INTERNATIONAL JOURNAL OF RESEARCHES IN BIOSCIENCES, AGRICULTURE AND TECHNOLOGY
© VISHWASHANTI MULTIPURPOSE SOCIETY (Global Peace Multipurpose Society) R. No. MH-659/13(N)
www.vmsindia.org

SCREENING OF ANTIFUNGAL *PSEUDOMONAS SPECIES* WITH EFFICIENCY TO PROMOTE GROWTH OF TOMATO (*SOLANUM LYCOPERSICUM*) PLANTS

B. M. Sandikar
Department of Microbiology, Maharashtra Udayagiri Mahavidyalaya, Udgir-413517 Dist. Latur (MS).
balkrishna64@rediffmail.com

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 *Fusarium* and *Pythium* species were used to study biocontrol and growth promotion of Tomato (*Solanum lycopersicum* L.) plants by pot culture experiments. Nutrient broth cultures of these *Pseudomonas* isolates were applied by soil amendment and foliar spray. Among the six isolates tested, four isolates i.e. *Pseudomonas aeruginosa*13, *P. aeruginosa* 58, *P. fluorescens*106, and *P. aeruginosa*154 showed considerable enhancement in wet weight, dry weight, shoot height and root length of tomato plants after 30 days. A direct correlation between the extent of antifungal activity and plant growth promotion was observed. Application of cultures by soil amendment was found more effective to promote tomato plant growth than the foliar spray. We conclude that, the bioformulations prepared by using these four *Pseudomonas* cultures will be significant to promote the growth and yield of tomato as well as other crops.

Keywords- *Pseudomonas*; Antifungal activity; Tomato; PGPR.

Introduction

Biological control of phytopathogens and plant growth promotion are the two facets of single coin. Indiscriminate use of chemical control agents has created many problems to the environment and public health. Hence, biological control has become an indispensable need of sustainable agriculture. Antagonistic activity of rhizobacteria against the soilborne phytopathogens protect the crops from infections, keep them healthy and enhance the yield of crops [1,2]. In addition to inhibit or kill the phytopathogens, the rhizosphere microbes promote the growth of host plants by enhancing soil fertility by conducting significant geochemical processes like mineralization, N_2 fixation, phosphate solubilization [3], etc., production of plant growth promoting substances like growth hormones (auxins, cytokinins, etc.), enhancing nutrient uptake by plants and supply of vitamins, enzymes and other significant metabolites to plant host [4]. These microbes in soil are so called 'PGPM'.

Among the PGPM, rhizobacteria are leading due to their characteristics like high population density per unit volume of soil, fast growth (short generation time, metabolic versatility, motility by means of flagella and ability of anaerobic growth and endospore formation by some genera. Among the rhizobacteria, the fluorescent *Pseudomonas species* are dominant in rhizosphere of crop plants and contribute a great in biocontrol and plant growth promotion [5,6,7]. Among the vegetables, tomato is widely used in food preparation worldwide.

Among the six antifungal *Pseudomonas* isolates, four i.e. *P. aeruginosa*13, *P. aeruginosa* 58, *P. fluorescens*106, and *P. aeruginosa*154 were found more effective to promote the growth of tomato plants in pot culture experiments conducted in three successive seasons.

MATERIALS AND METHODS

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

Six rhizosphere isolates of *Pseudomonas* showing potent antifungal activity against phytopathogenic *Fusarium* and *Pythium species* in dual culture method [8] were selected for the study of growth promotion of tomato plants.

Revival of *Pseudomonas* cultures and production of biomass

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

Study of growth promotion of tomato plants by *Pseudomonas* cultures by pot culture technique

Fertile soil was collected from field and sieved. It was filled in plastic pots. A set of nine pots was arranged in three rows, each containing three pots. The first row of three pots without any artificial inoculation was used as control for comparison. Second row of pots was inoculated by active NB cultures of *Pseudomonas* isolates by soil amendment @ 100ml per pot and third row by foliar spray @10ml per pot.

Sowing of seeds-

The pots filled with specifically treated soil were sown with healthy seeds of tomato (Mahabeej PKM-1) @10 seeds/pot, in triplicate sets. These were regularly irrigated and observed for development up to 30 days. The results obtained for Row-I (soil without any artificial inoculation) was used as control.

Calculations-

Plant growth after 30 days was recorded in the form of average wet weight, dry weight, shoot height and root length. Plant growth promotion abilities of the *Pseudomonas* cultures were calculated in terms of percent increase over control in wet weight, dry weight, root length and shoot length of plants, on 30th day.

$$\text{Percent increase over control} = \frac{(\text{Weight or length in test}) - (\text{Weight or length in control})}{\text{Weight or length in control}} \times 100$$

Results

Table-1 Effect of *Pseudomonas* cultures on wet weight and dry weight

***Pseudomonas* cultures**	**Average weight wet (mg)**		**Average dry weight (mg)**	
	Soil amendment	**Foliar spray**	**Soil amendment**	**Foliar spray**
*P. aeruginosa*13	3850 (23.20)	3780 (19.62)	275 (25.00)	262 (18.01)
*P. aeruginosa*58	3720 (19.04)	3625 (14.71)	270 (22.72)	250 (12.61)
*P. putida*71	3271 (4.67)	3168 (0.25)	226 (2.72)	224 (0.90)
*P. fluorescens*106	3665 (17.28)	3608 (14.17)	262 (19.09)	270 (21.62)
*P. aeruginosa*117	3320 (6.24)	3210 (1.58)	223 (1.36)	225 (1.35)
*P. aeruginosa*154	3930 (25.76)	3720 (17.72)	282 (28.18)	278 (25.22)
Control*	3125	3160	220	222

The values are average of triplicates. Control: No any artificial inoculations.
Values in parenthesis indicate percent increase over control.

Table-2 Effect of *Pseudomonas* cultures on shoot height and root length

***Pseudomonas* cultures**	**Shoot height**		**Root length**	
	Soil amendment	**Foliar spray**	**Soil amendment**	**Foliar spray**
*P. aeruginosa*13	260 (18.18)	255 (16.97)	158 (26.40)	162 (28.57)
*P. aeruginosa*58	256 (16.32)	252 (15.59)	155 (24.00)	157 (24.60)
*P. putida*71	228 (3.63)	222 (1.83)	125 (00.00)	128 (1.58)
*P. fluorescens*106	251 (14.09)	248 (13.76)	149 (19.20)	150 (19.04)
*P. aeruginosa*117	224 (1.81)	220 (0.91)	130 (4.00)	129 (2.38)
*P. aeruginosa*154	254 (15.45)	256 (17.43)	160 (28.00)	162 (28.57)
Control*	220	218	125	126

The values are average of triplicates. Control: No any artificial inoculations.
Values in parenthesis indicate percent increase over control.

Discussion:

Among the six *Pseudomonas* isolates tested, four i.e. *P. aeruginosa*13, *P. aeruginosa*58, *P. fluorescens*106 and *P. aeruginosa*154 were considerably successful to enhance the growth of tomato plants with respect to all parameters tested with varying efficiencies (about 12-28%), as compared to control set. The best *Pseudomonas* culture with respect to overall percent increase in growth of tomato plants was *P. aeruginosa*154, followed by *P. aeruginosa*13. A direct correlation between antifungal activity and growth promotion of tomato plants was observed that indicated major role of biocontrol in plant growth promotion.

Increase in tomato plant growth by *Pseudomonas* cultures was found to be higher by soil amendment than the foliar spray. Soil amendment of *Pseudomonas* cultures is a good inoculation method that supports the rapid colonization and growth of PGPR in the rhizosphere of crop plants. This allows production of a large biomass of PGPR for rapid enhancement of plant growth. Soil is a nutrient rich natural medium that supports the growth of microorganisms.

These *Pseudomonas* isolates may enhance tomato growth by different mechanisms such as control of phytopathogenic fungi [8], enhancing soil fertility by conducting significant geochemical processes such as phosphate solubilization [3], production of plant growth promoting substances, enhancing nutrient uptake by plants [9] and supply of vitamins, enzymes and other significant metabolites to plant host [1,2]. Crowley *et al.*, (1991) suggested that, siderophores produced by root colonizing microbes may provide Fe^{+++} to plants and

promote the plant growth [10]. Patten and Glick (2002) observed that, IAA production by *Pseudomonas putida* play important role in development of host plant root system [4].

Schipper *et al.*, (1987) observed that, seed treatment of fluorescent *Pseudomonas species* strains WCS 358, WCS 365 and WCS 374 improved the dry weight of potato plants in pots, by 128%, 131% and 123% respectively over the control plants [11]. Saikia *et al.*, (2004) observed improvement in shoot and root lengths of chickpea plants [12]. Jagadish and Jagadish (2008) obtained good biocontrol as well as yield improvements of tomato using *Pseudomonas gladioli* B-12 applied by combinations of delivery systems [13]. Similar results of biocontrol of phytopathogens, plant growth promotion and yield improvements were observed by in case of *Pseudomonas species* and other rhizobacteria with different crop plants.

Conclusion-

The antifungal *Pseudomonas* isolates *P. aeruginosa*13; *P. aeruginosa*58, *P. fluorescens*106 and *P. aeruginosa*154 were proved successful to promote the growth of tomato plants and are hopeful to apply on tomato as well as other crops to enhance growth and yield.

Acknowledgement

I am heartily thankful to- The Principal, Maharashtra Udayagiri College, Udgir and Mr. A. B. Nalgirkar, Assistant Cotton Research Officer, Agriculture Research Centre, Udgir to provide all necessary facilities for my research work.

References:

[1] Campbell R. (1989). Biological control of microbial plant pathogens. Cambridge University Press, New York.

[2] Pal V. and Jalali I. (1998). Rhizosphere bacteria for biocontrol of plant diseases (Review). Indian J. Microbiol. **38**: 187-204.

[3] Sandikar B. M. and Awasthi R. S. (2008). Phosphate solubilization by antifungal *Pseudomonas species* from tomato (*Solanum lycopersicum* L.) rhizosphere. J. Microb. World. **10** (2): 141-146.

[4] Patten C. L. and Glick B. R. (2002). Role of *Pseudomonas putida* indole acetic acid in development of host plant root system. Appl. Environ. Microbiol.**68:** 3795-3801.

[5] Bhatia S., Dubey R. C. and Maheshwari D. K. (2005) Enhancement of plant growth and suppression of coller rot of sunflower caused by *Sclerotium rolfsii* through fluorescent *Pseudomonas*. Indian Phytopath. **58** (1): 17-24.

[6] Costacurata A. and Vanderleyden J. (1995). Synthesis of phytohormones by plant associated bacteria. Critical Reviews in Microbiology. **21**(1): 1-18.

[7] Dowling N. D. and Fergal O'Gara (1994). Metabolites of *Pseudomonas* involved in biocontrol of plant diseases. (Review) Ti B Tech. **12**:133-141.

[8] Sandikar B. M. and Awasthi R. S. (2009). Studies on biological control agents against soilborne fungal pathogens of crop plants. Ph.D. Thesis. Submitted to Swami Ramanand Teerth Marathwada University, Nanded: 2009.

[9] Whipps J. M. (2001). Microbial interactions and biocontrol in the rhizosphere. J. Expt. Botany **52**: 487-511.

[10] Crowley D. E., Wang Y. C., Reid C. P. P. and Szaniszlo P. J. (1991). Mechanisms of iron acquisition from siderophores by microorganisms and plants. Plant and Soil **130**: 179-198.

[11] Schipper B., Bakker A. W. and Baker Peter A.H.M. (1987). Interactions of deleterious and beneficial rhizosphere microorganisms and the effect of cropping practices. Ann. Rev. Phytopathol. **25**: 339-358.

[12] Saikia R., Singh K. and Arora D. (2004). Suppression of Fusarium wilt and charcoal rot of chickpea by *Pseudomonas aeruginosa* RsB29. Indian J. Microbiol. **44**: 181-184.

[13] Jagadish D. R. and Jagadish K. S. (2008). Biological control of early blight of tomato by the growth promoting *Pseudomonas gladioli* B-25 strain: Evaluation of different delivery methods. Asian J. of Microbiol. Biotech. Env. Sc. **10**(1): 75-78.