wjpls, 2016, Vol. 2, Issue 6, 165-172. Research Article ISSN 2454-2229

World Journal of Pharmaceutical and Life Sciences WJPLS

www.wjpls.org

SJIF Impact Factor: 3.347

IN VITRO EFFICACY OF BIOAGENT AND ORGANIC AMENDMENT AGAINST DAMPING-OFF OF TOMATO PATHOGEN (*PYTHIUM APHANIDERMATUM*)

Manisha S. Shinde[1*], K. B. Rakholiya[2] and R. R. Waghunde[3]

[1]Ph. D. Scholar, Department of Plant Pathology, N. M. College of Agriculture, Navsari Agricultural University, Navsari-396450.

[2]Associate Professor, Department of Plant Pathology, N. M. College of Agriculture, Navsari Agricultural University, Navsari-396450.

[3]Assistant Professor, [1]College of Agriculture, Navsari Agricultural University, Bharuch.

Article Received on 25/09/2016 Article Revised on 15/10/2016 Article Accepted on 07/11/2016

***Corresponding Author**
Dr. Manisha S. Shinde
Ph. D. Scholar, Department of Plant Pathology, N. M. College of Agriculture, Navsari Agricultural University, Navsari-396450.

ABSTRACT

Studies on interaction of antagonists by dual culture method indicated that *Trichoderma harzianum (Navsari isolate), Pseudomonas fluorescens (Navsari isolate)* and *T. fasciculatum (Navsari isolate)* were found strong antagonistic against *P. aphanidermatum*. Organic extracts tested in vitro against *Pythium aphanidermatum* revealed that, castor cake had strong inhibitory effect on the growth of *P. aphanidermatum.*

KEYWORDS: *Bioagents, Organic amendment, Damping-off, Tomato* and *T. hazianum.*

INTRODUCTION

Damping-off is one of the destructive disease in the greenhouse, field and nursery, affecting germinating seeds and young seedlings. Damping-off is an important disease of in greenhouse and field bed of tomatoes, causing important losses in nurseries where young and susceptible transplants are produced. It is commonly caused by *Pythium* spp., /*Phytophthora* spp./*Rhizoctonia* spp./*Fusarium* spp./ *Macrophomina* spp. (Agrios, 2005). Among the fungal diseases, damping-off caused by *Pythium aphanidermatum* (Edson) Fitzpatrick in nurseries is a major constraint in vegetables production causing 62 % mortality of seedlings (Ramamoorthy *et al.* 2002). Rajagopalan (1961) reported that *P. aphanidermatum* was the

major species causing 75-80 per cent damping-off in tomato and chilli. Manoranjitham *et al*. (2001) also reported 60 % mortality of chilli seedlings both in nursery and main field. *Pythium* spp. is probably the most abundant and widespread plant pathogenic fungal species in soil (Pieczarka and Abawai, 1978). *P. aphanidermatum* and *P. ultimum*, a main causal agent of pre and post-emergence damping-off in tomatoes (Gravel *et al*., 2005). Continuous use of fungicides detoriate the soil health environmentl. Hence use of bioagent like *Trichoderma* spp. *Pseudomonas fluorescens, Bacillus spp.* and organic amendment like caster cake FYM help in protecting soil environment and prevent diseases. Hence in present study fungal and bacterial bioagents and organic amendments are used *in vitro* against *Pythium aphanidermatum.*

MATERIAL AND METHODS

Efficacy of bioagents against *Pythium aphanidermatum* under *in vitro* condition

Experiment was carried with Completely Randomized Design with three replication and seven treatments for bioagent and three replication and six treatments in organic amendment by dual culture method during 2014.

Treatment detail

Sr. No.	Bioagents (NAU, isolates)
T1	*Trichoderma viride*
T2	*T. harzianum*
T3	*T. virens*
T4	*T. fasciculatum*
T5	*Pseudomonas fluorescens*
T6	*Bacillus subtilis*
T7	Control

Various known native isolates *viz., Trichoderma viride, T. harzianum, Trichoderma fasciculatum, T. virens, Pseudomonas fluorescens,* and *Bacillus subtilis* was screened for their effectiveness against *Pythium aphanidermatum.* by dual culture technique as suggested by Kumar and Hooda (2007). The test organisms (*Trichoderma* spp.) and the pathogen was grown on PDA while bacterial bioagent was grown on nutrient agar. Sterilized PDA (20 ml) was poured aseptically in 90 mm diameter sterilized Petri plate for testing *Trichoderma* spp. and pathogen while bacterial bioagent was tested in media which contain PDA 10 ml + NA 10 ml which is poured in in 90mm sterilized Petri plate. Mycelial disc (5 mm) from 6 days

old actively growing culture of the bioagents and the test pathogen was cut separately with the help of sterilized cork borer and placed on solidified PDA at 4 cm away from each other. The experiment was replicated three times. Test pathogen and bioagent was subjected separately for growth and comparison. All inoculated plates was incubated at 27±1°C in BOD. The colony diameters of test pathogen in treated and control was observed periodically observation was recorded after 72 hrs. of incubation and Per cent inhibition of mycelial growth of the pathogen was calculated by using the formula given by Bell *et al*. (1982).

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

Where, I= Per cent inhibition (%)

C= Colony diameter in control plates (mm^2)

T= Colony diameter in treated plates (mm^2)

Efficacy of extract of organic amendments against *Pythium aphanidermatum* under *in vitro*.

To find out antifungal activity of organic amendment on pathogen, different amendment *viz*., farm yard manure (FYM), vermicompost, poultry manure, mustard cake, castor cake, and neem cake, was evaluated following the method suggested by Jha *et al*. (2007).

Treatment Detail

Treatment	Name of extract	Concentrations (%)	
T1	Neem Cake	10	20
T2	Castor Cake	10	20
T3	Mustard Cake	10	20
T4	FYM	10	20
T5	Vermicompost	10	20
T6	Control	-	-

Water extracts of above organic amendments was prepared by incorporating 25 g shade dried fine powder of each amendment into 250 ml of sterilized distilled water. The extracts was passed after 24 hours through muslin cloth and filtered through filter paper. This was constituted 10 per cent standard extract. In amendment oat meal powder and agar powder was added @ 2.0 per cent (w/v) to this extracts and then sterilized in autoclave for 15 min. Twenty millilitres sterilized oat meal agar medium was poured into sterilized Petri plates. Plates without addition of any amendment served as control. After solidification, the Petri

plates was inoculated with 5 mm diameter discs of 6 days old culture of the pathogen with the help of sterilized cork borer and placed at the centre and incubated for 3 days. Three replicates was maintained for each medium. Observations on colony diameter after 48 hrs. was recorded after incubation. Percent growth inhibition was calculated using the following formula suggested by Vincent, (1927).

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

Where,

PGI = Per cent Growth Inhibition (%)

C = Average diameter of mycelial growth in control (mm).

T = Average diameter of mycelial growth in treated (mm).

RESULT AND DISCUSSION

Antagonistic effect of bioagents against *Pythium aphanidermatum in vitro* by dual culture method.

In present study, attempts were made to identify antagonistic microorganisms for *Pythium aphanidermatum* in *in vitro*. Seven known native isolate were tested against *P. aphanidermatum* by dual culture method. Among them, the least growth of the pathogen was recorded in *T. harzianum* (Navsari isolate) (16.86 mm) which was significantly superior over the rest. Next best in order of merit was *P. fluorescens* (Navsari, isolate) (20.79 mm) which was followed by *T. fasciculatum* (Navsari isolate) (21.07 mm). The *T. viride* (Navsari isolate) (24.37 mm), *T. virens* (Navsari isolate) (44.27 mm) and *Bacillus subtilis* (Navsari isolate) (78.19 mm) were comparatively less effective in inhibiting the growth of *P. aphanidermatum.Trichoderma harzianum* (Navsari isolate) showed maximum (81.26 %) growth inhibition after 4 days of incubation and appeared to be the most superior over all the antagonists tested which was followed in sequence by *P. fluorescens* (Navsari isolate) (76.90 %), *T. fasciculatum* (Navsari isolate) (76.59 %), *T. viride* (Navsari isolate) (72.92 %), *T. virens* (Navsari isolate) (50.81 %) and *Bacillus subtilis* (Navsari isolate) (13.12 %) in decreasing order for per cent growth inhibition of *P. aphanidermatum.*(Table 1)

It was evident from these studies that among all the antagonists evaluated by dual culture method, *T. harzianum* (Navsari isolate), *P. fluorescens* (Navsari isolate), *T. fasciculatum* (Navsari isolate) and *T. viride* (Navsari isolate) consistently showed strong antagonistic activity against *P. aphanidermatum* as compared to other antagonists tested and hence

considered as potential antagonists against *P. aphanidermatum*. *T. virens* (Navsari isolate) and *B. subtilis* (Navsari isolate) have showed poor growth inhibition of the pathogen. This suggests that biological control of damping-off tomato using *T. harzianum* (Navsari isolate), *T. fasciculatum* (Navsari isolate) and *P. fluorescens* (Navsari isolate) were found very useful in South Gujarat area.

Muthukumar *et al.* (2011) recorded maximum growth inhibition of *P. aphanidermatum* by *Trichoderma* species. Malhotra *et al.* (2011) evaluated 13 isolates of biocontrol fungi and 4 bacterial strains against damping-off fungus. The results show that among the fungal species *Gliocladium virens* and *T. harzianum* (T8) are the most effective in inhibiting fungus mycelial growth 74.82 per cent and 73.33 per cent respectively. Among the bacterial strains maximum growth inhibition was recorded by *P. fluorescens* P.f.1 (73.33 %) followed by *P. fluorescens* P.f.2 (62.22 %). While, Yadav and Joshi (2012) found *Pseudomonas fluorescens* and *Bacillus subtilis* were more effective in inhibiting the growth of *P. aphanidermatum* than *Trichoderma sp.* These results are in harmony with earlier workers *viz*., Ramesh (2004), Kumar and Hooda (2007), Mishra, 2010) and Muthukumar *et al.* (2010) It may be due to production of viridin as reported by Brain (1951) or may be due to the penetration of antagonistic hyphae into hyphae of the pathogen at the place of contact as confirmed by Mukherjee *et al.* (2001). The antagonistic organisms act on the pathogen by different mechanisms *viz.*, competition, lysis, antibiosis, siderophore production and hyperparasitism (Vidyasekaran, 1999). The present findings are in complete agreement with the findings of the above workers.

Testing of organic extracts against *Pythium aphanidermatum in vitro*.

The aqueous extracts of different organics were evaluated for their inhibitory effect on *Pythium aphanidermatum*. Among all the organic extracts minimum growth was recorded in the extract of castor cake at 20 % (23.33 mm) followed by FYM at 20 % (25.33 mm). Next best in order of merit was mustard cake at 20 % (69.00 mm), Whereas, neem cake at 20 % (85.33 mm) and vermicompost at 20 % (74.67 mm) were poor in inhibiting growth of the pathogen. Maximum per cent growth inhibition of *Pythium aphanidermatum* was recorded in castor cake at 20 % (74.07 %) followed by FYM at 20 % (71.85 %). Next best in order of merit was castor cake at 10 % (61.00 %) followed by FYM at 10% (52.19 %) and mustard cake at 20 % (23.33 %). Whereas, vermicompost, at 20 % (17.04 %) and at 1 0% (5.19 %),

mustard cake at 10 % (11.48 %) and neem cake at 20 % (5.19 %) and at 10 % (2.96 %) were least effective in inhibiting the growth of the *P. aphanidermatum.* (Table 2)

From this study, it is clear indicated that castor cake and FYM were found effective in reducing the growth of *P. aphanidermatum* causing damping-off of tomato. The present investigation is more or less similar to the work done by earlier workers Yadav (2010) who evaluated different organic amendments against *P. aphanidermatum* and reported that castor cake significantly reduced mycelial growth (87 %).

Table 1: Antagonistic effect of bioagents against *Pythium aphanidermatum in vitro.*

Sr. No.	Name of antagonist	Average diameter of pathogen (mm)	Growth inhibition (%)
T1	*Trichoderma harzianum*	16.86	81.26
T2	*T. viride*	24.37	72.92
T3	*T. fasciculatum*	21.07	76.59
T4	*T. virens*	44.27	50.81
T5	*Bacillus subtilis*	78.19	13.12
T6	*Pseudomonas fluorescens*	20.79	76.90
T7	Control	90.00	-
	S.Em ± C. D. at 5% C.V. %	0.21 0.66 0.89	

Table 2: Effect of organic amendment against *Pythium aphanidermatum in vitro.*

Treatment	Name of extract	Average diameter of pathogen (mm)		Growth inhibition (%)	
		10%	20%	10%	20%
T1	Castor Cake	35.10	23.33	61.00	74.07
T2	Neem Cake	87.33	85.33	2.96	5.19
T3	Mustard Cake	79.67	69.00	11.48	23.33
T4	Vermicompost	85.33	74.67	5.19	17.04
T5	FYM	43.03	25.33	52.19	71.85
T6	Control	90.00	90.00	0.00	0.00
	S.Em ± C. D. at 5% C.V. %	0.13 0.41 0.33	0.38 0.41 0.38		

ACKNOWLEDGMENT

The author wish to thanks to the Department of Science and Technology, Ministry of Science and Technology, New Delhi, India for providing inspire fellowship during research work of Ph. D.

REFERENCES

1. Agrios, G. N. Plant disease caused by fungi. In: Plant Pathology IVth Ed. Academic Press, USA. 2005; pp. 266-270.
2. Bell, D. K., Wells, H. D. and Markham, C. R. *In vitro* antagonism of *Trichoderma* species against six fungal pathogens. *Phytopathology*, 1982; 72: 379-382.
3. Brain, P. W. Antibiotics produced by fungi. *Botanical Rev.,* 1951; 17: 357-370.
4. Echobichon, D. J. Pesticide use in developing countries. *Toxicology*, 2001; 160: 27-33.
5. Gravel, V., Martinez, C., Antoun, H. and Tweddell, R. J. Antagonist microorganisms with the ability to control *Pythium* damping-off of tomato seeds in rockwool. *BioControl*, 2005; 50: 771- 786.
6. Jha, A. K., Upadhayay, J. P. and Lal, H. C. Evaluation of organic amendments against white mold of *Phaseolous vulgaris*. *J. Mycol. Pl. Pathol.*, 2007; 37(1): 141-142.
7. Kumar, M. R. and Hooda, I. Evaluation of antagonistic properties of *Trichoderma* species against *Pythium aphanidermatum* causing damping-off of tomato. *J. Mycol. Pl. Pathol*., 2007; 37(2): 240-243.
8. Mahrotra, R. S. and Agrawal, A. Rots, damping-off, downy mildews and white rusts. In: Plant Pathology, IInd Ed. Tata McGraw-Hill Publishing Company Limited, New Delhi. 2004; pp: 312-368.
9. Manoranjitham, S. K., Prakasam, V. and Rajappan, K. Biocontrol of damping-off of tomato caused by *Pythium aphanidermatum*. *Indian Phytopath*., 2001; 54(1): 59-61.
10. Mishra, V. K. *In vitro* antagonism of *Trichoderma* spp. against *Pythium aphanidermatum*. *J. Phytology*., 2010; 2(9): 28-35.
11. Mukherjee, S., Tripathi, H. S. and Rathi, Y. P. S. Integrated management of wilt complex in frenchbean (*Phaseolus vulgaris* L.). *J. Mycol. Pl. Path*., 2001; 31(2): 213-215.
12. Muthukumar, A., Eswaran, A. and Sangeeta, G. Occurance, virulence and pathogenicity of species of *Pythium* inciting damping-off disease in chilli. *J. Mycol. Pl. Pathol*., 2010; 40(1): 67-71.
13. Muthukumar, A., Eswaran, A. and Sanjeevkumar, K. Exploitation of *Trichoderma* spp. on the growth of *Pythium aphanidermatum* in chilli. *Brazilian J. Microbiol*., 2011; 42: 1598-1607.
14. Pieczarka, D. J. and Abawi, G. S. Populations and biology of *Pythium* species associated with snap bean roots and soils in New York. *Phytopathology*, 1978; 68: 409-416.
15. Rajagopalan, C. K. S. (1961). Studies on Phycomycetes in agricultural soils with special reference to Pythiaceae. A Ph. D Thesis submitted to University of Madras. India.

16. Ramamoorthy, V., Raguchander, T. and Samiyappan, R. Enhancing resistant of tomato and hot pepper to *Pythium* disease by seed treatment with Fulorescent pseudomonads. *Eur. J. Plant Pathol*., 2002; 108: 429-441.
17. Ramesh, R. Management of damping-off in brinjal using biocontrol agent. *J. Mycol. Pl. Pathol*., 2004; 34(2): 666-669.
18. Vidyasekaran, P. (1999). Biotechnological approaches to suppress seed to plant transmission of seed-borne pathogens. *In: National Symposium on Seed Science and Technology*. 5-7 August, University of Mysore, Manasagangotri, Mysore, India, pp.41. (Fide: Kumar P. N., M.Sc. Thesis (2009) U.A.S., Dharwad).
19. Vincent, J. M. Distortion of fungal hyphae in the presence of certain inhibitors. Nature, 1927; 150: 850.
20. Yadav, D. L. (2010). Biochemical variability in isolates of *Pythium* spp. Incitant of damping-off in bidi tobacco and it's management. M.Sc. (Ag.) Thesis submitted to Anand Agricultural University, Anand.
21. Yadav, D. L. and Joshi, K. R. Cultural, morphological, pathogenic and biochemical variability in isolates of *Pythium aphanidermatum*, incitant of damping-off in bidi tobacco. *Indian Phytopath*., 2012; 65(4): 386-390.