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Impact of Fungicides and Biocontrol Agents in Managing Peduncle Blight of Tuberose Caused by *Lasiodiplodia theobromae* (Pat.) Griffon and Maubl

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Abstract: Peduncle blight, hitherto an unknown disease, was found to be a major limiting factor to the cultivation of tuberose, as the disease incidence was noticed up to 42.60% in pockets of Madurai district. Though, *Lasiodiplodia theobromae* is an ubiquitous pathogen, its occurrence on tuberose is a new record. The fungus induced confounding symptoms which included blossom blight, peduncle blight and leaf blight at tips as well. The causal agent of the disease was identified as *Lasiodiplodia theobromae*. The efficacy of fungicides and biocontrol agents effective in *in vitro* was evaluated in pot culture experiment to manage peduncle blight of tuberose. Foliar application of carbendazim 0.1% at 60, 90 and 110 Days After Planting (DAP) was found to be highly effective in reducing the disease incidence up to 95.50%. Among the bioconrol agents, bulb treatment at 10 g kg^{-1} followed by three foliar sprays at 0.5% on 60, 90 and 110 DAP using the combination of Tv_1, Pf_1 and Bs_{10} was equally effective as that of Pf_1 and Bs_{10} with 65.68 and 64.45% disease reduction, respectively.

Key words: *Lasiodiplodia theobromae*, fungicides, bio-control agents, tuberose

INTRODUCTION

Tuberose (*Polianthes tuberosa* Linn.) is one of the most important ornamental plants which is extensively cultivated in many sub-tropical and tropical areas of the world (Biswas *et al*., 2002). Tuberose is commercially cultivated for cut and loose flower trade and also for the extraction of its highly valued natural flower oil. Its blooms, besides cutflower, are extensively used in preparation of garlands, bouquets and floral ornaments for bridal make up. Apart from domestic consumption, cut spikes of tuberose have a good export potential. Diseases appear to be the major constraints to the production of tuberose. Peduncle blight, hitherto an unknown disease, was found to be a limiting factor in the cultivation of tuberose. The causal organism *Lasidiplodia theobromae* was found to be associated with this disease, producing blossom blight, peduncle blight and blighting of leaf tips (Durgadevi and Sankaralingam, 2012). This fungus has a wide host range and is found throughout the tropics and subtropics (Punithalingam, 1980). The present study was undertaken to manage peduncle blight of tuberose using fungicides.

MATERIALS AND METHODS

Isolation of pathogen: The pathogen causing peduncle blight in tuberose was isolated from the samples by tissue segment method on Potato Dextrose Agar (PDA) and the fungus was purified by single spore isolation and maintained on PDA. The causal organism was identified based on spore morphology and confirmed further (ID.NO. 6751/11) by Indian Type Culture Collection Centre (ITCC) of Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi.

Pathogenicity in glasshouse: The pathogenicity of the fungus was confirmed by Koch's postulates using five numbers of four-month-old healthy plants. Plants were inoculated by making a vertical cut (3 mm) in the peduncle region below the calyx using a sterilized needle and placing a fungal disc over the wound. The inoculated area was covered with moist cotton and wrapped with parafilm. The plants were covered with polythene bags to maintain humidity and monitored for symptom expression. Proper controls were maintained with PDA plugs.

Screening of fungicides *in vitro*: The relative efficacy of six fungicides viz., Kocide 1011 (35% metallic copper), copper oxychloride, cholorothalonil, carbendazim, azoxystrobin and tebuconazole each at four concentrations (0.05, 0.1, 0.15, 0.2%) was tested against *B. theobromae* under laboratory condition. The name of fungicides and their chemical names and doses are given in Table 1.

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Table 1: Fungicides used for *in vitro* evaluation against *Lasiodiplodia theobromae*

Fungicides	Chemical name	Dose (%)
Kocide	Cupric hydroxide	0.2
BLITOX	Copper oxy choloride	0.2
Cholorothalonil	Tetrachloroisophthalonitrile	0.2
Tebuconazole	(RS)- 1-(4-Chlorophenyl)- 4,4-dimethyl-3-(1H, 1, 2, 4-triazol-1-ylmethyl)pentan- 3-ol	0.1
Azoxystrobin	Methyl (2E)-2-(2-{[6-(2-cyanophenoxy)pyrimidin-4-yl]oxy}phenyl)-3-methoxyacrylate	0.1
Carbendazim	Methyl benzimidazol-2-ylcarbamate	0.1

For this purpose, poisoned food technique devised by Schmitz (1930) was followed. The requisite quantities of fungicides (0.05, 0.1, 0.15, 0.2%) were incorporated into two percent sterilized unsolidified potato dextrose agar and shaken well make it homogenous, medium was then poured into 90 mm sterilized petri dishes with three replications of each treatment with proper control and allowed to solidify. These dishes were inoculated with 9 mm diameter culture discs of 5 days old culture and these discs were placed in the center of the petri dishes. The petri dishes were incubated at 28±1°C for 5 days. The fungal growth was measured after 5 days and percentage inhibition was calculated.

Isolation of biocontrol agents

Rhizosphere: One isolate of biocontrol agent each in *Bacillus* spp., fluorescent pseudomonads and *Trichoderma* spp. was isolated from the rhizosphere of tuberose and the other isolates were obtained from the Depatment of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore. Rhizosphere soil from tuberose was collected and the biocontrol agents were isolated by serial dilution (Pramer and Schmidt, 1956) using Trichoderma selective medium for *Trichoderma* spp., King's B medium for fluorescent pseudomonads and Nutrient Agar (NA) for *Bacillus* spp.

Screening of biocontrol agents *in vitro*: The antagonistic effect of the biocontrol agents viz., three isolates in each of fluroescent pseudomonads, *Bacillus* spp. and *Trichoderma* spp., were tested against *L. theobromae* by dual culture technique (Dennis and Webster, 1971). Five millilitre diameter mycelial disc of the pathogen was placed at one end of the petri plate containing PDA and the bacterial antagonist was streaked at the opposite end. Inoculation of the pathogen without antagonist served as control and each treatment was replicated three times. When the fungus attained full growth in the control plate, growth of the pathogen and inhibition zone were measured and percentage reduction in growth over control was calculated.

Screening of *Trichoderma* spp. against *L. theobromae*: *Trichoderma* spp. were screened by placing a 5 mm mycelial disc of *L. theobromae* at one end of the petri dish and placing a 5 mm diameter mycelial disc of *Trichoderma* at the opposite end. Simultaneous inoculation of pathogen and antagonists were followed. Inoculation of the pathogen without antagonist served as control and each treatment was replicated three times. When the fungus attained full growth in the control plate, growth of the pathogen and inhibition zone were measured and percentage reduction in growth over control was calculated.

Compatibility between biocontrol agents: The compatibility of antagonistic bacteria among themselves was tested by streaking the test bacterium vertically on one side of the NA medium and streaking the other bacterium horizontally. The growth of the bacteria were observed and recorded as positive or negative.

Formulation of biocontrol agents

Bacteria: The isolate of *P. fluorescens* viz., Pf1 and the isolate of *B. subtilis* viz., Bs10 that were found to be effective *in vitro*, were used to prepare talc based formulations. Four hundred millilitre of 72 h old bacterial culture in their respective medium with a population of 9×10^8 CFU mL^{-1} were mixed with 1 kg of talc containing 15 g of calcium carbonate and 10 g of CMC. Moisture content of the product was reduced to 20% by shade drying and it was packed in polythene bags for further use (Vidhyasekaran and Muthamilan, 1995).

Fungi: The isolate of *Trichoderme viridae* viz., Tv1 was cultured in sterilized molasses yeast medium for 10 days. The fungal biomass and broth containing spore concentration of 1×10^7 CFU mL^{-1} were mixed with talc at 1:2 ratio. The formulation was air dried and packed in polythene covers (Jeyarajan *et al*., 1994) and used for further study.

Glasshouse experiment: A pot culture experiment using three biocontrol agents viz., TV1, Pf1, Bs10, their combinations and the fungicides viz., carbendazim and tebuconazole was laid out with 11 treatments replicated three times in completely randomized design. A single bulb of tuberose was planted in each pot containing sterile potting medium (red soil:sand:manure at 1:1:1 w/w/w). The method of application included Bulb

Table 2: Effect of biocontrol agents and fungicides on peduncle blight of tuberose under glasshouse condition

Treatments	Disease incidence (%)	Reduction over control (%)
Tv_1 (BT+FS)	42.00	54.44
Pf_1 (BT+FS)	40.50	52.75
Bs_{10} (BT+FS)	38.00	57.25
Tv_1+Pf_1 (BT+FS)	37.40	57.92
Tv_1+Bs_{10} (BT+FS)	35.10	60.51
Pf_1+Bs_{10} (BT+FS)	31.60	64.45
Tv_1+Pf_1 + Bs_{10} (BT+FS)	30.50	65.68
Carbendazim (0.1%)-FS	4.00	95.50
Tebuconazole (0.1 %)-FS	8.00	91.00
Healthy control	0.00[a]	-
Infected control	88.89	-
CD	2.34	

BT: Bulb treatment, FS: Foliar spray at 60, 90 and 110 days after planting

Treatment (BT) and Foliar Spray (FS). Talc based bioformulations were applied at 10 g kg^{-1} bulb followed by three foliar sprays at 0.5% on 60, 90 and 110 DAP. The fungicides were applied at 0.1% on 60, 90 and 110 DAP. *B. theobromae* was inoculated by wound inoculation at the peduncle region in all the treated plants. Plants inoculated with the pathogen alone served as control. Healthy controls were also maintained. Disease incidence was recorded on 120 DAI and percentage disease incidence was calculated (Table 2).

Treatments:

- *Trichoderma viride* (Tv1): BT+FS
- *Pseudomonas fluorescens* (Pf1): BT+FS
- *Bacillus subtilis* (Bs10): BT+FS
- *Trichoderma viride* (Tv1)+*Pseudomonas fluorescens* (Pf1): BT+FS
- *Trichoderma viride* (Tv1)+*B. subtilis* (Bs10): BT+FS
- *P. fluorescens* (Pf1) + *B. subtilis* (Bs10): BT+FS
- *Trichoderma viride* (Tv1)+*P. fluorescens* (Pf1) +*B. subtilis* (Bs10): BT+FS
- Carbendazim (0.1%): FS
- Tebuconazole (0.1%): FS
- Pathogen-inoculated control
- Healthy control

RESULTS AND DISCUSSION

The efficacy of biocontrol agents and fungicides to manage peduncle blight was evaluated in a pot culture experiment (Fig. 1a, b).

Out of six chemicals screened *in vitro* systemics, tebuconazole and carbendazim inhibited the fungal growth completely. Both were effective even at 500 ppm. However, azoxystrobin at 500 and 2000 ppm was inhibitory to *L. theobromae* by 54.44 and 67.74%, respectively. There was a significant interaction between fungicide and concentration. Among the non-systemic fungicides, chlorothalonil was found to be effective against the fungal growth at all the concentrations.

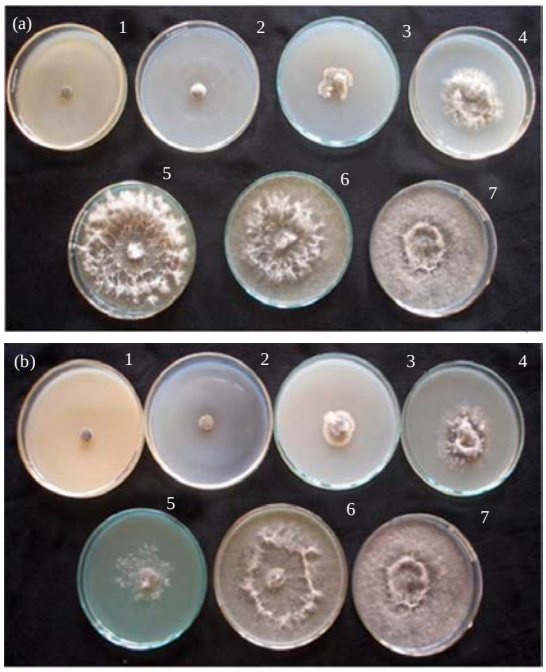

Fig. 1(a-b): Efficacy of fungicides, 1: Carbendazim, 2: Tebuconazole, 3: Azoxystrobin, 4: Cholorotahlonill, 5: Copper oxy choloride, 6: Kocide and 7: Control on the growth of *Lasiodiplodia theobromae* on, (a) 500 ppm and (b) 1500 ppm

The inhibition at 500 ppm was 67.00% while, the same at 2000 ppm was 78.56% (Table 3). Tebuconazole and carbendazim were found to be significantly superior compare to control in inhibiting the fungal growth. These observation are similar to the findings of Rakholiya *et al.* (2009) and Shah and Verma (2009) for *B. theobromae*.

Among the 11 treatments, carbendazim 0.1% (T_8) was found to be highly effective with the least disease incidence of 4.00% which indicated 95.50 reduction over control. Carbendazim was followed by tebuconazole

Table 3: Inhibitory effect of various fungicides against the growth of *Lasiodiplodia theobromae* in bio assay test

	Mycelial growth (cm)/Reduction over control				
	500	1000	1500	2000	
Fungicides	----------(ppm)----------				Mean
Kocide	9.00	8.20	8.00	7.80	2.94
	(3.00)	(2.96)	(2.92)	(2.89)	
	0.00	8.89	11.11	13.33	
Copper oxychloride	9.00	9.00	4.83	4.70	2.59
	(3.00)	(3.00)	(2.19)	(2.16)	
	0.00	0.00	46.33	47.78	
Cholorothalonil	2.97	2.57	2.10	1.93	1.54
	(1.72)	(1.60)	(1.44)	(1.38)	
	67.00	71.14	76.67	78.56	
Tebuconazole	0.00	0.00	0.00	0.00	
	(0.70)	(0.70)	(0.70)	(0.70)	
	100.00	100.00	100.00	100.00	0.70
Azoxystrobin	4.10	3.37	3.03	2.93$^{\backslash}$	
	(2.02)	(1.83)	(1.74)	(1.71)	
	54.44	62.56	66.33	67.74	1.82
Carbendazim	0.00	0.00	0.00	0.00	
	(0.70)	(0.70)	(0.70)	(0.70)	
	100.00	100.00	100.00	100.00	0.70
Control	9.00	9.00	9.00	9.00	
	(3.00)	(3.00)	(3.00)	(3.00)	3.00
Mean	2.02	1.97	1.81	1.79	-

Figures in parentheses are square root transformed values, CD (p = 0.05), Fungicide = 0.01, Concentration = 0.84, Fungicide×Concentration = 0.03

Table 4: Biocontrol agents used for *in vitro* evaluation against *Lasiodiplodia theobromae*

Isolates	Source
Fluorescent pseudomonads	
Pf_1	Department of Plant Pathology, TNAU, Coimbatore
FP_7	Department of Plant Pathology, TNAU, Coimbatore
Pf_2	Rhizosphere, tuberose, Madurai
Bacillus subtilis	
Bs_{10}	Department of Plant Pathology, TNAU, Coimbatore
Bs_1	Rhizosphere, tuberose, Madurai
Bs_2	Rhizosphere, tuberose, Nilakottai
Trichoderma viride	
Tv_1	Department of Plant Pathology, TNAU, Coimbatore
Tv_2	Rhizosphere, tuberose, Madurai
Tv_3	Rhizosphere, tuberose, Nilakottai

(T_9) wherein disease incidence was 8.00% with a disease reduction of 91.00%. Both the treatments differed significantly from others (Table 4). The results pertaining to relative efficiency of spray fungicides and biocontrol agents in pot culture experiment indicated that all the fungicides and biocontrol agents proved superior to un sprayed check in reducing the peduncle blight infection. However, carbendazim 0.1% proved most effective in reducing the disease intensity followed by tebuconazole. These findings are in agreement with the observation made by Rakholiya *et al.* (2009). In field experiment, carbendazim was found to be highly effective in reducing the infection of *B. theobromae* in mango, suppressing dieback and wilt. Carbendazim 0.1% spray was found to protect custard apple against the infection by *B. theobromae*. Triazoles viz., difenconazole and tebuconazole at 0.1% were observed to reduce the twig infection and bark canker of pear incited by *B. theobromae* (Shah and Verma, 2009).

Among the biocontrol agents, treatment T_7 (Tv_1+Pf_1+Bs_{10}) recorded 30.50% disease incidence followed by T_6 (Pf_1 +Bs_{10}) with 31.60% incidence and both the treatments were on par. Bulb treatment and foliar spray with Tv_1 (T_1) recorded 42.00% disease incidence with the least disease reduction of 54.44%. Though, all the three isolates of *Bacillus* spp. inhibited the growth of *L. theobromae*, Bs_{10} was found to be more effective. Strains of *B. subtilis* have been reported to be inhibitory to *B. theobromae* by earlier workers (Okigbo, 2005; Swain and Ray, 2009; Swain *et al.*, 2008). The antagonistic activity of the three isolates of *P. fluorescens* against *L. theobromae* was found to vary and two isolates viz., Pf_1 and FP_7 were highly inhibitory to the pathogen. The isolate Pf_1 of *P. fluorescens* was the most effective bacterial biocontrol agent which inhibited the growth of *B. theobromae* up to 84.8% (Govindaiah *et al.*, 2003; Sharma *et al.*, 2009). Isolates of *T. viride* were highly inhibitory to *L. theobromae*. *T. harzianum* and *T. atroviridae* have been exploited in

the management of fruit rots caused by *B. theobromae* (Kexiang *et al.,* 2002). Pramod *et al.* (2007) observed that *Trichoderma* spp. was found to be effective against post-harvest rot of papaya caused by *B. theobromae.*

CONCLUSION

The current study concludes that fungicide like carbendazim and tebuconazole were found to be effective against peduncle blight disease of tuberose.

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