	INDOFIL

Stock preparation of the fungicide

Inhibitory action of the fungicides 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. Concentrations of 100, 200, 400, 800 and 1600 ppm were uniformly selected for all the fungicides.

Determination of antifungal activity of fungicides

Required quantity of the stock solution was incorporated into sterile, molten, and cooled Potato Dextrose Agar medium (HIMEDIA) or Carrot Agar (CA) (for *P. colocasiae)* to get final concentrations of 100, 200, 400, 800 and 1600 ppm. Fungicide amended medium was mixed gently and dispensed into Petri dishes. All three pathogens were cultured either on PDA (Potato Dextrose Agar) or CA (Carrot Agar) for 48 h and 96 h respectively and mycelial discs (1cm diameter) were cut from the growing edges of the culture and placed in the centre of plates containing PDA/CA amended with various chemicals at different concentrations. Plates with media were inoculated with mycelial discs of pathogens without fungicide served as control.

Three plates were maintained for each concentration of the fungicides. The plates were incubated at 28±2ºC. The growth of the colony was measured from 48h onwards. 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 was compared with that of various concentrations and the difference was converted into percent inhibition. The percent inhibition of various pathogens was calculated based on the diameter of growth of the colony by using the formula of Vincent (1947).

$$I = \frac{C-T}{C} \times 100 \qquad \text{... Eqn (1)}$$

Where, I was the percent inhibition, C was the growth of isolates pathogens in control plates (without fungicide) and T was the growth of isolates of pathogens in test plates (with fungicide).

Determination of activity at lower concentrations of fungicides

The fungicides, which completely arrested the growth of the organisms at the concentrations 100, 200 and 400 ppm were evaluated again at lower concentrations to find out the exact concentration at which complete inhibition of mycelial growth occurs. For that all those fungicides which showed complete inhibition at 100 ppm were first tested at 50 ppm, then 25 ppm etc. The concentration was reduced to 50% in next step based on the result. Similar protocol was followed for 200 and 400 ppm also.

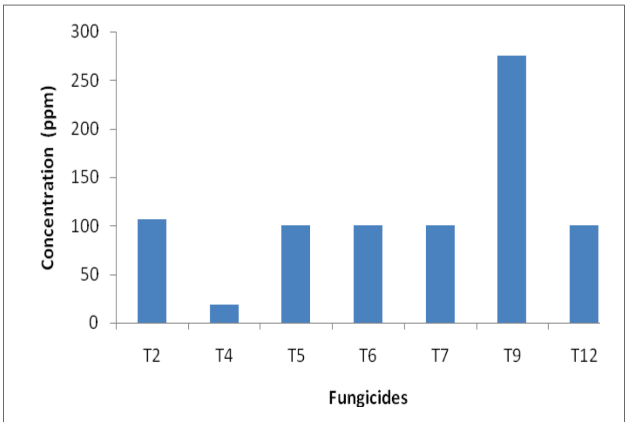
Statistical analysis

The data on mycelial growth of pathogens 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

Sensitivity of *C. gloeosporioides* to various fungicides

The inhibition of mycelial growth of *C. gloeosporioides* at 100 ppm ranged from 4.79 to 100.00% (Table. 2). The fungicides, difenoconazole (Score), hexaconazole (Contaf), metalaxyl-M+ Mancozeb (Ridomil Gold), mancozeb (Indofil M-45) and carbendazim+ mancozeb (Sprint) completely inhibited the growth of *C. gloeosporioides* at 100 ppm. The inhibition of mycelial growth at 200 ppm ranged from 8.07 to 100.00%. Apart from those five fungicides, which completely arrested the growth at 100 ppm, metalaxyl-M+ chlorothalonil (Folio Gold) also exhibited 100% inhibition. The inhibition of mycelial growth at 400 ppm ranged from 30.72 to 100.00%. In addition to the fungicides which showed complete inhibition at 200 ppm, the fungicide propineb (Antracol) also showed 100% inhibition at 400 ppm. Inhibition at 800 ppm ranged from 42.44 to 100.00% and in this concentration, the fungicide carbendazim (Tagstin) also could completely check the mycelia growth of *C. gloeosporioides*. Barring the fungicides, azoxystrobin (Amistar) (71.77%) and cymoxanil+famoxadone (Equation Pro) (67.61%), all other fungicides completely inhibited the growth of *C. gloeosporioides* at 1600 ppm.



T2- Metalaxyl + Chlorothalonil; T4- Difenoconazole; T5- HexaconazoleT6- Metalaxyl + Mancozeb; T7- Mancozeb; T9- Propineb; T12- Carbendazim + Mancozeb

Fig.1. Concentration of most effective fungicides for complete inhibition of mycelial growth of *C. gloeosporioides*

The evaluation of fungicides at lower concentrations showed that *C. gloeosporioides* was most sensitive to difenoconazole (Score). The fungicides, hexaconazole (Contaf), metalaxyl-M+ Mancozeb (Ridomil Gold), mancozeb (Indofil M-45) and carbendazim+ mancozeb (Sprint) could yield complete inhibition of the growth of *C. gloeosporioides* at concentrations below 100 ppm (Fig. 1).

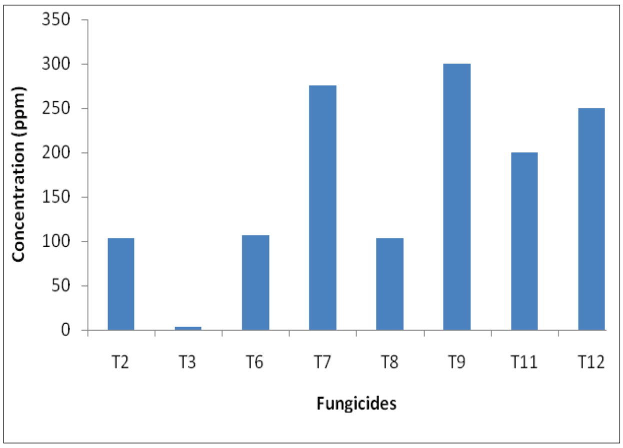
The fungicide, hexaconazole showed 100% inhibition of *C. gloeosporioides* (Pruthviraj et al., 2024; Kadlag et al., 2024). Hexaconazole @ 0.1% completely inhibited the growth of *C. capsici,* causing anthracnose in chilli (Tiwari et al., 2023). The effectiveness of the fungicide difenoconazole in inhibiting mycelial growth of *Colletotrichum* sp causing anthracnose in orange was verified (Guillén-Carvajal et al., 2023). High efficiency of foliar application of carbendazim + mancozeb (@0.15-0.2%) was reported by many workers (Nandeesha, 2023; Kadlag et al., 2024). The present study differs with few earlier reports regarding the inhibitory action of the fungicides propineb, mancozeb and copper oxy chloride. When thirteen fungicides were tested at different concentrations against *C. capsici*, the minimum inhibition at 0.05% was recorded with Propineb (Padghan et al., 2023). Likewise, relatively low activity in field was also reported with Propineb is reported (Nandeesha, 2023). In the present study, propineb at 400 ppm could offer complete inhibition of mycelial growth. But, in contrast to the result of the study, low activity of Mancozeb (0.25%) is reported by Kadlag et al. (2024). Copper oxychloride was found effective against different species of *Colletotrichum* (Tiwari et al., 2023; Pruthviraj et al., 2024; Kadlag et al., 2024). In our study, only with the highest concentration (1600 ppm), copper oxychloride could give 100% mycelial growth inhibition. Sensitivity varies with the species and isolates and that may be the reason for getting contradicting results.

Sensitivity of *P. colocasiae* to various fungicides

At 100 ppm, the mycelial growth inhibition by different fungicides ranged from 2.98% (hexaconazole) to 100% (cymoxanil+famoxadone). At the lowest concentration, only cymoxanil+famoxadone (Equation Pro) was able to completely inhibit the mycelial growth of *P. colocasiae* (Table.3). At 200 ppm, four more fungicides, metalaxyl- M+ chlorothalonil, metalaxyl - M+ mancozeb, cymoxanil+mancozeb and copper oxychloride completely inhibited the growth. At 400 ppm, 75% of the fungicides tested completely arrested the growth and the least inhibition was with carbendazim (57.5%). The same trend continued with 800 ppm also and at highest concentration of 1600 ppm, none of fungicides allowed *P. colocasiae* to grow.

The evaluation of eight fungicides at lower concentrations showed that *P. colocasiae* was most sensitive to cymoxanil+ famoxadone (Equation Pro). Complete arresting of mycelial growth was observed at very low concentration (3.125 ppm). It was followed by metalaxyl-M+ chlorothalonil and cymoxanil +mancozeb (103.125

ppm) (Fig. 2). The efficiency of these fungicides to combat different species of *Phytophthora* on various crops was reported. Bio-efficacy of metalaxyl M+ chlorothalonil (Folio-Gold) was on par with the recommended schedule involving metalaxyl- mancozeb to tackle *P. parasitica* var. *nicotianae* causing black shank in FCV tobacco nurseries and can form as an alternative to recommended schedule (Ramakrishnan et al., 2018). Highest inhibition of both mycelial growth and sporangia formation of *P. infestans* were noted with chlorothalonil + metalaxyl (Islam et al., 2018).



T2- Metalaxyl + Chlorothalonil; T3- Cymoxanil + Famoxadone; T6- Metalaxyl + Mancozeb, T7- Mancozeb; T8- Cymoxanil + Mancozeb; T9- Propineb; T11- Copper oxychloride; T12- Carbendazim + Mancozeb

Fig. 2. Concentration of promising fungicides for complete inhibition of mycelial growth of *P. colocasiae*

In the study, the fungicides with metalaxyl-M were highly inhibitory to the pathogen and showed 100% inhibition at 200 ppm. Metalaxyl has been used regularly for the management of Phytophthora diseases (Dann and McLeod, 2021). The treatment of raspberry plants with metalaxyl-M was more effective compared to other fungicides in reducing Phytophthora root rot and wilt and improving plant vigor (Sapkota et al., 2023). Metalaxyl showed very negligible or little effect on *P. infestans.* However, metalaxyl when associated with mancozeb inhibited mycelial growth and sporangia formation (Islam et al., 2018a). All isolates of *P. sojae* causing Phytophthora root and stem rot (PRR) in soy bean were sensitive to mefenoxam and metalaxyl (Daniel et al., 2023).The fungicide cymoxanil (CMX) is used in the treatment of plant pathogen, *P. infestans (*Kazmirchuk et al., 2024). Minimum late blight incidence was observed in mancozeb-cymoxanil + mancozeb based scheduling which was found on par with chlorothalonil-famoxadone + cymoxanil based scheduling (Mhatre et al., 2020).

The fungicides with mancozeb alone and its combinations showed 100% inhibition at 200-400 ppm. Mancozeb and copper are commonly used to protect taros from damage (Singh et al., 2012). Mancozeb controls numerous fungal diseases in a wide range of field crops, fruits, nuts, vegetables, and ornamentals, including late blight in potatoes and tomatoes (Naim et al., 2023). The result of the study with the fungicides azoxystrobin and carbendazim indicated its low efficacy in checking the mycelial growth of *P. colocasiae.* Azoxysrobin failed to inhibit mycelial growth of *P. cactorum* causing major damage to strawberry plants worldwide (Ali et al., 2022) and carbendazim showed very negligible or little effect on *P. infestans* (Islam et al., 2018b).

Sensitivity of *S. rolfsii* to various fungicides

Compared to other two pathogens, *S. rolfsii* was less sensitive to many of the fungicides evaluated (Table 4). The highest fungicidal action was exhibited by the fungicides, difenoconazole (Score) and hexaconazole (Contaf). Up to 800 ppm, only these two fungicides could completely arrest the mycelial growth of *S .rolfsii.* At the highest concentration, the fungicides, metalaxyl-mancozeb, cymoxanil+mancozeb and carbendazim+ mancozeb showed 100% inhibition. Copper oxychloride was the least effective one which showed only 47.39% inhibition even at 1600 ppm. All other fungicides showed >75% inhibition at a concentration of 1600 ppm. The fungicides, difenoconazole (Score) and hexaconazole (Contaf) were tested at lower concentrations (50, 25, 12.5, 6.25 and 3.125 ppm). Even at the lowest concentration of 3.125 ppm, these two fungicides completely inhibited mycelial growth of *S. rolfsii.* The findings of the study endorse the previous reports. Hexaconazole showed 100% mycelial growth suppression of *S. rolfsii* causing diseases like foot rot of finger millet (Bharath and Raveendra, 2021; Kamthe et al., 2023). Difenoconazole 25% (Score) was effective in controlling damping off and root rot diseases in Sugarbeet (Elnaggar and Yassin, 2023). Complete growth suppression by Carbendazim 12% + Mancozeb 63% was reported with many diseases (Kamthe et al., 2023), viz., collar rot in chick pea (Divyashree et al., 2024); seedling rot in rice (Nath and Patel., 2023) and foot rot of finger (Bharath and Raveendra, 2021). Metalaxyl + mancozeb inhibited germination of *S. rolfsii,* the pathogen causes white rot of tomato (Penghaing et al., 2022). Mancozeb 75% WP at recommended concentration was highly effective in suppressing radial expansion as well as percent inhibition of *S. rolfsii,* the pathogen responsible for damping off of seedlings in yard long bean (Kukulevithana et al.,2023). The commonly used fungicides, carbendazim and copper fungicides were almost ineffective in checking the growth of *S. rolfsii.* The less efficiency of Carbendazim 50% WP (Kukulevithana et al., 2023), copper hydroxide (Penghaing et al., 2022) and other copper fungicides (Singh et al.,2021) in checking *S. rolfsii* was reported.

Conclusion

As per the findings of the study, the fungicides, difenoconazole, hexaconazole and metalaxyl-M+ chlorothalonil can be used as alternatives to manage various diseases in tuber cops due to the pathogen, *C.gloeosporioides.* Management of diseases caused by *Phytophthora* spp under favourable condition remains as a difficult task. Use of fungicides, metalaxyl-M+ chlorothalonil (Folio Gold) and cymoxanil+ famoxadone (Equation Pro) can overcome the difficulty in managing taro leaf blight. Inhibition pattern shown by the fungicides, hexaconazole and difenoconazole against *S. rolfsii* looks promising and timely application of the fungicide can provide higher rate of protection than the existing management strategy.

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