Biological Forum – An International Journal **15(8): 546-556(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

A New Disease of Mulberry, Pythium Soft Root Rot Managed through Biological, Botanical and Fungicidal Approaches, both *In-vitro* and Field Condition

Ravichandra[1*], Somasekhara Y.M.[2], Shalini M.[3] and Thimmareddy H.[4]

[1]Department of Plant Pathology, College of Agriculture, UAS,
GKVK, Bangalore (Karnataka), India.
[2]Professor Department of Plant Pathology, UAS,
GKVK, Bangalore (Karnataka), India.
[3]Assistant Professor (Horticulture), Agricultural Technology Information Center,
GKVK, UAS, Bangalore (Karnataka), India.
[4]Division Chief mariculture division KSSRDI,
Thalaghattapura, Bangalore (Karnataka), India.

(Corresponding author: Ravichandra[*])
(Received: 06 June 2023; Revised: 23 June 2023; Accepted: 22 July 2023; Published: 15 August 2023)
(Published by Research Trend)

ABSTRACT: Mulberry (*Morus* sp.) is an important commercial crop grown under varied climatic conditions ranging from temperate to tropical region of the world but mulberry cultivation has many hindrances in its cultivation, one such constraint is diseases that to soft root rot disease. Symptoms of the soft root rot disease manifests in yellowing sudden withering and drying of leaves starting from the bottom branches and spreads upwards, followed by defoliation and finally resulting in death of plants. The disease initiates in isolated patches later spreading throughout the mulberry field. Bark of root peels off easily and the plants die. The cortex of infected roots first turns brown and rot followed by darkening of xylem, conspicuous mucilaginous matrix covers the mulberry root and hence the name soft root. Disease incidence was 27.17 % in major mulberry growing area *i.e.*, Ramanagar district of Karnataka. Mulberry soft root rot disease, effective management is needed. Hence, *in vitro* and *in vivo* studies on efficacy of fungicides (contact, systemic and combi-product) having different mode of action was taken. The biological control method is contemplated as promising approach to management of soil borne diseases. Among the fungicides evaluated *in vitro* condition Captan (86.29 %), Mancozeb + Metalaxyl, Carbendazim + Mancozeb, Fenamidone + Mancozeb, Azoxystrobin + Mancozeb (94.44 %), and Tebuconazole (100 %) were found effective. Among botanicals, neem extract (14.02 %) had highest inhibition of the pathogen. *Trichoderma viride*-1 (95.54 %) and *Bacillus pumilis* (58.88 %) were effective in vitro conditions. The bio-agents *T. viride*-1 and *T. viride*-2 found effective in glasshouse and field conditions. The effective fungicides Mancozeb + Metalaxyl (0.1 %) and Captan (0.2 %) reduced disease under field conditions up to 31.96 and 27.45 per cent, respectively. These fungicides, botanicals and bio-agents can be exploited for the management of soft root rot disease of mulberry.

Keywords: Mulberry, Soft root rot disease, *Pythium* sp. Botanicals, bioagents.

INTRODUCTION

Mulberry (*Morus* sp.) is an important commercial crop grown under varied climatic conditions ranging from temperate to tropical region of the world. Mulberry plant has got a great economic importance due to its wide range of uses. The important economic part of the mulberry plant *i.e.* leaves are sole source of silkworm, Bombyx mori for the production of silk yarn. Mulberry leaves, quality and quantity decide the production of cocoon and silk by silkworms. Leaves of mulberry plant used as animal feed. It is bestowed with many nutritional benefits and medicinal values. Mulberry leaves are the rich source of protein, vitamin-C and vitamin-K. Also contains phytochemicals such as caumarins, flavonoid and phenols which helps in reducing cholesterol, blood pressure level in human body (Sheng-qin *et al.*, 2003 and Zhang *et al.*, 2009). Symptoms of the disease manifests in yellowing sudden withering and drying of leaves starting from the bottom branches and spreads upwards, followed by defoliation and finally resulting in death of plants. The disease initiates in isolated patches later spreading throughout the mulberry field. Bark of root peels off easily and the plants die. The cortex of infected roots first turns brown and rot followed by darkening of xylem. A Studies on Pythium Soft Root Rot Disease of Mulberry 3 conspicuous mucilaginous matrix covers the mulberry root and hence the name soft root. The exact cause of soft root rot disease in Karnataka is identified as Pythium sp. and further studies on etiology and management of soft root rot is yet to be established. Whereas, mortality of cuttings due to root rot reported up to 44.40 per cent (Gupta *et al*., 1997).

As the soft root rot disease is spreads rapidly in mulberry gardens and damage the crop, so an attempt was conducted to know the occurrence and causal agent of soft root rot disease in Karnataka *viz*., Kolar, Mandya, Ramanagara and Chikkaballapur districts. Disease incidence was 27.17 % in major mulberry growing area *i.e.*, Ramanagar district of Karnataka (Ravichandra *et al.*, 2021). Mulberry soft root rot disease, effective management is needed. Hence, in vitro and in vivo studies on efficacy of fungicides (contact, systemic and combi-product) having different mode of action was taken. The biological control method is contemplated as promising approach to management of soil borne diseases was concerned due to continuous use of fungicides causes imbalance in the soil ecosystem, threat to silkworm and environmental pollution. Emphasise is also given on management of soft root rot of mulberry by using bio-agents and botanicals.

MATERIAL AND METHODS

Evaluation of different botanicals, bio-agents and chemicals against *Pythium* sp.

***In vitro* evaluation of fungicides.** The efficacy of four non-systemic, eight systemic fungicides and six combi-products were tested against *Pythium* sp. by poisoned food technique. The fungicides were evaluated at three concentrations. The required quantity of fungicides was added to sterilized potato dextrose medium so as to get desired concentration. Twenty ml of poisoned medium was poured to sterilized Petridishes. The test fungal disc of five mm was taken from actively growing culture and was placed on center of Petri plate. The control plate was maintained without any fungicides. Each treatment was replicated for three times. These plates were incubated at room temperature till the fungal growth reached to periphery of Petri plate in case of control plate and at the same time the colony diameter of test fungus was recorded in treatment plates. The efficacy of different fungicides was expressed as per cent inhibition of mycelial growth over control was calculated by using the formula suggested by Vincent (1947) as follows

$$I = \frac{C - T}{C} \times 100$$

Where I = Per cent inhibition of mycelial growth
C = Growth of mycelium in control.
T = Growth of mycelium in treatment

***In vitro* evaluation of bio-agents against *Pythium* sp.** Eight fungal bio agents and eight bacterial bio agents were tested against *Pythium* sp. by dual culture technique. In order to get fresh and active growing bio agents as well as test organism, these were cultured on potato dextrose agar medium.

The inhibition zone between test organism and antagonistic microorganism was measured and compared with control. The per cent inhibition of growth of the pathogen was calculated by using the formula suggested by Vincent (1947).

***In vitro* evaluation of botanicals against *Pythium* sp.** The efficiency of plant extract or botanicals were tested against *Pythium* sp. on PDA medium by using poisoned food technique The per cent inhibition of mycelial growth of test fungus was calculated by using the formula suggested by Vincent (1947).

Glasshouse evaluation of fungal bio-agents against soft root rot disease of mulberry

Mass multiplication of *Pythium* sp. The test pathogen *Pythium* sp. was mass multiplied in sterilized carrot broth for the pot culture studies. For this, 50 g of healthy and cleaned broken rice grain was taken in polypropylene bags (10″×12″, transparent) and 50 ml of 2 per cent dextrose solution was added and plugged by using cotton plug. This was covered with brown paper and rubber band. The bags were sterilized in autoclave at 121.6 ºC temperature at 15 psi pressure for 15-20 minutes and allowed to cool. Seven days old culture of *Pythium* sp. on potato dextrose agar medium was used for inoculation. The fungal disc (5 mm) was inoculated in each bag and incubated for 15 days at 27 ± 1 ºC.

Mass multiplication of Trichoderma isolates. The Trichoderma isolates were mass multiplied on broken rice grain and dextrose mixture prepared as before and the bags were incubated at 27 ± 1 ºC for 15 days.

Pot culture experiment. Pot culture experiment was designed and carried out to evaluate the effectiveness of the fungal bio-agents against soft root rot of mulberry under glasshouse condition at the department of Plant Pathology, UAS, GKVK, Bengaluru.

Plastic pots were filled with two kg of sterilized soil. Inoculum of *Pythium* sp. multiplied in rice grain medium was added at 20 g/kg soil and mixed thoroughly and then seedlings of mulberry (V1) were transplanted in individual pot and watered regularly. After 2 weeks, the infected mix were inoculated separately with each antagonist and placed in a glasshouse. In control, the infected mix was not inoculated with antagonist. This experiment included eight treatments and one control with three replications. Ten pots per treatment arranged in a completely randomized design were used in this experiment. The disease incidence was recorded at 10 weeks after application.

$$\text{Per cent Disease Incidence} = \frac{\text{Number of plants infected}}{\text{Total number of plants observed}} \times 100$$

Experiment details
Design: CRD Test crop: Mulberry
No. of treatments: 9
Variety: V1 Application level: 20 g/kg of soil No. of replications: 3

In vivo evaluation of fungal bio-agents for the management of soft root rot disease

An experiment was conducted in the farmer's field located at Halaguru village in order to manage the soft rot disease of mulberry. The variety, V1 was used and soil application of different fungal bio-agents. The experiment included 8 treatments and one check with three replications.

Details of experiment

Details	Orchard 1
Location	Halaguru village
Variety	V-1
Treatment	8
Replication	3
Number of plants /treatments	25
Year	2019-2020 (Nov- Mar)

Observation on per cent disease incidence was recorded at 150 day after application of fungal bio-agents and the data was analysed statistically.

***In vivo* evaluation of fungicides for the management of soft root rot disease.** Effective treatment from *in vitro* experiment was evaluated in mulberry garden. An experiment was conducted in the farmer's field located at Halaguru village in order to manage the soft rot disease of mulberry. The variety, V1 was used and soil drenched with different fungicides. The experiment included ten treatments and one check with three replications.

Details of experiment

Details	Orchard 2
Location	Halaguru village
Variety	V1
Treatment	10
Replication	3
Number of plants /treatments	43
Year	2019-2020 (Nov- Mar)

Statistical analysis. The data obtained in the present investigation for various parameters were subjected to ANOVA for a completely randomized design for *in vitro* studies and randomized complete block design for *in vivo* studies.

RESULTS

Evaluation of fungicides, botanicals and bio-agents against *Pythium* sp.

***In vitro* evaluation of fungicides *Pythium* sp.** Availability of different fungicides were evaluated under *in vitro* condition in order to provide useful and preliminary information regarding their efficacy and quick action therefore, serves as a guide for field evaluation. *In vitro* evaluation of different systemic, non-systemic fungicides and combi-products against *Pythium* sp. and results were presented in this chapter. Four non-systemic, eight systemic and six comi-product fungicides were evaluated against *Pythium*sp.at three concentrations in laboratory by poisoned food technique.

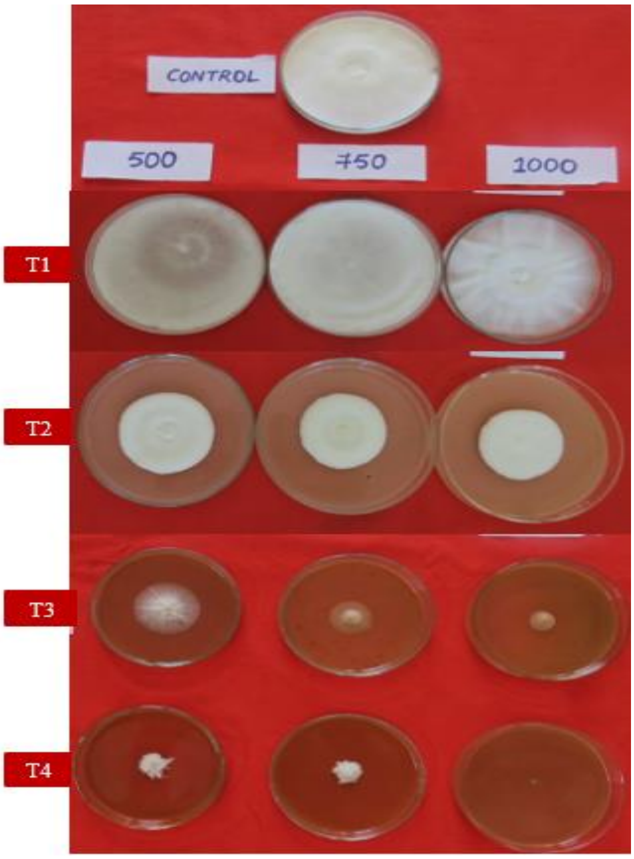
***In vitro* evaluation of non-systemic and combi-product fungicides against *Pythium* sp.** Four non-systemic and six combi-product fungicides tested against *Pythium* sp. in three different concentrations 500, 750 and 1000 ppm and 250, 500 and 750 ppm respectively. The per cent mycelial inhibition of soft root rot pathogen *Pythium* sp. was calculated and presented in Table 1 and 2 and Fig. 1 and 2.

The results presented in table revealed that, statistically difference between non-systemic fungicides in per cent mycelial inhibition. Captan was most effective and statistically superior over all fungicides which inhibited 86.29 per cent. Second most effective fungicide was Mancozeb (72.16 %) and followed by Chlorothalonil (48.24 %) and Propineb 5.55 per cent.

Among combi-product fungicides, Metalaxyl + Mancozeb (94.44 %), Fenamidone + Mancozeb (94.44 %), Carbendazim + Mancozeb (94.44 %) and Azoxystrobin + Mancozeb (94.44 %), were found most effective and which is followed by Hexaconazole + Zineb (80.20 %) whereas least inhibition of mycelial growth was recorded in Hexaconazole + Captan (61.01 %). At 1000 ppm highest inhibition was seen these results were similar with Muthulakshmi and Seethapathy (2019).

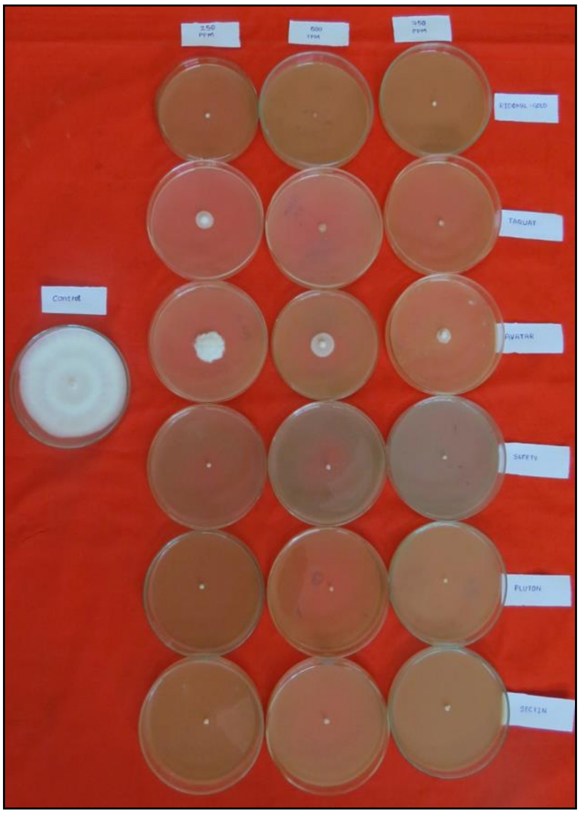
Table 1: *In vitro* evaluation of non-systemic fungicides against *Pythium* sp.

Sr. No.	Contact fungicides	Percent inhibition over control			
		Concentration			
		500 ppm	750 ppm	1000 ppm	Mean
1.	Captan 50% WP	79.63	84.82	94.44	86.29
2.	Mancozeb 75% WP	53.15	71.48	91.85	72.16
3.	Chlorothalonil 75% WP	42.22	50.30	52.22	48.24
4.	Propineb 70% WP	5.55	5.55	5.55	5.55
	Mean	**43.13**	**53.03**	**61.01**	**53.06**
		Fungicides (F)	**Concentration (C)**	**Interaction (F×C)**	
SEm ±		**0.10**	**0.039**	**0.006**	
CD @ 1%		**0.86**	**0.540**	**0.212**	



T1: Propineb T2: Chlorothalonil T3: Mancozeb T4: Captan

Fig. 1. *In vitro* evaluation of non-systemic fungicides against *Pythium* sp.



T1: Mancozeb+ Metalaxyl; T2: Hexaconazole + Captan; T3: Hexaconazole + Zineb; T4: Carbendazim + Mancozeb; T5: Fenamidone + Mancozeb; T6: Azoxystrobin + Mancozeb

Fig. 2. *In vitro* evaluation of combi-product fungicides against *Pythium* sp.

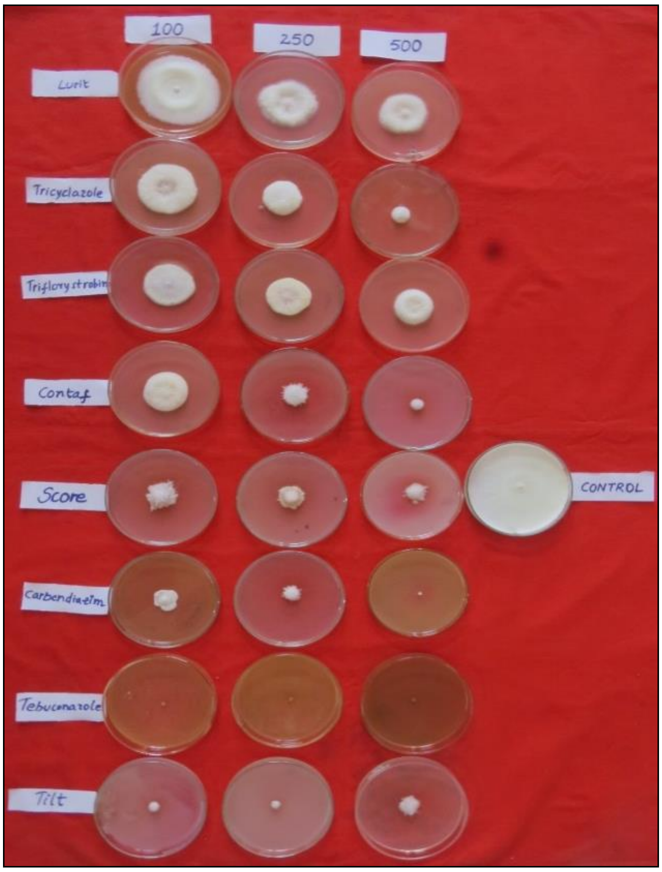
Table 2: *In vitro* evaluation of combi-product fungicides against *Pythium* sp.

Sr. No.	Combi-product fungicides	Percent inhibition over control			
		Concentration			
		250 ppm	500 ppm	750 ppm	Mean
1.	Mancozeb 64 % w/w + Metalaxyl-M 4% w/w	94.44	94.44	94.44	94.44
2.	Hexaconazole 5 % + Captan 70% WP	82.22	91.48	93.33	61.01
3.	Hexaconazole 4 %+ Zineb 68% WP	74.82	79.63	86.16	80.20
4.	Carbendazim 12% + Mancozeb 63 % WP	94.44	94.44	94.44	94.44
5.	Fenamidone 10% + Mancozeb 50 % WG	94.44	94.44	94.44	94.44
6.	Azoxystrobin 11.5 % + Mancozeb 30 % WP	94.44	94.44	94.44	94.44
	Mean	**89.47**	**91.47**	**92.87**	**86.49**
		Fungicides (F)	**Concentration (C)**	**Interaction (F×C)**	
SEm ±		**0.144**	**0.003**	**1.562**	
CD @ 1%		**0.946**	**0.136**	**3.117**	

Dohroo *et al.* (2015) study showed thatthe soil drenching of Mancozeb at 0.25 per cent was most effective in reducing the incidence of soft rot on ginger besides have its significant response in improving the growth and yield.

***In vitro* evaluation of systemic fungicides against *Pythium* sp.** The per cent inhibition *Pythium* sp. mycelial growth at three different concentrations (100, 250 and 500 ppm) of seven systemic fungicides were recorded.

Among eight systemic fungicides tested, 100 per cent inhibition of *Pythium* sp. was recorded in Tebuconazole at three concentration tested (100, 250 and 500 ppm), mean of these show the statistically superior to all other fungicides followed by Carbendazim (86.79 %) Hexaconazole (79.70 %), Propiconazole (75.35 %), Difenoconazole (74.69 %), Tricyclazole (65.80 %) and Thiophanate methyl (60.37 %) whereas least growth was recorded in Dimethomorph (47.04 %), Table 3 and Fig. 3. The similar findings were reported by Chavan *et al.* (2017) evaluated the different fungicides in that significantly inhibited growth of *P. aphanidermatum*, over control. The similar findings were reported by Chavan *et al.* (2017).



T1: Dimethomorph, T2: Tricyclazole, T3: Thiophanate methyl, T4: Hexaconazole, T5: Difenoconazole, T6: Carbendazim, T7: Tebuconazole, T8: Propiconazole

Fig. 3. *In vitro* evaluation of systemic fungicides against *Pythium* sp.

Table 3: *In vitro* evaluation of systemic fungicides against *Pythium* sp.

Sr. No.	Systemic fungicides	Per cent inhibition over control			
		Concentration			
		100 ppm	**250 ppm**	**500 ppm**	**Mean**
1.	Dimethomorph	36.30	48.52	56.30	47.04
2.	Tricyclazole	47.41	60.74	89.26	65.80
3.	Thiophanate methyl	48.15	63.71	69.26	60.37
4.	Hexaconazole	69.66	78.15	91.30	79.70
5.	Difenoconazole	66.30	76.30	81.47	74.69
6.	Carbendazim	78.52	87.41	94.44	86.79
7.	Tebuconazole	100.00	100.00	100.00	100.00
8.	Propiconazole	65.93	75.61	84.52	75.35
	Mean	**64.03**	**73.80**	**83.31**	**73.71**
		Fungicides (F)	**Concentration (C)**	**Interaction (F×C)**	
SEm ±		**0.02**	**0.011**	**0.008**	
CD @ 1%		**0.3372**	**0.25**	**0.213**	

In vitro evaluation of botanicals against *Pythium* sp.

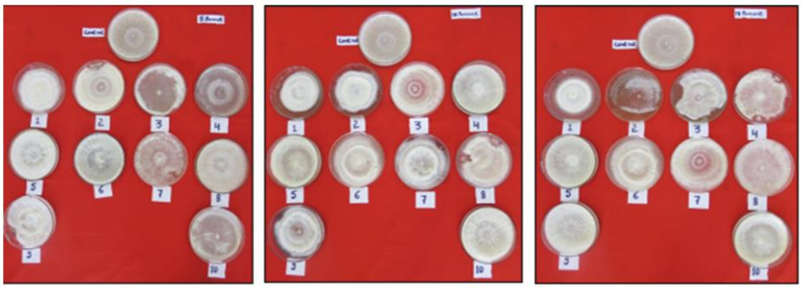
The study was carried out to know the fungitoxic nature of different plant extracts against *Pythium* sp. The effectiveness of different plant extracts in reducing the mycelial growth of *Pythium* sp. is varied greatly. The results thus obtained are presented here under (Table 4 and Fig. 4).

The results presented in table 8 revealed that, statistical difference between plant extracts when per cent inhibition of three replication concerned. Neem (14.02 %) was found to be most effective and statistically superior over other treatments and it as followed by garlic (11.93 %), tulasi (1.93 %) and onion (0.90 %). There was no inhibition of mycelial growth in other treatments of plant extracts like subabul, lemon grass, calotropis, agave, lantana and pongamia.

At 15 per cent concentration of plant extracts, maximum inhibition of mycelial growth was recorded in neem (23.58 %), followed by garlic (19.3 %), tulasi (5.80 %) and onion (2.72 %). Similar observation was recorded by Ramraj *et al.* (2017).

Table 4: *In vitro* evaluation of botanicals against *Pythium* sp.

Sr. No.	Botanicals	Per cent inhibition over control			
		Concentration			
		5 per cent	**10 per cent**	**15 per cent**	**Mean**
1.	Neem	1.15	17.35	23.58	14.02
2.	Garlic	0.00	16.50	19.30	11.93
3.	Onion	0.00	0.00	2.72	0.90
4.	Tulasi	0.00	0.00	5.80	1.93
5.	Subabul	0.00	0.00	0.00	0.00
6.	Lemon grass	0.00	0.00	0.00	0.00
7.	Calotropis	0.00	0.00	0.00	0.00
8.	Agave	0.00	0.00	0.00	0.00
9.	Lantana	0.00	0.00	0.00	0.00
10.	Pongamia	0.00	0.00	0.00	0.00
	Mean	**0.11**	**3.38**	**5.14**	**2.87**
		Fungicides (B)	**Concentration (C)**	**Interaction (B×C)**	
SEm ±		**1.782**	**0.001**	**0.01**	
CD @ 1 %		**0.0098**	**0.734**	**0.06**	



1: Neem, 2: Garlic, 3: Onion, 4: Tulasi, 5: Subabul, 6: Lemon grass, 7: Calotropis, 8: Agave, 9: Lantana, 10: Pongamia

Fig. 4. *In vitro* evaluation of botanicals against *Pythium* sp.

***In vitro* evaluation of bio-agents against *Pythium* sp.**

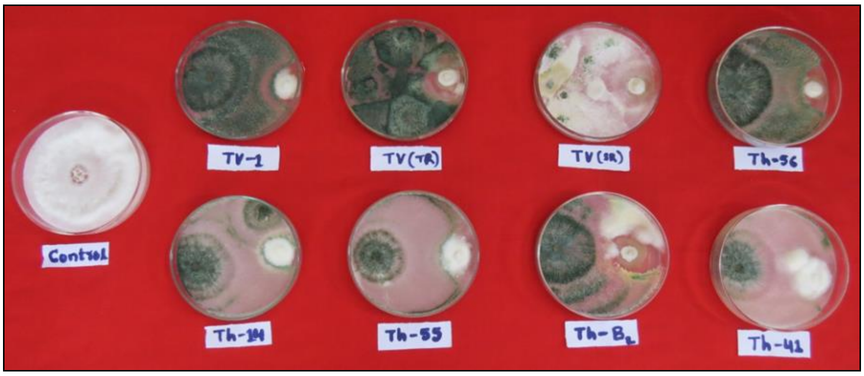
***In vitro* evaluation of fungal bio-agents against *Pythium* sp.** The fungal antagonistic microorganisms were evaluated against *Pythium* sp. by dual culture technique to know their antagonistic effect.

The per cent inhibition of mycelial growth of fungus was calculated and results are noted in Table 5 and Fig. 5. Statistically difference among the bio-agents (Tv-1, Tv-2, Tv-3, Th-56, Th-14, Th-55, Th-B2, Th-41) evaluated with respect to per cent inhibition of mycelia of *Pythium* sp. The highest 95.52 per cent inhibition of mycelial growth was observed in *T. viride*-1 (Tv-1) found to be statistically superior when compared to other treatments. It was followed by Tv-2 (93.15 %), Tv-3 (92.55 %), Th-56 (91.68 %), Th-55 (89.78 %), Th-14 (89.47 %), and Th-B2 (84.22 %) whereas lowest inhibition noticed in Th-41 (83.85 %).

Table 5: *In vitro* evaluation of fungal bio-agents against *Pythium* sp.

Sr. No.	Fungal bio-agents	Per cent inhibition over control*
1.	*Trichoderma viride*- 1(Tv-1)	95.52
2.	*T. viride*-2(Tv-2)	93.15
3.	*T. viride*- 3 (Tv-3)	92.55
4.	*T. harzianum*-56 (Th-56)	91.68
5.	*T. harzianum*-14 (Th-14)	89.47
6.	*T. harzianum*-55 (Th-55)	89.78
7.	*T. harzianum*-B2 (Th-B2)	84.22
8.	*T. harzianum*-41 (Th-41)	83.85
	SEm±	**0.029**
	CD @ 1%	**0.4061**

*Mean of three replications



T1: *T. viride*- 1, T2: *T. viride*-2, T3: *T. viride*- 3, T4: *T. harzianum*-56, T5: *T. harzianum*-14, T6: *T. harzianum*-55, T7: *T. harzianum*-B2, T8: *T. harzianum*-41

Fig. 5. *In vitro* evaluation of fungal bio-agents against *Pythium* sp.

***In vitro* evaluation of bacterial bio-agents against *Pythium* sp.** *In vitro* evaluation of bacterial bio-agents *viz., Bacillus subtilis, Pseudomonas fluorescence, Bacillus megatherium, Bacillus velezensis* (P42), *Bacillus velezensis* (A6) and *Bacillus pumilis.*

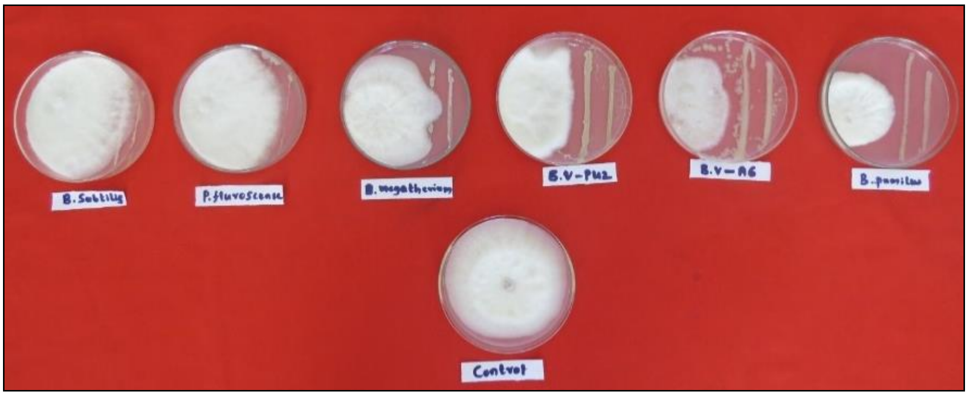
Experiment was carried out by following dual plate culture method and results are presented in Table 6 and Fig. 6. There was statistically difference among the bacterial bio-agents evaluated with regarding to mycelial growth of *Pythium* sp. Among bio-agents evaluated, *Bacillus pumilis* (58.88 %) was found very effective in inhibiting the mycelial growth of *Pythium* sp. and this was superior over all other treatments. Then this was followed by *Bacillus velezensis* strain A6 (52.66 %), *Bacillus velezensis* strain P42 (50.74 %), *Bacillus megatherium* (39.33 %) and *Pseudomonas fluorescence* (15.33 %). The minimum inhibition was recorded in *Bacillus subtilis* (9.88 %).

Above results on par with Chavan *et al.* (2017) evaluated bio-fungicide, *T. viride* was most effective, showed significantly highest mycelia inhibition (94.97%) while the bacterial bio-fungicide *Pseudomonas fluorescens* showed least inhibition (54.76%).

Table 6: *In vitro* evaluation of bacterial bio-agents against *Pythium* sp.

Sr. No.	Bacterial bio-agents	Per cent inhibition over control*
1.	*Bacillus subtilis*	9.88
2.	*Pseudomonas fluorescence*	15.33
3.	*Bacillus megatherium*	39.33
4.	*Bacillus velezensis* (P42)	50.74
5.	*Bacillus velezensis* (A6)	52.66
6.	*Bacillus pumilis*	58.88
	SEm±	**0.033**
	CD @ 1%	**0.4531**

*Mean of three replications



T1: *Bacillus subtilis*, T2: *Pseudomonas fluorescence*, T3: *Bacillus megatherium*, T4: *Bacillus velezensis* (P42), T5: *Bacillus velezensis* (A6), T6*: Bacillus pumilis*

Fig. 6. *In vitro* evaluation of bacterial bio-agents against *Pythium* sp.

Glasshouse evaluation of fungal bio-agents against incidence of soft root rot disease of mulberry

Mulberry soft root rot disease pot culture was conducted in a glasshouse to know the effect of fungal bio-agents on per cent disease incidence of mulberry root rot and data presented in Table 7 and Fig. 7a and b. The results revealed that, at 7 weeks after inoculation, highest per cent soft root rot disease reduction seen in *Trichoderma viride*-1 (30.81 %) and least disease reduction was recorded in *Trichoderma harzianum*-B2 (22.23 %) which was followed by Th-14 (23.21 %), Th-55 (23.79 %), Th-56 (27.24 %), Tv-3 (27.73 %), Tv-2 (30.65 %), and in untreated control 100 per cent disease seen, these results on par with Chavan *et al.* (2017).

Table 7: Glasshouse evaluation of fungal bio-agents against incidence of soft root rot disease of mulberry.

Sr. No.	Fungal bio-agents	Per cent recovered plants
1.	*T. harzianum*-56 (Th-56)	27.24
2.	*T. harzianum*-14 (Th-14)	23.21
3.	*T. viride*-1(Tv-1)	30.81
4.	*T. viride*-3 (Tv-3)	27.73
5.	*T. viride*-2(Tv-2)	30.65
6.	*T. harzianum*-55 (Th-55)	23.79
7.	*T. harzianum*-41 (Th-41)	24.80
8.	*T. harzianum*-B2 (Th-B2)	22.23
9.	Control (*Pythium* sp.)	0.00
	SEm±	**0.258**
	CD @ 1%	**0.387**

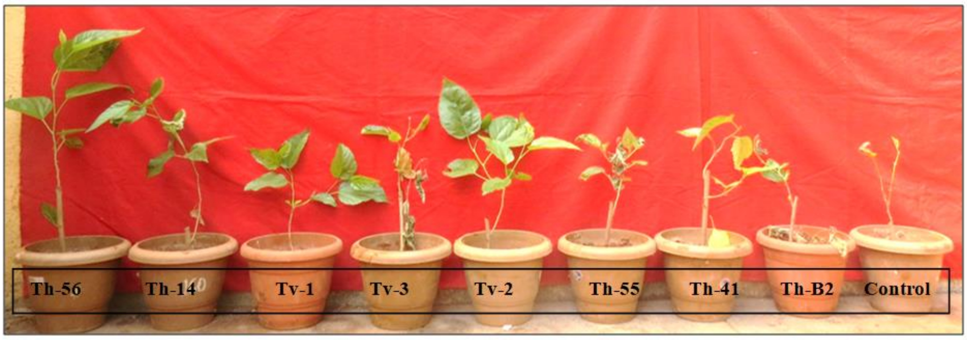


Fig. 7a. Infected mulberry samplings before application of fungal bio-agents under glasshouse condition.

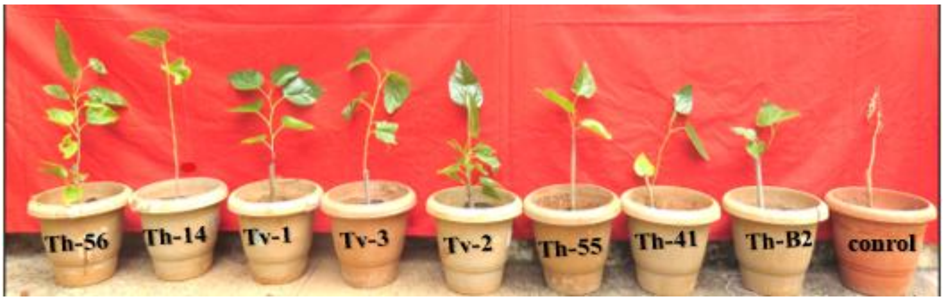


Fig.7b. Recovered infected mulberry samplings after application of fungal bio-agents under glasshouse condition.

***In vivo* evaluation of fungal bio-agents for the management of soft root rot disease of mulberry.** Field experiment was done to evaluate the relative bio-efficacy of bio-agents against soft root rot disease of mulberry at Halaguru village of Malavalli taluk, Mandya district. The results noted in Table 8, Fig. 8a, 8b).

The experiment was conducted in farmer's field which includes nine treatments including one untreated control. The observation on mulberry soft root rot incidence was recorded before and 150 days after application of bio-agents. Results showed that the incidence of disease before the treatment application was non-significant. The highest per cent disease incidence was noticed in untreated control (34.15 %).

Among the eight treatments, the highest per cent disease decrease over untreated control was observed in T7 (22.12 %) and other treatments *viz.*, T8 (21.96 %), T6 (21.63 %), T5 (21.25 %), T3 (20.50 %), T4 (19.08), T2 (12.29 %) and least reduction was observed in T1 (11.79 %). The results were similar with Muthukumar *et al.* (2011).

Table 8: *In vivo* evaluation of fungal bio-agents against soft root rot disease of mulberry.

Treatments		Per cent root rot disease incidence		Per cent reduction over control
		Before application	150 days after application	
T1	*T. harzianum*-41(Th-41)	24.59	21.69	11.79
T2	*T. harzianum*-B2 (Th-B2)	25.45	22.32	12.29
T3	*T. harzianum*-55 (Th-55)	15.85	12.60	20.50
T4	*T. viride*-1(Tv-1)	17.55	14.20	19.08
T5	*T. harzianum*-56 (Th-56)	20.98	17.03	21.25
T6	*T. harzianum*-14 (Th-14)	21.08	16.52	21.63
T7	*T. viride*-2 (Tv-2)	22.10	17.21	22.12
T8	*T. viride*-3 (Tv-3)	25.18	19.65	21.96
T9	Control	21.57	34.15	-
SEm ±		**0.129**	**0.180**	-
CD @ 5%		**0.303**	**0.387**	-

Fig. 8a. Infected mulberry garden before application of fungal bio-agents at Halaguru, Mandya.

Application of different fungal bio-agents in field condition.

Fig. 8b. Field evaluation of fungal bio-agents for management of soft root rot of mulberry at Halaguru, Mandya.

***In vivo* evaluation of fungicides for the management of soft root rot disease of mulberry/** Field experiment was laid to know the effective fungicides against soft root rot disease of mulberry. This field experiment was conducted in farmer's field at Halaguru village of Malavalli taluk, Mandya district (Table 9 and Fig. 9a and 9b).

Which includes ten treatments and one untreated control. The observation on mulberry soft root rot incidence was recorded before and 150 days after application of fungicides. Incidence of disease before the treatment application was non-significant. The highest per cent disease incidence was noticed in untreated control (32.67 %).

Among the ten treatments, the highest per cent disease decrease over untreated control was observed in T9 (31.96 %) and followed by other treatments T1 (27.45 %), T2 (26.78 %), T10 (25.58%), T7(25.46 %), T4 (22.57 %), T8 (21.90 %), T6 (21.69 %), T3 (19.26 %), and least reduction was observed in T5 (18.43 %). These findings on par with Gholve *et al.* (2014).

Table 9: *In vivo* evaluation of fungicides against soft root rot disease of mulberry.

Treatments		Per cent root rot incidence		Per cent reduction over control
		Before application	150 days after application	
T1	Captan 50% WP	30.23	21.93	27.45
T2	Mancozeb 75% WP	27.25	19.95	26.78
T3	Tebuconazole 25%EC	32.25	29.35	19.26
T4	Hexaconazole 5% SC	31.89	24.69	22.57
T5	Thiophanate methyl 70% WP	27.18	22.17	18.43
T6	Propiconazole 25%EC	23.46	18.37	21.69
T7	Difenoconazole 25 %EC	22.78	16.98	25.46
T8	Carbendazim 50 % WP	29.45	23.00	21.90
T9	Mancozeb 64% w/w + Metalaxyl-M 4% w/w	21.90	14.90	31.96
T10	Carbendazim 12% + Mancozeb 63% WP	21.45	15.96	25.58
T11	Control	20.74	32.67	-
SEm ±		**0.167**	**0.351**	-
CD @ 5%		**0.201**	**0.423**	-

Fig. 9a. Infected mulberry garden before application of fungicides at Halaguru, Mandya.

Fig. 9b. Recovere infected mulberry plants after application of fungicides at Halaguru, Mandya.

DISCUSSION

In vitro studies of non-systemic fungicides indicated that Captan (86.29 %) and Mancozeb (76.16 %) were found effective against *Pythium* sp. In systemic fungicides, Tebuconazole (100 %) and Carbendazim (86.79 %) and in case of combi-product, Mancozeb+ Metalaxyl (94.44 %) Carbendazim + Mancozeb (94.44 %), Fenamidone + Mancozeb (94.44 %) and Azoxystrobin + Mancozeb (94.44 %) found effective against the *Pythium* sp. Ten plant extracts were tested, highest mean inhibition *Pythium* sp. of (14.02 %) was observed in neem extract followed by garlic bulb (11.93 %).

Among the *in vitro* fungal bio-agents tested, effective one's were *Trichoderma viride*-1 (95.52 %) and *Trichoderma viride*-2 (93.15 %), whereas in bacterial bio-agents *Bacillus pumilis* showed maximum (58.88 %) mycelial growth inhibition.

In glasshouse evaluation the effective fungal bio-agents against soft root rot pathogen was *Trichoderma viride*-1 (30.81 %), wherein treated plants showed per cent decrease in soft root rot incidence compared to control. Eight Trichoderma isolates were used for management of mulberry soft root rot disease in farmer's field and found that *Trichoderma viride*- 2 (Tv-2) and *Trichoderma viride*-1(Tv-1) were found effectively in reducing the disease up to 22.12 and 21.96 per cent respectively.

Of the ten fungicides were used for management of soft root rot disease in the farmer's field in that Mancozeb + Metalaxyl (31.96 %) and Captan (27.45 %) were effective against Pythium soft root rot disease of mulberry under filed condition.

FUTURE SCOPE

1. Molecular confirmation of mulberry soft root pathogen *Pythium* sp.
2. Large scale demonstration of effective fungicides against soft root rot disease of mulberry in farmer's field.
3. Large scale mass production of bio-agents along with organic amendments would be helpful for the management of mulberry soft root rot disease

Acknowledgement. University of agriculture sciences Bnagalore-560065, conducting the research work. Division Chief mariculture division KSSRDI, Thalaghattapura, Bangalore-560109. Survey and sample collection work.

REFERENCES

Chavan, P. G., Apet, K. T. And Borade, R. S. (2017). Efficacy of fungicides and bio-agents against *Pythium aphanidermatum* causing rhizome rot of turmeric. *International Journal of Current Microbiology and Applied Sciences, 6*(1), 4312-4320.

Dohroo, N. P., Kansal, S. And Ahluwalia, N. (2015). Studies on eco-farmer-friendly practices for management of soft rot of ginger (*Zingiber officinale*). *Indian Phytopathology, 68*(1), 93-96.

Gholve, V. M., Tatikundalwar, V. R., Suryawanshi, A. P. And Dey, U. (2014). Effect of fungicides, plant extracts/botanicals and bio-agents against damping off in brinjal. *African Journal of Microbiology Research, 8*(30), 2835-2848.

Gupta, V. R., Govindaiah and Raju, H. V. (1997). Diseases and associated pathogens of mulberry nurseries, *Indian Phytopathology, 50*, 402-407.

Muthukumar, A., Eswaran, A. And Sanjeevkumar, K. (2011). Exploitation of *Trichoderma* species on the growth of *Pythium aphanidermatum* in chilli. *Brazilian journal of microbiology, 42*(4), 1598-1607.

Muthulakshmi, P. And Seethapathy, P. (2019). Evaluation of fungicides and bio-control agents against foliar and soil borne diseases of *Meliadubia*. *International Journal of Current Microbiology and Applied Sciences, 8*(9), 2476-2486.

Ramraj, C., Kakraliya, S. S., Mukesh, K. S. And Bajiya, M. R. (2017). Bio-efficacy of few plant extracts/botanicals against damping off of brinjal (*Pythium ultimum*). *International Journal of Chemical Sciences, 1*(1), 1-3.

Ravichandra., Somasekhara, Y, M. and Thimmareddy, H (2021). New Report of Pythium Soft Root Rot in Mulberry and its Cultural and physiological Studies. *International Journal of Current Microbiology and Applied Sciences, 10*(2), 1500-1510.

Sheng-Qin, Z. O. U., Wu and Chen. (2003). A review on chemical constituents, pharmological activity and application of mulberry leaves. *Journal of Chemical Industry For Products, 1*, 1-8.

Vincent, J. M.., (1947). Distribution of fungal hyphae in the presence of certain inhibitors. *Nature, 159*, 850.

Zhang, M., Chen, M., Zhang, H. Q., Sun, S., Xia, B. And Wu, F. H. (2009). *In vivo* hypoglycemic effects of phenolics from the root bark of *Morus alba*. *Fitoterapia of journal, 80*(8), 475-477.

How to cite this article: Ravichandra, Somasekhara Y.M., Shalini M. and Thimmareddy H. (2023). A New Disease of Mulberry, Pythium Soft Root Rot Managed through Biological, Botanical and Fungicidal Approaches, both *In-vitro* and Field Condition. *Biological Forum – An International Journal, 15*(8): 546-556.