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Morpho-molecular characterisation and *in vitro* analysis of chemical fungicides and bioagents for the integrated management of foliage fungal pathogens of *Philodendron* spp.

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Philodendron spp. are excellent ornamental foliage plants, which are well known for their attractive glossy leaves. Fungal infections on foliage plants affect the leaf quality and marketability of their leaves. Since these foliage plants are much valued for their leaves it is necessary to know about the etiology of disease and develop an integrated approach for disease management to reduce pesticide residues on these crops as well as in ecosystem environment. This led to the comparative analysis of chemical fungicides and bioagents for the integrated management of foliage fungal pathogens of *Philodendron* spp. in Kerala. The fungal isolates, obtained from diseased samples of *Philodendron burle-marxii* and *Philodendron bipinnatifidum* were identified as *Colletotrichum dracaeonophilum* and *Phytophthora nicotianae* based on morpho molecular characterisation. The *in vitro* evaluation studies revealed Bordeaux mixture as the best contact fungicide and hexaconazole 5EC and tebuconazole 25.9EC as the efficient systemic fungicides with complete inhibition against the pathogens. Analysis of combination fungicides recorded carbendazim 12% + mancozeb 64% and tebuconazole 50% + trifloxystrobin 25% as the potential inhibitors of *C.dracaeonophilum*. The oomycete member, *P. nicotianae* showed complete inhibition on treatment with all tested fungicides except chlorothalonil 75%WP. Dual culture analysis revealed *Trichoderma asperellum* as the most competing antagonist against pathogens with cent per cent inhibition. Plant growth promoting rhizobacteria (PGPR II, KAU) showed complete control over *P. nicotianae* and plant growth promoting microorganism (PGPM, KAU) exhibited an inhibition of 78.88 per cent against *C. dracaenophilum*.

Keywords: Dual culture, *In vitro* evaluation, per cent inhibition, *Philodendron*, poisoned food technique

INTRODUCTION

Philodendron, the second largest genus of the Araceae family, comprises 489 plant species that are widely distributed in the humid tropical forests of America and the West Indies.

It is an epiphytic, hemi-epiphytic or terrestrial plant that survives low light conditions and grows well in a temperature range of 15 to 18^{0}C. The word 'Philodendron' is derived from two Greek words, 'Philo' and 'dendron', which means 'love' and 'tree' respectively. The morphological diversity among different species and their growth habits make them a potential component in the ornamental foliage industry. These foliage plants, with glossy, elegant shapes and brilliant colours are greatly valued and suitable as desk plants, hanging baskets, totems, or floor plants (Mc Connell *et al*. 2003). Among different foliage plants, Philodendron stands in first place in production and shares 50 and 36 per cent of wholesale values in 1956 and 1967 respectively. Since ornamental foliage plants have wide acceptance, the presence of diverse diseases which rapidly diminishes their quality is major constraint in their trade. Hence being an export oriented industry, it is highly pertinent to investigate the etiology of foliar disease to develop suitable management measures for these diseases.

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Xue *et al*. (2020) reported *Colletotrichum* spp. as the emerging pathogen in diverse *Philodendron* species. *Colletotrichum philodendricola*, *Colletotrichum pseudoboninense* and *Colletotrichum orchidearum* are the prominent species isolated from *Philodendron tatei* cv. Congo. *Colletotrichum siamense* was reported for the first time as the leaf spot causing pathogen on *Philodendron bipinnatifidum* in Guangxi province of China by Ning *et al*. (2022). Zhang *et al*. (2023) described a leaf spot disease in *Philodendron bipinnatifidum* with irregular sunken, necrotic lesions with 80 per cent disease incidence and identitifed *Colletotrichum karsti* and *C. endophytica* as the causal organisms. The oomycete member, *Phytophthora nicotianae* var. *parasitica* is reported as a major pathogen which infect the leaves and stem cuttings of *Philodendron oxycardium*, *P. selloum* and *P. panduraeforme* with characteristic water soaking and subsequent drying. Cundom and Cabrera (2004) reported *Phytophthora capsici* as the pathogen causing leaf spot symptoms on *Philodendron scandens* subsp. *oxycardium* in Argentina. Since foliar fungal pathogens were recognised as the potential threat to the largescale production of *Philodendron*, efficient and ecofriendly measures should be adopted to manage these pathogens. The specific knowledge on the etiology is necessary for the development of pathogen specific, cost effective eco-friendly management measures.Therefore, this study aims to identify the etiology of foliar diseases through morpho-molecular analysis and forms baseline research to identify the most effective chemical fungicides and biocontrol agents against the foliar pathogens of *Philodendron* plants.

MATERIALS AND METHODS

Collection, isolation and pathogenicity studies

Philodendron spp. with characteristic foliar disease symptoms were collected from commercial nurseries of Thrissur (10^{0}C56 8.9 N / 76^{0}C26' 8.4" E), Ernakulam (10°56'8.9" N / 76°26'8.4" E) and Thiruvananthapuram (8^{0}C39' 0.8" N / 77^{0}C04' 4.5" E) districts of Kerla during April-June of 2022. Symptoms of leaf blights and leaf spots were recorded and symptomatic plants were collected for the isolation of causal agents.

Pathogens associated with diseased samples were isolated by tissue segmentation method (Rangaswamy, 1958). The surface sterilized leaf bits were transferred to potato dextrose agar (PDA) medium (3 bits/ dish) in sterile Petri plates and incubated at room temperature (25 ± 2^{0}C). Further purification of each isolate was carried out by transferring a single hyphal tip of them to sterilised PDA medium (Brown, 1924). For long term storage, pure cultures of the pathogens were maintained by repeated subculturing at specific intervals and were stored at 4^{0}C under refrigerated conditions.

Pathogenicity of each isolate was confirmed by proving Koch's postulates by artificial inoculation of the pathogen on healthy detached Philodendron leaves of respective cultivars. The pathogen was reisolated from symptomatic leaves and its identity was confirmed by comparing the cultural and morphological characteristics and alsoby sequencing LSU regions.

Characterization of pathogens

Individual fungal pathogens of Philodendron were identified up to genus level based on the cultural and morphological features and further species level confirmation was done by amplifying the LSU sequences. Culture discs (8mm) of pathogens were aseptically transferred to Potato Dextrose Agar medium (PDA) in sterile Petri dishes and kept for incubation at 25 ± 2^{0}C. Cultural characters like colony colour, texture, pigmentations, growth rate, formation of fruiting bodies and sporulation were recorded. Microscopic observations of hyphal colour, hyphal septation, spore shape, spore colour and septations were observed and compared with the Commonwealth Mycological Institute's descriptions of pathogenic fungi (CMI, 1964). Species level identification of pathogens was done by comparing the amplified Large Sub Unit sequences of fungal pathogens with the nucleotide sequences in NCBI BLASTn database. The DNA isolation was carried out and Large Sub Unit nucleotide sequences of the pathogen were amplified using LROR (5'ACCCGCTGAACTTAAGC3') and LR7

(5'TACTACCACCAAGATCT3') forward and reverse primers and *in silico* analysis of the retrieved sequences performed for the species level identification of pathogen.

In vitro efficacy assay of chemical fungicides against fungal pathogens

The inhibitory effect of different contact, systemic and combination fungicides on colony growth of the fungal pathogens of Philodendron was assessed by poisoned food technique using different concentrations of fungicides as given in Table1. Mycelial growth inhibition of pathogen in the media amended with different concentrations of toxicants were recorded separately. Different test dosages of chemical fungicides were mixed separately in 100 ml sterilized molten PDA and 8 mm mycelial plugs of the test pathogen was transferred to the fungicide amended media.

In vitro evaluation of Oomycete pathogen was done against selected fungicides viz., mancozeb 75%WP (Indofil M-45), copper hydroxide 53.8% DF (Kocide), chlorothalonil 75% WP (Kavach) and cymoxanil 8% + mancozeb 64% WP (Curzate) and Bordeaux mixture (Table 2). The experiment was conducted in completely randomized design (CRD) with four replications. Per cent inhibition of the mycelial growth of test pathogen was worked out as per Vincent *et al*. (1927): Percent inhibition of growth $= \frac{C-1}{C}$

where C – Growth of fungus in control plate (cm) , T – Growth of fungus in treatment (cm)

Evaluation of potential biocontrol agents

The efficacy of fungal biocontrol agent, *Trichoderma asperellum* (KAU reference culture) and the bacterial antagonist, *Pseudomonas fluorescens* (KAU reference culture) were evaluated by dual culture assay (Johnson and Curl, 1972).The inhibition of the growth of the pathogen by the antagonist was calculated as per Vincent *et al*. (1927). The interaction between pathogen and antagonist was detailed in comparison with the keys suggested by Webber and Hedger (1986).

Evaluation of PGPM and PGPR- II microbial consortia against fungal pathogens

Efficacy of the microbial consortium of PGPM (Plant Growth Promoting Microorganism, KAU) and PGPR II (Plant Growth Promoting Rhizobacteria, KAU) were evaluated against the pathogens of *Philodendron* by poisoned food technique (Nene and Thapliyal, 2018). Observations on the radial growth of pathogens was recorded and inhibition in the growth of pathogen were computed using above mentioned formula.

RESULTS AND DISCUSSION

During April to June of the year 2022, *Philodendron* leaf blight and leaf spot symptoms were frequently recorded from the home gardens and commercial nurseries of Thrissur and Thiruvananthapuram districts of Kerala.

The leaf blight sample of *Philodendron burle-marxii* collected from Thrissur appeared as initial small, brown, water-soaked necrotic lesion with prominent halo and resulted in subsequent defoliation (Fig. 1A). These symptoms were in accordance with the descriptions of anthracnose of *P. tatei* and *P. bipinnatifidum* (Xue *et al*. 2020; Ning *et al*. 2022; Zhang *et al*.2023). The *Philodendron bipinnatifidum* samples collected from Thiruvanathapuram, Kerala was distinguished by dark, water-soaked spots which gradually spread over larger area of the leaf lamina (Fig. 1C). The leaf spot documented were identical to the observations of earlier workers such as Baysal-Gurel *et al*. (2022) during Phytophthora infestation on *Philodendron oxycardium, Catharanthus roseus* and in different vegetables. Pathogens associated with each symptom were isolated by tissue segmentation method as mentioned in materials and methods and pathogenicity was established on healthy detached Philodendron leaves of the respective cultivars. Symptoms similar to the natural condition were developed within 3 to 4 days of incubation on inoculated leaves (Fig 1B, 1D) and symptomless in control.

Characterization of pathogens

Based on colony characteristics and morphological features, foliar pathogens of

Table1: Concentrations of chemical fungicides used for in vitro evaluation

Fungicide	Concentration (Per cent)
Mancozeb 75% WP (Indofil)	0.2, 0.25, 0.3
Copper hydroxide 53.8% DF (Kocide)	0.1, 0.2, 0.3
Propineb 70% WP (Proximain)	0.1, 0.2, 0.3
Chlorothalonil 75% WP (Kavach)	0.1, 0.2, 0.3
Hexaconazole 5% EC (Contaf)	0.1, 0.15, 0.2
Tebuconazole 25.9% EC (Orius)	0.05, 0.1, 0.15
Difenoconazole 25.0% EC (Score)	0.1, 0.15, 0.2
Carbendazim 50%WP (Bavistin)	0.05, 0.1, 0.2
Carbendazim 12% + Mancozeb 63% W (SAAF)	0.1, 0.2, 0.3
Tebuconazole 50% + Trifloxystrobin 25% WG (Nativo)	0.03, 0.04, 0.05
Azoxystrobin 18.2% + Difenoconazole 11.4% SC (Amistar top)	0.05, 0.1, 0.2
Bordeaux mixture	1

Table. 2 : Fungicides and concentrations for *in vitro* evaluation studies against oomycete

Fungicide	Concentration (Per cent)
Mancozeb 75WP (Indofil M-45)	0.2,0.25,0.3
Copper hydroxide 53.8DF (Kocide)	0.1, 0.2, 0.3
Chlorothalonil 75WP(Kavach)	0.1, 0.2, 0.3
Cymoxanil 8%+ Mancozeb 64WP (Curzate)	0.1,0.25,0.3
Bordeaux mixture	1

Table 3: Inhibition of mycelial growth of fungal pathogens by biocontrol agents

Fungal pathogens	Per cent inhibition of fungal pathogens by			
	Trichoderma asperellum	*Pseudomonas fluorescens*	PGPM	PGPR
Colletotrichum gloeosporioides	100	6.11	78.88	75.55
Phytophthora nicotianae	100	0	80.00	100

Philodendron burle-marxii and *Philodendron bipinnatifidum* samples were identified as *Colletotrichum* sp. and *Phytophthora* sp. and further confirmation and species level identification were done by *in silico* analysis of amplified LSU sequences.

The fungal colony of *Colletotrichum* sp. on PDA medium formed greyish white, woolly, aerial mycelia initially which subsequently turned grey on upper side, greyish black on underside of the Petridish and recorded a growth rate of 1.12 cm per day (Fig 2A, 2B).Acervuli produced were black, globular with salmon orange coloured spore mass on the mycelial surface. Hyphae hyaline, septate, branched and conidia were non-septate, hyaline, bullet shaped with dimensions of 6.4 - 7.1 µm x 2.5 - 3.1 µm. The characters of this leaf blight pathogen was identical to micro-morphological features and cultural characteristics of pathogen described by Weir *et al*.(2012) and Xue *et al*. (2020). Amplified LSU sequences (1315bp) of pathogen were analysed with the nucleotide sequences in nBLAST database and observed 99.24 per cent similarity with *C. dracaenophilum* with accession number DQ286210.1 and 99.16% similarity with DQ286208.1. The sequences were deposited in NCBI database with accession number OR617090. Pathogenic nature of *C. dracaenophilum* causing anthracnose disease in plants of Agavaceae family was described by Farr *et al*. (2006), Bobev*et al*. (2008) and Macedo and Barreto (2016).

Colonies of *Phytophthora* sp. on PDA medium were delicate, bright white with fluffy aerial

Fig. 1: Leaf blight and leaf spot symptoms under natural and artificial conditions. Marginal necrosis with yellow halo symptom on *Philodendron burl max* (**A**), necrotic lesion with halo produced under artificial condition on *P. burle-marxii*(**B**), dark water soaked lesion on *P. bipinnatifidum* (**C**), water soaked lesion on artificially inoculated leaves of *P. bipinnatifidum*

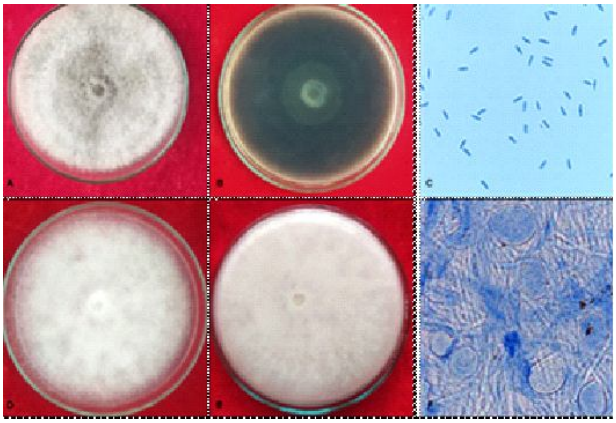

Fig. 2: Fungal colony on PDA medium and morphological features. Upper surface of *Colletotrichum* sp. colony on PDA (**A**), reverse side of the culture plate (**B**), hyaline, single celled and guttulate conidia at x 40 (**C**), upper surface of *Phytophthora* sp (**D**), reverse side of the culture plate (**E**), hyaline, globose to sub globose, papillate to non-papillate sporangia at x 40 (**F**)

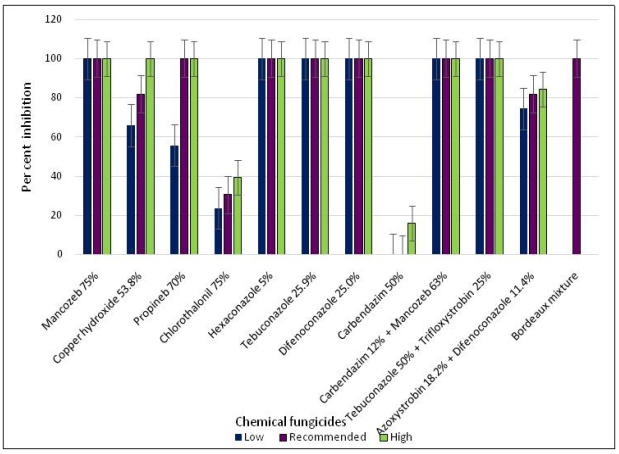

Fig. 3: Efficacy of chemical fungicides against *Colletotrichum dracaenophilum*

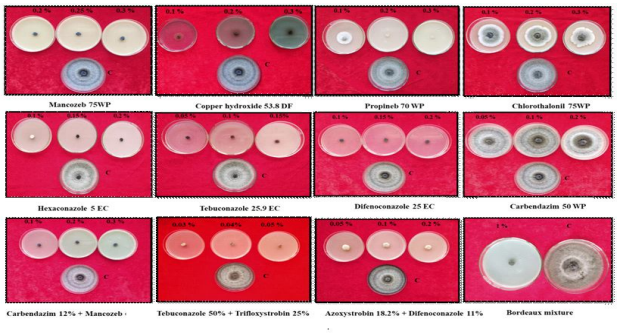

Fig 4: *In vitro* evaluation of chemical fungicides against *Colletotrichum dracaenophilum*

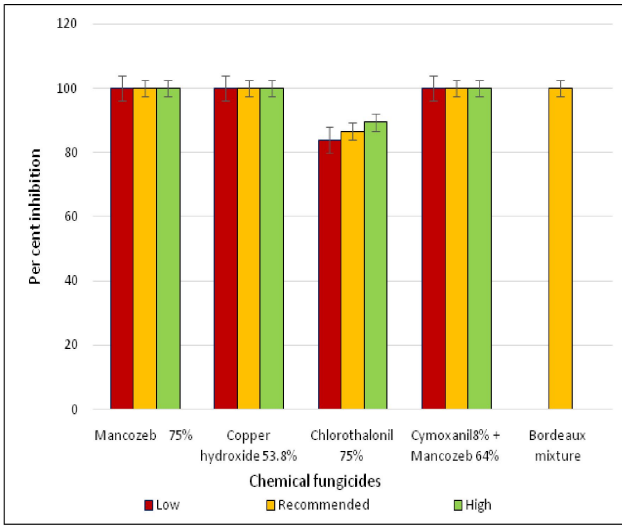

Fig.5: Efficacyof fungicides against *Phytophthora nicotianae*

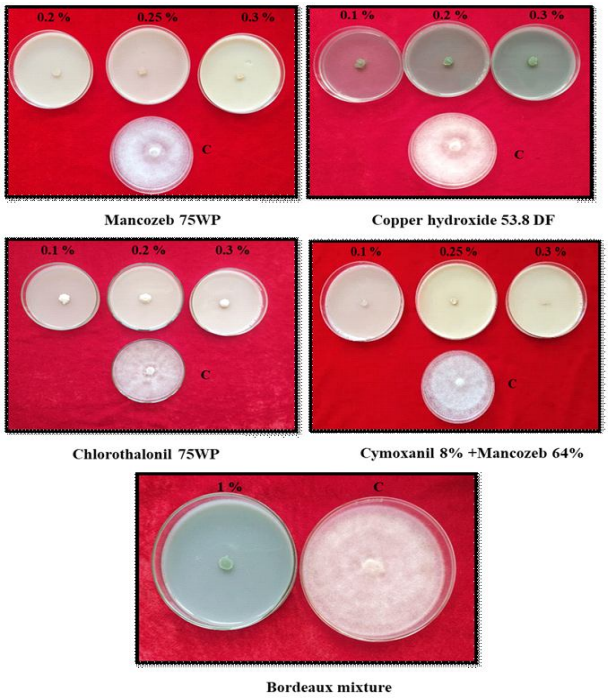

Fig. 6: *In vitro* evaluation of chemical fungicides against *Phytophthora nicotianae*

mycelium. Hyphae hyaline, aseptate, branched and sporangia were hyaline, globose to sub globose, papillate to non-papillate along with sporangiophores and 18-25µm × 12-16µm dimension. Similar morphological and cultural features were recorded by previous authors in Phytophthora leaf spot pathogen of *Philodendron oxycardium*. Species level identification was done by comparing the retrieved LSU nucleotide sequences (836bp) with the sequences available in NCBI database. The results of the BLAST analysis data showed 99.64 per cent identity and 100 per cent query coverage with the different *Phytophthora nicotianae* isolates viz., KX250521.1, KX250514.1, OP738518.1, OP738517.1, and OP738516.1 and deposited the LSU sequences with accession number OQ402226.

In vitro efficacy assay of chemical fungicides against C. dracaeonophilum

The fungicidal action of 11 chemical fungicides at three different test dosages and Bordeaux mixture at 1% concentration were assessed against *C. dracaenophilum* by poisoned food technique. The chemicals viz. mancozeb 75WP, copper hydroxide 53.8DF, propineb 70WP, chlorothalonil 78.12WP, difenoconazole 25EC, hexaconazole 5EC, tebuconazole 25.9EC, carbendazim 50WP, carbendazim 12% + mancozeb 64% WP, cymoxanil 8% + mancozeb 64% WP, tebuconazole 50% + trifloxystrobin 25% WG, azoxystrobin 18.2% + difenoconazole 11.4% SC and Bordeaux mixture (1%) at specified concentrations. Significant reduction in the mycelial growth of *C. dracenophilum* was recorded during the *in vitro* evaluation of chemical fungicides at different test concentrations (Fig.3).

Complete inhibition over the pathogen was achieved by all the combination fungicides under study, except azoxystrobin 18.2% + difenoconazole 11.4% (74-85%) and by all triazole fungicides (hexaconazole 5EC, tebuconazole 25.9 EC and difenoconazole 25 EC). Sterol biosynthesis pathway of fungal pathogens were inhibited by the triazole fungicides which is an integral component in fungal cell membrane (Nabi *et al*. 2017). Among the contact fungicides, mancozeb 75WP (0.2, 0.25 and 0.3 %), Bordeaux mixture (1%) and propineb at 0.2 and 0.3 per cent concentrations exhibited cent per cent inhibition of pathogen in fungicide amended medium. Chlorothalonil was least effective contact fungicide with an inhibition of 23.75 per cent at 0.3 per cent concentration (Fig.4). Dithiocarbamate fungicides like mancozeb inhibit the fungal growth by disrupting the sulphydryl groups of several enzymes which subsequently interfere several biochemical processes taking place in the cytoplasm (Gullino *et al*. 2010).

Compared to the other chemical fungicides, carbendazim 50WP at 0.05 and 0.1 per cent dosages recorded zero inhibition and showed only 16.11 per cent inhibition in 0.2 per cent concentration of the chemical. Rulin *et al*. (2005) also recorded the insensitivity of carbendazim fungicide against *C. gloeosporioides* isolates of mango fruit under *in vitro* conditions which was in line with present result. Resistance in *Colletotrichum coffeanum* against the fungicides belonging to methyl benzimidazol carbamate (Carbendazim) have also been reported earlier during the field studies conducted in coffee plantation of Kenya.

In vitro efficacy assay of fungicides against Phytophthora nicotianae

Being oomycete, five chemical fungicides previously reported as inhibitory to the pathogen were selected for evaluation of their efficacy. The pathogen recorded cent per cent inhibition in all the tested chemicals at all concentrations except chlorothalonil 75 WP with per cent inhibition of 83.89 to 89.44 per cent at tested concentrations (Fig.5 and Fig 6). Corroborating with this findings Gupta and Jarial (2010), recorded cent per cent inhibition in the growth of the pathogen on treatment with mancozeb and cymoxanil 8% + mancozeb 64%. The efficacy of the Bordeaux mixture and copper hydroxide was in confirmation with the studies of Thomas and Naik (2017) and Ajayi (2019) respectively.

In vitro assessment of Trichoderma asperellum and Pseudomonas fluorescens against fungal pathogens

The antagonistic activity of the fungal biocontrol agent, *T. asperellum* (KAU reference culture) and

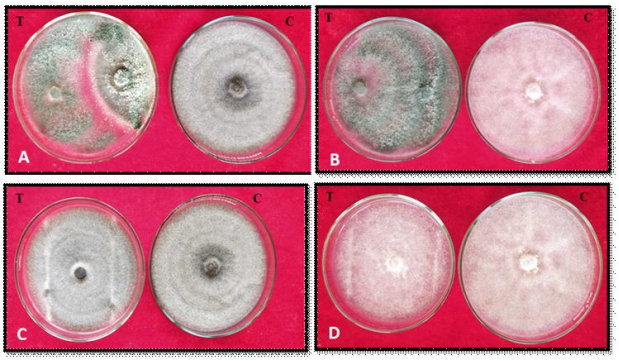

Fig.7: *In vitro* efficacy of *Trichoderma asperellum* against *Colletotrichum dracaenophilum* (A),*Phytophthora nicotianae* (B),*In vitro* efficacy of *Pseudomonas fluorescens* against *Colletotrichum dracaenophilum* (C) and *Phytophthora nicotianae* (D)

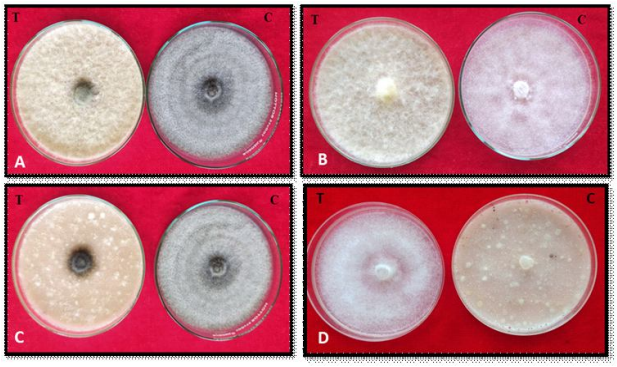

Fig 8: *In vitro* efficacy of PGPM against *Colletotrichum dracaenophilum* (A), *Phytophthora nicotianae* (B). *In vitro* efficacy of PGPR II against *Colletotrichum dracaenophilum* (C) and *Phytophthora nicotianae* (D)

bacterial antagonist *P. fluorescens* (KAU reference culture) were evaluated against the pathogens of *Philodendron* spp. (Table3). Complete inhibition in the growth of *Colletotrichum dracaenophilum* and *Phytophthora nicotianae* was recorded with *T. asperellum* in which antagonist inhibited pathogens by the mechanism of overgrowth (Fig.7). In congruence with the present study Rafi (2021) and Mohan (2020) recorded complete inhibition in the mycelial growth of *C. gloeosporoides (*anthurium*)* and *C. brevisporum (*passion fruit*)* on treatment with *T. asperellum.* Dev *et al*. (2016), also recorded complete inhibition in the growth of anthracnose pathogen of pomegranate by different *T. viride* isolates.

The bacterial biocontrol agent, *Pseudomonas fluorescens* proved ineffective against *Phytophthora nicotianae* (0%) and recorded only 6.11 per cent inhibition against *Colletotrichum dracaenophilum*. On contrary, several researchers have reported the inhibitory effect of *P. fluorescens* against the fungal pathogens. Jagpat *et al*. (2012) and Chlebek *et al*. (2020) recorded 81.25 and 39.99 per cent inhibition against *Phytophthora* spp. and *Colletotrichum dematium* respectively. The difference in the bacterial biocontrol agent and pathogens used may have influenced, the results of the present *in vitro* studies.

In vitro assessment of microbial consortia against fungal pathogens

In vitro evaluation of consortium of plant growth promoting microorganism (PGPM) and plant growth promoting rhizobacteria (PGPR) against *Colletotrichum dracaenophilum* and *Phytophthora nicotianae* reavealed significant mycelial growth reduction (Table 3). PGPM found as the potential biocontrol agent against *C. dracaenophilum* with 78.88 per cent inhibition followed by PGPR (75.55%) (Fig.8).

Phytophthora *nicotianae* recorded complete inhibition with PGPR and 80 per cent inhibition with PGPM. According to the studies, plant growth promoting rhizobacteria act as a potent inhibitor of plant pathogens by the mechanism of parasitism, hyper parasitism, competition, and by inducing resistance in plants (Kaymak, 2010; Al- Ani, 2017; Verma *et al*. 2019).

CONCLUSION

The research work enlightened the knowledge about etiology of common fungal infections of *Philodendron* spp., *Philodendron bipinnatifidum* and *P. burle-marxii.C. draceophilum* and *P. nicotiane* were identified as the pathogens responsible for leaf blight symptoms. The *in vitro* evaluation of chemical fungicides and biocontrol agents against the pathogens reflected Bordeaux mixture, hexaconazole 5EC, tebuconazole 25.9 EC, carbendazim 12% + mancozeb 64% and tebuconazole 50% + trifloxystrobin 25% as the potential inhibitors of *C. dracaenophilum* and all the fungicides assessed except chlorothalonil were identified as the promising fungicide against the oomycete pathogen, *P. nicotianae*. Among the bioagents, *Trichoderma asperellum* was the most potential antagonist followed by PGPM and PGPR.

The integrated disease management approach with chemicals and bioagent after identifying the pathogen should be adopted as a viable and ecofriendly strategy to combat the pathogen. The current piece of work can be utilised for the identification of promising fungicides for disease management in *Philodendron* under field conditions and can make significant progress in disease management in ornamental foliage industry against devastating diseases of foliage plants.

DECLARATIONS

Conflict of Interest. Authors declare no conflict of interest.

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