WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 14, 1185-1191. Research Article ISSN 2277– 7105

EFFECT OF TIME OF INOCULATION AND DELIVERY SYSTEMS ON BIOLOGICAL CONTROL OF FUNGAL INFECTIONS OF TOMATO (*SOLANUM LYCOPERSICUM*) BY *PSEUDOMONAS SPECIES*

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Article Received on
21 Sept. 2017,

Revised on 11 Oct. 2017,
Accepted on 01 Nov. 2017

DOI: 10.20959/wjpr201714-10060

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ABSTRACT

Six *Pseudomonas* isolates i.e. *P. aeruginosa*13, *P. aeruginosa* 58, *P. putida*71, *P. fluorescens*106, *P. aeruginosa*117, *P. aeruginosa*154 obtained from rhizosphere of healthy tomato plants with potent antifungal activity against the phytopathogenic *Pythium* and *Fusarium* species were used to study biocontrol of tomato (*Solanum lycopersicum*) plants by pot culture experiments. Active nutrient broth cultures of these *Pseudomonas* species were applied by three delivery systems- soil amendment, seed coating and foliar spray, at three different periods, i.e. before sowing, at the time of sowing and after the sowing of seeds. All the *Pseudomonas* cultures were proved successful to control tomato plants from *Pythium* and *Fusarium* infections, although with different efficiencies. Biocontrol of fungal infections of tomato was found to be more effective by soil amendment (>50%) while least effective by foliar spray (34-60%). Application of *Pseudomonas* cultures before sowing of seeds was found to be more effective in case of all the isolates. Biocontrol efficiency was found to be higher in case of *P. aeruginosa* 154, *P. aeruginosa*58 and *P. aeruginosa*13. We conclude that, application of these *Pseudomonas* cultures is more significant for biocontrol of fungal infections of tomato plants especially by soil amendment, before sowing of seeds.

KEYWORDS: Biocontrol, *Pseudomonas*, Phytopathogens, *Pythium*, *Fusarium*.

INTRODUCTION

The Indian farmers have to suffer great economic losses per year due to weather irregularities and crop diseases. Among the phytopathogenic microbes, fungi are the most potent and

destructive. Many of the serious plant diseases are caused by soil-borne fungi such as root rot, crown or collar rot, damping-off, blights, fruit decay, wilts, etc. These diseases show necrosis type of symptoms indicated by decay or rotting of tissues due to derangement of cells.[1] The soil-borne fungal pathogens mainly include the species of- *Phytopthora*, *Pythium*, *Fusarium*, *Phymatotrichum, Colletotrichum, Macrophomina, Gauemannomyces*, *Corticium*, *Verticillium*, *Armillaria, Sclerotium*, etc. Among the soil-borne fungal pathogens *Fusarium species* are considered to be very dynamic and taxonomically notorious.[2] The *Pythium* species are relatively fast growing and aggressive fungal pathogens, particularly at moist and low temperature conditions.[3]

Indiscriminate use of chemical agents to control the plant diseases since last few decades has created great harm to human beings, animals, vegetation and the complete environment. Biological control of crop plants has now become an indispensable need of sustainable agriculture. Biocontrol microorganisms also function to promote crop growth by different mechanisms and so called 'PGPM'. Among the bacteria, species of *Pseudomonas* and *Bacillus* while among the fungi, species of *Trichoderma* and *Gliocladium* are the most widely studied and found successful. Different biocontrol products are available in market with trade names in either liquid or solid form. These are applied before, after or at the time of seed sowing, using the delivery systems like seed coating, soil amendment and foliar spray.[4] Our antifungal *Pseudomonas* isolates were found considerably effective to control the infections of tomato plants by *Fusarium* and *Pythium* species, especially by soil amendment before the sowing of seeds.

MATERIAL AND METHODS

Screening of potent antifungal *Pseudomonas* isolates against phytopathogenic *Fusarium* and *Pythium species*

Six isolates of *Pseudomonas* species showing good antifungal activity against phytopathogenic *Fusarium* and *Pythium species* in dual culture method in PDB and on PDA [5] were selected for the study of biocontrol of fungal infections of tomato plants.

Revival of cultures and production of biomass

100 µl of each of the six *Pseudomonas* cultures was separately inoculated in 100ml nutrient broth (NB) and phytopathogenic *Fusarium* and *Pythium species* in 100ml potato dextrose broth (PDB) and incubated for 24 and 48 hours respectively at 28^{o}C, on a rotary shaker.

Study of biocontrol efficiency of *Pseudomonas* cultures by pot culture technique

Ability of six *Pseudomonas* cultures to control the fungal diseases caused by *Pythium* and *Fusarium* species was tested by pot culture experiments.[7,8] Fertile soil was collected from field and sieved. It was filled in polyethylene bags and sterilized in autoclave at 121^0C for 30min. The *Pseudomonas* cultures were applied by three delivery systems: Soil amendment (100ml/kg), Seed coating and Foliar spray (10ml/pot). Seed coating was done 24hrs before sowing of seeds using sterilized carboxy methyl cellulose @ 10g in 100ml broth culture (10%) as sticking agent. Soil amendment and foliar spray was applied at three times separately, i.e. before, after and at the time of seed sowing.

Arrangement of pots and inoculations

A set of twelve pots was arranged in four rows, each containing three pots as follows.

Row-I: Three pots inoculated with each phytopathogen separately, 1hr before the seed sowing (Control set). **Row-II:** Three pots inoculated with each *Pseudomonas* species separately, 7days before seed sowing. **Row-III:** Three pots inoculated with each *Pseudomonas* species separately, at the time of seed sowing. **Row-IV:** Three pots inoculated with each *Pseudomonas* species separately, 7days after the seed sowing.

Sowing of seeds: The pots filled with specifically treated soil were sown with healthy seeds of tomato (Mahabeej PKM-1) @10 seeds/pot, in triplicates pots. They were regularly irrigated and observed for development up to 15 days. The experiment was repeated thrice with each fungal pathogen and *Pseudomonas* culture.

Calculations

Percent disease incidence (PDI) and Percent biocontrol efficiency (PBE) values were calculated using formula.[1,6,9]

$$\text{P. D. I.} = \frac{\text{(Average number of infected plants/pot) X 100.}}{\text{Total number of plants/pot}}$$

i.e. P. D. I. = (Average number of infected plants/pot) X 10.

Percent biocontrol efficiency (PBE) = (Average number of healthy plants/pot) X 10.

i.e. PBE = 100 - PDI.

RESULTS

Table. 1: PDI in case of soil amendment of *Pseudomonas* cultures.

***Pseudomonas* cultures**	**PDI for *Pythium species***			**PDI for *Fusarium species***		
	BSS	**ATSS**	**ASS**	**BSS**	**ATSS**	**ASS**
*P. aeruginosa*13	20	28	40	26	30	35
*P. aeruginosa*58	22	32	37	20	28	38
*P. putida*71	28	40	50	35	42	50
*P. fluorescens*106	27	40	52	32	38	47
*P. aeruginosa*117	30	45	58	28	35	45
*P. aeruginosa*154	18	26	35	20	38	35
Control	100	100	100	100	100	100

Values are average of triplicate tests. ***PDI****- Percent disease incidence;****BSS****- Before seed sowing;* ***ATSS****- At the time of seed sowing;* ***ASS****- After seed sowing.*

Table. 2: PDI in case of foliar spray of *Pseudomonas* cultures.

***Pseudomonas* cultures**	**PDI for *Pythium species***			**PDI for *Fusarium species***		
	BSS	**ATS**	**ASS**	**BSS**	**ATSS**	**ASS**
*P. aeruginosa*13	32	35	52	35	42	65
*P. aeruginosa*58	35	42	50	42	54	68
*P. putida*71	40	40	45	48	58	76
*P. fluorescens*106	50	58	90	55	60	90
*P. aeruginosa*117	48	55	86	57	62	90
*P. aeruginosa*154	32	38	70	45	42	66
Control	100	100	100	100	100	100

Values are average of triplicate tests. ***PDI****- Percent disease incidence;* ***BSS****-Before seed sowing;* ***ATSS****- At the time of seed sowing;* ***ASS****- After seed sowing.*

Table. 3: PBE of *Pseudomonas* cultures by different delivery systems.

***Pseudomonas* cultures**	**Average PBE for *Pythium species* by three delivery systems**			**Average PBE for *Fusarium species* by three delivery systems**		
	SA	**FS**	**SC**	**SA**	**FS**	**SC**
*P. aeruginosa*13	70.67	**60.34**	62.33	69.67	**52.67**	55.00
*P. aeruginosa*58	69.67	57.67	**63.66**	**71.34**	45.34	**60.33**
*P. putida*71	60.67	58.34	58.00	57.67	39.34	42.00
*P. fluorescens*106	60.34	34.00	50.00	61.00	31.67	52.00
*P. aeruginosa*117	55.67	37.00	50.00	64.00	30.34	47.33
*P. aeruginosa*154	**73.67**	53.34	60.66	69.00	49.00	52.66
Control*	00	00	00	00	00	00

Values are average of triplicate tests. ***PBE****- Percent biocontrol efficiency;* ***SA****- Soil amendment;* ***FS****- Foliar spray;* ***SC****- Seed coating.*

DISCUSSION

All the pots inoculated with phytopathogenic fungal cultures (Row-I) in all experiments showed 100% infection, PDI = 100%, i.e. PBE = Zero. Percent disease incidence (PDI)

values in case of soil amendment (table-1) before, at the time and after seed sowing for *Pythium* infection ranges between 18-30, 25-45, and 35-58%, respectively whereas for *Fusarium* infection 20-35, 28-42, and 35-50%, respectively. PDI values in case of foliar spray (table-2) before, at the time and after seed sowing for *Pythium* infection ranges between 32-50, 35-58 and 50-90%, respectively whereas for *Fusarium* infection 35-57, 42-62 and 65-90%, respectively.

Percent biocontrol efficiency (PBE) of *Pseudomonas* cultures by different delivery systems (table-3) revealed that, it was best with soil amendment (55.67-73.67% and 57.67-71.34%) followed by seed coating (50.00-63.66% and 42.00-60.33%) and least in case of foliar spray (34.00-60.34% and 30.34-52.67%), in case of *Pythium* and *Fusarium species*, respectively. It may be due to the fact that, most of the fungi are soilborne phytopathogens[1,4] and infect the crop plants below the soil surface where the foliar spray is not much effective.

Soil amendment of *Pseudomonas* cultures especially before sowing of seeds is a good inoculation method that supports the rapid colonization and growth of biocontrol bacteria in the rhizosphere of crop plants. This allows production of a large biomass of biocontrol organism to fight against the population of phytopathogen. Soil is a nutrient rich natural medium that supports the growth of microorganisms. However, Dal Bellow *et. al*.,(2002) observed that, seed coating of antifungal bacterial culture was especially effective to control *Fusarium* infections.[10] An inverse proportion between the extents of antifungal activity of *Pseudomonas* isolates (as observed by *in vitro* co-culture method in PDB and PDA) and PDI values was observed, i.e. values of percent biocontrol efficiency (PBE) were found directly proportional to the extent of antifungal activity. This indicated that, the *in vitro* antifungal activity tested in laboratory by co-culture method is the primary criterion for screening of biocontrol organism.[11] Expert and Digit (1995) observed similar correlation in case of *Pseudomonas putida* and *P. fluorescens* isolates.[12]

All the six potent antifungal *Pseudomonas species* tested in present study for control of *Pythium* and *Fusarium* infections of tomato proved successful, although with varying efficiencies (PDE values 30.34-73.67%). Among the six *Pseudomonas* cultures tested, *P. aeruginosa*154 was the best followed by *P. aeruginosa*58 and then *P. aeruginosa*13, with respect to their maximum PBE values.

Jeyalakshmi *et. al*., (1998) observed 33.2% control of fruit rot and die back of chili by *P. fluorescens*.[6] Manoranjitham *et al*., (2001) observed that, soil application of *Trichoderma viride* and *Pseudomonas fluorescens* effectively controlled damping-off of tomato caused by *Pythium aphanidermatum* under pot culture experiments.[7] Rangeshwaran and Prasad (2000) observed good disease suppression by *P. fluorescens* PDBCAB2 and *P. putida* PDBCAB19.[13] Maheswari *et. al*., (2001) observed that the *Pseudomonas* isolates F11, F13 and F14 were successful to reduce damping-off of cotton.[14] Anith *et al*., (1999) observed that, *Pseudomonas species* EM85 isolated from cotton rhizosphere showed *in vitro* antifungal activity against number of soil-borne pathogens and protected plants in pot culture experiment, with production of multiple antifungal compounds.[8] Ansari M. M.(2004) observed biocontrol of collar rot of soybean by *P. fluorescens.*[15] Jambulkar and Sharma observed that, seed and seedling treatment of talc based bioformulations of *P. fluorescens* RRb-11 to rice were found more effective than soil drenching. A combination of different delivery systems reduced the disease by 92.3 and 88.5% over control in 2009 and 2010, respectively.[16]

We conclude that, application of these *Pseudomonas* cultures will be significant for biological control of fungal infections of tomato plants in field, especially by soil amendment before sowing of seeds.

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