The Pharma Innovation Journal 2022; 11(12): 452-454

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(12): 452-454

www.thepharmajournal.com
Received: 18-10-2022
Accepted: 23-11-2022

RS Gaonkar
Department of Plant Pathology, Dr. BSKKV, Dapoli, Maharashtra, India

RR Rathod
Assistant Professor, Department of Plant Pathology, Dr. BSKKV, Dapoli, Maharashtra, India

MS Joshi
Head of the Department, Department of Plant Pathology, Dr. BSKKV, Dapoli, Maharashtra, India

MM Patil
Department of Plant Pathology, Dr. BSKKV, Dapoli, Maharashtra, India

NV Chaure
Department of Plant Pathology, Dr. BSKKV, Dapoli, Maharashtra, India

VP Kharbadkar
Department of Plant Pathology, Dr. BSKKV, Dapoli, Maharashtra, India

Corresponding Author:
RS Gaonkar
Department of Plant Pathology, Dr. BSKKV, Dapoli, Maharashtra, India

In vitro management of stem rot disease of papaya caused by *Pythium aphanidermatum*

RS Gaonkar, RR Rathod, MS Joshi, MM Patil, NV Chaure and VP Kharbadkar

Abstract
In the present study, one bacterial and three fungal bio-agents *viz*., *Pseudomonas fluorescens* (Pf5), *Trichoderma harzianum*, *T. longibrachiatum* and *T. koningii* and seven fungicides *viz*., Bordeaux mixture 20% WP, Propiconazole 25% EC, Metalaxyl 35% WS, Metalaxyl (4%) + Mancozeb (64%), Copper oxychloride 50% WP, Fosetyl-Al 80% WP and Chlorothalonil 75% WP were evaluated *in vitro* to study their efficacy against *Pythium aphanidermatum*. From all the bio-agents evaluated *in vitro*, *P. fluorescens* (Pf5) showed an effective inhibition of the pathogen after 7 days of inoculation with highest mycelial growth inhibition of 76.11%. The least mycelial growth inhibition was observed in case of *T. koningii* with 69.44 percent inhibition over control. Among all the fungicides evaluated *in vitro* Metalaxyl 35% WS (0.3%) exhibited highest percent growth inhibition (79.7%) whereas, fungicide Chlorothalonil 75%WP (0.1%) recorded significantly least mycelial inhibition (33%) against the test pathogen.

Keywords: *Pseudomonas fluorescens*, *T. longibrachiatum*, *T. koningii*, bio-agents, metalaxyl, fungicides

Introduction
Papaya (*Carica papaya*), commonly referred to as pawpaw, is a delicious fruit belonging to the *Caricaceae* family is grown throughout the tropical regions of the world and is attacked by a number of fungal, bacterial and viral diseases. However, major fungal disease of papaya crop causing considerable damage in this area is stem rot disease which is caused by *Pythium aphanidermatum* (Edson Fitzp). It may also lead to unpredictable incidences in field ranging up to 100% in a single growing season (Gupta and Choudhary, 2015) [4]. Stem rot disease of papaya is widespread in India, Sri Lanka, Hawaii, Cuba, and South Africa. In India, the disease generally manifests during the rainy season (June to August) and becomes the cause of heavy damage to papaya in different parts of the state. When the severity of the disease is high, it devastates the entire plantations within the same growing season and makes the soil unfit for replanting. *P. aphanidermatum* is one of the most prevalent plant pathogens of various crop plants in warmer regions of the world. It is known to infect a variety of plant species. *P. aphanidermatum* produces oospores, sexual spores that serve as resting bodies and a means of survival in unfavorable environments. Considering the economic importance of papaya stem rot disease, it is necessary to find out the integrated management of the disease.

Materials and Methods
The *in vitro* experiments were conducted at the Department of Plant Pathology, College of Agriculture, (DBSKKV) Dapoli, Maharashtra, during the year 2021-22. Pure cultures of three *Trichoderma* strains and one strain of *Pseudomonas fluorescens* (Pf5) were obtained from Department of Plant Pathology, College of Agriculture, DBSKKV, Dapoli. The antagonistic potential of four bio-agents was evaluated *in vitro* by Dual culture technique (Dennis and Webster, 1971) [3] on PDA medium. Four replications of inoculated PDA plates per treatment were maintained in Completely Randomized Design. Seven fungicides from different fungicidal groups *viz*., Bordeaux mixture 20% WP, Propiconazole 25% EC, Metalaxyl 35% WS, Metalaxyl (4%) + Mancozeb (64%), Copper oxychloride 50% WP, Fosetyl-Al 80% WP and Chlorothalonil 75% WP were evaluated for their efficacy against *P. aphanidermatum* by using "Poisoned Food Technique" with three replications in Completely Randomized Design. All these plates were incubated at 28±2 °C in an incubator. Observations on inhibition of mycelial growth of the test fungus was measured and percent inhibition of the test fungus was calculated by applying formula given by Vincent (1947) [6].

$$\text{Per cent Inhibition (I)} = \frac{C - T}{C} \times 100$$

Where,
I = Percent inhibition
C = Growth (mm) of test fungus in untreated control plate
T = Growth (mm) of test fungus in treated plates

Results and discussion

Results (table 1, plate 1, fig. 1) revealed that among the fungal bio-agents evaluated, *T. longibrachiatum* showed an effective inhibition of the pathogen after 7 days of inoculation with highest mycelial growth inhibition of 74.44 percent. The second inhibitory antagonist found was *T. harzianum* with inhibition of 72.83%, followed by *T. koningii* with 69.44 percent inhibition over control. *P. fluorescens* strain Pf5 evaluated against *P. aphanidermatum* exhibited percent inhibition of 76.11% which was found to be the most effective of all the antagonists evaluated against the test pathogen.

The results of current investigation are in close conformity with earlier findings of Jeyaseelan *et al.* (2012) [5] who determined *in vitro* antagonistic efficacy of two different species of *Trichoderma* and nine different species of *Bacillus* against tomato damping off causing fungi *P. aphanidermatum*. In dual culture assay and assay for volatile metabolites, *T. harzianum* revealed significantly (p<0.05) higher effect at 48 hours of incubation. Similarly, the efficacy of a bacterial agent, *Pseudomonas fluorescence* (Pf) and yeast *Saccharomyces cerevisiae* (Sc) against *P. aphanidermatum* fungus was determined by Abdalmoohsin *et al.* (2019) [1] in which *Pf* caused significant growth inhibition of 88.66 and 100% at the concentrations of 5 × 10^6 and 5 × 10^7 Cfu, respectively.

Table 1: *In vitro* evaluation of bio agents against *P. aphanidermatum*

Tr. No.	Bio agents	Radial growth (mm)*	% Inhibition
T_1	*Trichoderma longibrachiatum*	23	74.44
T_2	*Trichoderma harzianum*	24.45	72.83
T_3	*Trichoderma koningii*	27.5	69.44
T_4	*Pseudomonas fluorescens* (Pf5)	21.5	76.11
T_5	Control	90	0.00
	S.E.±	0.49	
	C.D.	2.06	

(*mean of four replications)

Results (table 2, plate 2 and fig.2) revealed that all fungicides evaluated against *Pythium aphanidermatum* were effective in inhibiting the mycelial growth. Metalaxyl 35% WS (0.3%) emerged as most effective fungicide with highest percent growth inhibition of 79.7% over control. The systemic fungicide Fosetyl-Al 80 WP (0.1%) was the next best in order of merit with percent growth inhibition of 72.2%. Whereas, Copper oxychloride 50%WP (0.2) exhibited 56.2% inhibition, Metalaxyl (4%) + Mancozeb (64%) (0.125%) recorded 49.7% inhibition. The fungicide Chlorothalonil 75%WP (0.1%) recorded significantly least mycelial inhibition (33%), followed by Bordeaux mixture 20%WP (0.05%) (42%) and Propiconazole 25% EC (0.075%) with percent mycelial growth inhibition of 45.4%.

The present findings are in accordance with earlier report of Zamanizadeh and Hatami (2011) [7] who investigated fungicides Metalaxyl (5% WG) and Metalaxyl MZ (72% WT) against *P. aphanidermatum* which causes damping off disease in cucumber seedlings in greenhouses. Metalaxyl WG was found to be the most effective fungicide, with a growth inhibition rate of 85% for *P. aphanidermatum*. Metalaxyl MZ came in second with a 78% growth inhibition rate. Similarly, Apet *et al.* (2018) [2] evaluated systemic fungicides at 1000 and 1500 ppm whereas non-systemic and combo fungicides were evaluated at 2000 and 2500 ppm concentration. Copper oxychloride was found to be most effective with highest mean mycelial inhibition (93.28%) and least mycelial inhibition was by Propiconazole (35.05%).

Table 2: *In vitro* evaluation of non-systemic, systemic and combi fungicides against *P. aphanidermatum*.

Treatment No.	Treatment	Concentration (%)	Radial growth (mm)*	% Inhibition
T_1	Bordeaux mixture 20%WP	0.05	52.2	42.0
T_2	Propiconazole 25% EC	0.075	49.1	45.4
T_3	Metalaxyl 35% WS	0.3	18.3	79.7
T_4	Metalaxyl (4%) +Mancozeb (64%)	0.125	45.3	49.7
T_5	Copper oxychloride 50%WP	0.2	39.4	56.2
T_6	Fosetyl-Al 80%WP	0.1	25	72.2
T_7	Chlorothalonil 75%WP	0.1	60.3	33.0
T_8	Control	-	90	0.0
	S.E(m) ±		0.96	
	C.D (@ 1%)		3.97	

(*mean of three replications)

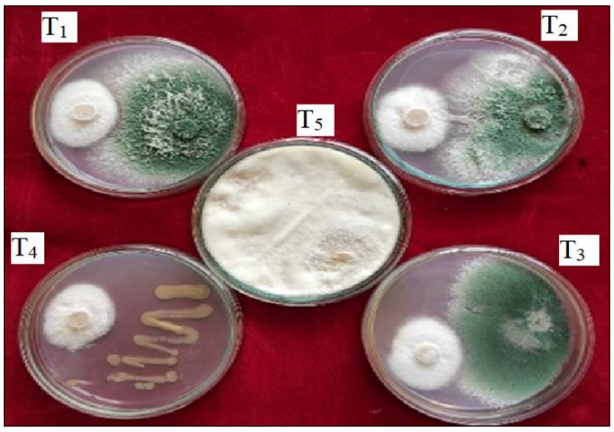

Plate 1: *In vitro* efficacy of bio-agents against *P. aphanidermatum*

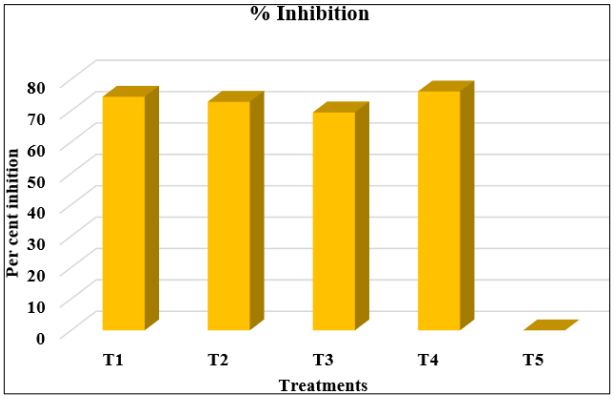

Fig: 1 *In vitro* efficacy of bio-agents against *P. aphanidermatum*

Plate 2: *In vitro* efficacy of fungicides against *P. aphanidermatum*

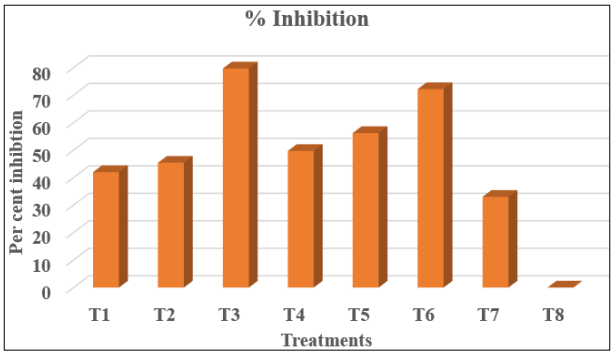

Fig 2: *In vitro* efficacy of fungicides against *P. aphanidermatum*

Conclusion

From the results of the present study it is concluded that the stem rot disease of papaya caused by *Pythium aphanidermatum* can be effectively controlled by the bacterial bioagent *Pseudomonas fluorescens* (Pf5) and fungicide Metalaxyl 35% WS at 0.3%.

Acknowledgement

I consider myself fortunate in availing this golden opportunity to express my heartiest sense of gratitude and humble indebtedness towards my chairman Dr. R. R. Rathod, Assistant Professor of Plant Pathology, DBSKKV, Dapoli, Dist. Ratnagiri MH-415712 for his kind and valuable guidance, also express my sincere thanks to Dr. M. S. Joshi, Head, Dept. of Plant Pathology for providing all necessary facilities during the course of investigation, suggestions and comments during investigation and completion of Research work.

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