Journal of Agricultural Technology 2012 Vol. 8(2): 693-697
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

Bioefficacy of *Pseudomonas fluorescens* in management of damping-off disease in papaya (*Carica papaya* L.)

Dar, W.A.[1], Bhat, J.A.[2], Rashid, R.[3*], Rehman, S.[4] and Bhat, Z.A.[5]

[1]Department of Plant Pathology, Allahabad Agricultural Institute Deemed University, Allahabad, U.P. India, [2]Department of Plant Pathology, PAU-Ludhiana, India, [3]Central Institute of Temperate Horticulture, Srinagar, India,[4] Department of Plant Pathology, Skaust- Kashmir India, [5]Department of Horticulture, PAU-Ludhiana, India

Dar, W.A., Bhat, J.A., Rashid, R., Rehman, S. and Bhat, Z.A. (2012) Bioefficacy of *Pseudomonas fluorescens* in management of damping-off disease in papaya (*Carica papaya* L.). Journal of Agricultural Technology 8(1): 693-697.

Damping-off of papaya caused by *Pythium debaryanum* was reduced by soil and seed application of talc based formulations of *Pseudomonas fluorescens* in pot experiment. A chemical metalaxyl also reduces the damping-off as compared control. Besides the reducing the damping-off the antagonist and chemical increased the seed germination, shoot length, root length, fresh and dry weight of papaya seedlings. *Pseudomonas fluorescens* significantly reduced the population of *P. debaryanum* in soil.

Key words: Papaya, *Pseudomonas fluorescens, Pythium debaryanum*, metalaxyl.

Introduction

Papaya is cross pollinated plant. Seeds are sown in raised nursery beds at a depth of 1 cm in rows spaced at 15 cm with 21 cm spacing is a row. Papaya can also be propagated by cuttings or budding however methods have little value for commercial cultivation. A number of diseases have been reported from nursery stage to maturity and damping-off disease is most common during nursery stage. It is caused by *Pythium debaryanum.* (Bose, 1996) and occurs in two phases i.e pre-emergence and post-emergence damping-off. The widely accepted practice for the control of diseases among the farmers is the application of chemical fungicides. Their indiscriminate and excessive use, however results in environmental degradation, decline of beneficial micro and macro organisms and accumulation of chemical residues in the food chain. In view of this the control of pathogens by microbiological control agents has attained increasing interest, coupled with the legislative withdrawal of certain agro-chemicals. A wide range of isolated natural bacteria and fungi with the

* Corresponding author: Rashid, R.; e-mail: rizwan60@gmail.com

potential for bio-control of seed and soil borne plant pathogens have been reported. *Pseudomonas fluorescens* has received overview helming attention because of their capacity to inhibit wide range of soil-borne as well as foliar pathogens. The important mechanism is the indirect inhibition of the pathogen by bacterial stimulation of defense responses in the plant host. *Pseudomonas* can indirectly suppress fungal pathogen by scavenging iron in the rhizosphere environment through the release of siderophores which can trap traces of insoluble iron and form stable complexes. Additional factors such an aggressive root colonization play an important role in the rhizosphere competence and associated biocontrol ability of a *Pseudomonas fluorescens* (Nielson, 1990). In view of the above, the present study entitled "Bio-efficacy of *Pseudomonas fluorescens* in the management of damping-off disease of papaya (*Carica papaya* L.) in nursery" was conducted with following objectives: to evaluate the effect of *Pseudomonas fluorescens* against the seed -ling disease (damping –off) in nursery and study the effect of *Pseudomonas fluorescens* on percentage germination, seedling growth and vigour.

Materials and methods

The *Pseudomonas fluorescens* was isolated from soil on Kings B Medium and mixed with talc powder in the ratio of 1:2. Talc based formulation of *P. fluorescens* and carbendazim were used in the present study. The isolated *Pseudomonas fluorescens* were identified on the basis of morphological character and bio-chemical tests (as per description in Bergey's Mannual of Systemic Bacteriology 2001). Infected seedlings of papaya showing the characteristic symptoms of damping–off were collected from the Research field. The isolation of pathogen *Pythium debaryanum* was done on potato dextrose agar as per the standard method and identified on the basis of cultural and morphological character.

The experiment was conducted in the earthen pots of 30 cm in diameter and pots washed with tap water and treated with formalin (4%) one day before filling of soil in pots. The pots were filled next day with the mixture of soil and farm yard manure in the ratio of 3:1. The 10 day old culture of *Pythium debaryanum* was mixed in a mixer by making uniform spore suspensions. The above layer of soil in pots (6 cm) was removed and spore suspension was inoculated uniformly at 4 x 10^6 spores/ ml (Fatah, 2004) and the pots were re filled with this inoculated soil as per the treatment. Seeds were sown in earthern pots at 60 seeds/pot in 30 cm diameter. Papaya seeds were treated with *Pseudomonas fluorescens* formulation at 4 g/kg of seed (Georgeakopoulos *et. al*., 2002) and with metalaxyl at 2 g/kg of seed (Mazzola, *et.al*., 2002). The soil application was done mixing with 1.0 kg talc based formulation of

Pseudomonas fluorescens with 50 kg of FYM at one kg/pot. The observation of germination percentage at 20 and 30 DAS, damping -off incidence, shoot length (cm), root length (cm), fresh and dry weight (g) were recorded at 20 ,30 and 45 DAS. The data were analyzed with Randomized Block Design.

Result and discussion

Identification of Pseudomonas fluorescens (Bio – chemical test)

Bio-chemical test	Result	Bio-chemical test	Result
Mcconkey Agar	+ve	Indole Test	-ve
Methyl red test	+ve	Voges Proskour Test	-ve
Citrate utilization test	+ve	Gas production from glucose	-ve
Casein hydrolysis	-ve	Starch hydrolysis	-ve
Urea hydrolysis	-ve	Nitrate reduction	-ve
Nitrite reduction	+ve	H_2S Production	+ve
Catalase test	+ve	Oxidation / Fermentation	+ve
Gelatin hydrolysis	-ve	Argimine dihydrolase test	-ve
Cyvine decarboxylase test	-ve	Armthine decarboxylase test	-ve

The above table indicates colonies were identified *as Pseudomonas fluorescens* on the basis of bio-chemical test per description in Bergey's Mannual of Systemic Bacteriology (2001).All treatments significantly increased the seed germination as compared to control. The highest germination percentage at 25 and 40 DAS was recorded in seed + soil treated with *Pseudomonas fluorescens* (88.3% and 93.4%), soil treated with *Pseudomonas fluorescens* (78.7% and 88.3 %) followed by seed treatment with *Pseudomonas fluorescens* (72.9% and 83.7%) and seed treatment with metalaxyl (68.7% and 77.5%) respectively. At 30 DAS the lowest germination percentage was recorded (48.3% and 57.0%) in control. Damping-off incidence was significantly reduced by different treatments of *Pseudomonas fluorescens* as compared to control. The lowest damping-off incidence was recorded in seed + soil treatment with *Pseudomonas fluorescens* (20.0% and 24.1%) and soil treated and seed treated with *Pseudomonas fluorescens* (25.83%, 32.91%, 28.58% and 38.3%) followed by seed treatment with metalaxyl (32.9% and 42.0%) respectively at 25 and 40 DAS. The highest damping-off incidence percentage was recorded in control (37.91% and 66.6%) respectively. The results are in agreement with (Nielson, 1990) ; Kraus *et. al.,* 1992, Doddi *et. al.,*

(1993) and Handelsman and Stabb (1996) and revealed that the reduction of disease incidence may be due to direct inhibition of the pathogen by bacterial stimulation of defense responses in the plant host and can indirectly suppress fungal pathogen by scavenging iron in the rhizosphere environment through the release of siderophores which can trap traces of insoluble iron and form stable complexes. Muthukumar *et.al.,* (2008) also suggest the reduction in damping-off may be due to increase in the density of population of antagonist. In addition to that the antibiotics produced by antagonist might have reduced the population of pathogen. Campbell (1989) also suggest that *Pythium* spp. are poor competitors and thus their population may be replaced due to the competitive effect of antagonist. It was evaluated that treatments seed treatment with *Pseudomonas fluorescens* and seed treatment with metalaxyl were at par with each other. The maximum shoot length (15.0 cm) was recorded in seed + soil treatment with *Pseudomonas fluorescens* followed by soil and seed treatments with biocontrol agent (*Pseudomonas fluorescens*) and metalaxyl at 25 DAS.

The minimum shoot length (7.25 and 11.75cm) was recorded in control. At 40 DAS the highest shoot length was observed in seed + soil treated with *Pseudomonas fluorescens* (33.75 cm) and lowest shoot length was recorded in control (11.75 cm). The maximum root length (5.52 and 12 cm) was recorded in seed + soil treatment with *Pseudomonas fluorescens* followed by soil (4.20 and 10.0 cm) and seed (3.60 and 9.75 cm) treatments with *Pseudomonas fluorescens* and seed treatment with metalaxyl (3.0 and 8.75cm) at 25 and 40 DAS respectively. The minimum root length (2.45 and 7.0cm) was recorded in control. The maximum fresh weight (2.04g) was recorded in seed + soil treatment with *Pseudomonas fluorescens* followed by other treatments i.e soil and seed treatments with *Pseudomonas fluorescens* and seed treatment with metalaxyl. The minimum fresh weight was (1.24g) was recorded in control. The maximum dry weight was recorded (0.39 g) in seed + soil treatment with *Pseudomonas fluorescens* and the minimum dry weight (0.16 g) was recorded in control at 25 DAS. At 40 DAS the maximum dry weight (2.25 g) was recorded in seed + soil treatment with *Pseudomonas fluorescens* followed by other treatments. The minimum dry weight (0.30 g) recorded control Table1. These findings were in confirmation with the findings of Muthukumar *et. al.,* (2008) and he suggested several possible mechanisms to explain this phenomenon including control of minor pathogens, production of plant hormones, production of vitamins, conversion of non utilizable materials into a form that can be utilized by the plant and increased uptake and translocation of minerals, increases the efficiency of nutrient uptake solubilizing certain insoluble nutrient elements like rock phosphate. This is actually one of the

reason due to which growth rate of papaya is increased in *Pseudomonas fluorescens* treated pots.

Table 1. Effect of *Pseudomonas fluorescens* and carbendazim on seed germination %, damping off, plant growth vigor at 20 and 30 DAS

Treatments	Seed germination		Damping off incidence		Shoot length		Root length		Fresh weight		Dry weight	
	20 DAS	30 DAS	20 DAS	30 DAS	30 DAS	45 DAS	30 DAS	45 DAS	30 DAS	45 DAS	20 DAS	30 DAS
Control	48.3	57.0	37.91	66.6	7.25	11.75	2.45	7.00	1.24	2.15	0.16	0.30
Seed treatment with *Pseudomonas fluorescens*	72.9	83.7	28.58	38.3	10.92	25.0	3.60	9.75	1.51	3.32	0.21	0.85
Soil treatment with *Pseudomonas fluorescens*	78.7	88.3	25.83	32.9	13.22	29.5	4.20	10.0	1.68	4.25	0.27	1.45
Seed + Soil treatment with *Pseudomonas fluorescens*	88.3	93.7	20.0	24.1	15.0	33.75	5.52	12.0	2.04	6.30	0.39	2.25
Seed treatment with carbendazim	68.7	77.5	32.9	42.0	9.45	22.25	3.00	8.75	1.36	2.77	0.17	0.70
F test	S	S	S	S	S	S	S	S	S	S	NS	S
SE	1.74	1.23	1.62	1.28	0.56	1.34	0.33	0.45	.078	0.25		0.18
CD	3.80	2.69	3.53	2.81	1.22	2.93	0.73	0.99	0.171	.55		0.41

Each value indicates mean value of four replications

References

Bose, T.K. (1996). Tropical Horticulture - 1. Naye Prakesh. 308-317.

Cambell, R. (1989). Biological control of microbial plant pathogens. Cambridge University Press. p-213.

Doddi, S., L. and Stewart, A. (1993). Biological control of *Pythium* induced damping-off of Beetrot (*Beeta vulgaris*) in glass house. New Zealand Journal of Crop and Horticulture *Science*. 20 : 421-426.

Fatah, S., Indra, S. and Singdan, G.S. (2004). Biological control of tomato damping-off. Journal of Mycology and Plant Pathology 34 : 568-570.

Georgakopoulos, D.G., Fiddaman, P., Leifert, C. and Malathrakis, N.E. (2002). Biological control of cucumber and sugar beet damping-off caused by *Pythium aphanidermatum* with bacterial and fungal antagonists. Journal of Applied Microbiology 92: 1078–1086.

Handelsman, J. and Stab, E.V. (1996). Bio-control of soil borne plant pathogens. Plant Cell 8: 1855-1869.

Kraus, J. and Leopper, J.E. (1992). Lack of evidence of antifungal metabolites produced by plant in biological control of damping-off of Cucumber. Phytopathology 82: 264-271.

Mazzola, M., Andrews, P.K., Reganold, J.P. and Lévesque, C.A. (2002). Frequency, virulence and metalaxyl sensitivity of *Pythium* spp. isolated from apple roots under conventional and organicpr oduction systems. Plant Diseases 86: 669-675.

Muthukumar, A., Eswaran, A. and Sanjeev, K. (2008). Bilogical control of *Pyhtium aphanidermatum*. Mysore Journal of Agricultural Science. 42: 20-25.

Neilson, M.N., Sorenesion, J. and Fel, S. (1990). Secondary metabolite and endochitinase dependent antagonism towards plant pathogenic fungi of *Pseudomonas fluorescens* from Sugerbeet rhizosphere. Applied and Environmental Phicrobiology. 64: 3363-3369.

(Published in March 2012)