Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2013, 5(9):10-14

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Antagonistic effect of tomato rhizospheric microbes against some pathogens

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ABSTRACT

The present research deals with the study of antagonistic effect of microorganism against black root rot disease caused by Fusarium oxysporum and collar rot disease caused by Rhioctonia solani. The antagonistic soil bacteria, Bacillus species Pseudomonas fluorescens were isolated from tomato field soil by serial dilution method. The tomato pathogens were isolated from the infected plants which were collected from tomato field. The antagonistic microorganisms against the fungal pathogens were observed by Dual Culture Technique and antibiotic interaction. The growth of the pathogen in the treated plate and control plate were measured and the efficiency of antagonistic organisms control the pathogens were expressed as % inhibition of mycelia. Among two organisms, Bacillus spp (36.7%) was found to have more antagonistic activity against test pathogens. The Pseudomonas spp showed 35.5% of growth inhibition against test pathogens. In this study, both the antagonistic bacteria effectively control the Fusarium oxysporum than Rhizoctonia solani.

Key words: Biocontrol agent, *Fusarium oxysporum, Rhizoctonia solani, Bacillus subtilis and Pseudomonas fluroscens.*

INTRODUCTION

Tomato (*Lycopersicon esculentum*) is one of the most popular and important commercial vegetable crop grown throughout the world ranking second in importance next to potato in many countries. It is rich in vitamin A, B and C and has very high potential for developing value added products like soap, puree, juice, ketchup and powder through processing. It is also economically important for its edible fruits which can be consumed either raw or cooked. In India, it occupies an area of 0.54 million with a production of 7.60 million tones with an average yield of 14.07 tonnes per ha [1].

Tomato is affected by a number of diseases causing substantial losses in yields. Besides fungal, bacterial and phytoplasmal infections, it is also affected by a large number of viral diseases including tomato leaf curl and tomato mosaic in India. The tomato mosaic virus cause reduction in weight of tomato fruits upto 59^{o}C percent with a mean disease incidence of 55.98 percent.

Biological control by antagonistic organism has been studied extensively and rhizobacterial strains have as potential biocontrol agents for the control of root and foliar diseases [2]. It has been proved that microorganisms isolated from roots or rhizosphere of a specific crop adapted better to that crop and provided effective control of diseases than organisms isolated from other plant species.

The natural control of several phyto pathogens is based on the presence of suppressive soils where several biocontrol microorganisms rhizosphere soil tomato plants and screen them in relation with the control of *Fusarium oxysporum*, which can cause tomato root rot disease. Also tomato plants treated by *Rhizoctonia solani* have shown biocontrol activity against damping-off and root rot disease and gave hign yield of tomato.

Antagonism

The killing, injury or inhibition of growth of one species of microorganism by another when one organism adversely affects the environment of the other [3]. Non pathogenic to plants, human beings and animals. It should be broad spectrum of activity with fast growth and sporulation. It must be easily available and cheap to produce long shelf life and must be efficient in different environmental conditions. Must be compatible with biofertilizers and should be susceptible to action with seed treating chemicals.

Symptoms of Tomato [4]

Tomato which produced clearing of veins and veinlets on the younger leaves. In the early stages, the symptoms of the disease included stunting of whole plant and folding or rolling of leaves along with midrib. In the later stages, some of the leaflets showed pale green or yellow areas with different mottling between the veins of the leaf lets[5]. observed a strain of TMV on tomato, which produced vein clearing, occasional foliar necrosis and interveinal chlorosis in the field. The virus under green house conditions produced systemic mottling on tomato.

Most of the biofertilizers are based on bacteria and fungi that are living in a symbiotic way with plants; this is a positive way to organic fertilization of different Crops like leguminuous, grains and others, the use of antagonistic microorganisms for biological control of pothogenic fungi like *Rhizoctonia solani and Fusarium oxysporum* and many other is a viable option. Therefore, this organic approach may represent an effective solution where other chemical options had been unsuccessful [6]. Hence, the present study under taken following objectives,

Collection of sample from tomato rhizosphere soils at Sundarakkotai, Thiruvarur District, Tamil Nadu, South India. Isolation and identification of antagonistic microorganisms from the soil sample. Isolation and identification of plant pathogens. Identification of test pathogen. Antagonistic effect of isolated microorganisms against *Rhizoctonia solani* and *Fusarium oxysporum.* Determination of antibiotic interaction.

EXPERIMENTAL SECTION

Sample collection

Samples were collected from Rhizospheric soil of tomoto plant, Sundarakkottai, Mannargudi, Thiruvarur district,TamilNadu,South India. The collected samples were taken in a sterile container and were transferred to the laboratory for microbiological analysis.

Isolation of test organisms

The infected parts of tomato plant was selected for isolation of test pathogens. Infected tissues of leaves, stem and roots were sterilized with 0.1 mercuric chloride and washed with distilled water in 3times. Potato dextrose agar (PDA) was prepared. The surface sterilized tissues were aseptically transferred to potato dextrose agar media and incubated at 24^0C for three days.

Identification of soil fungi and bacteria

The soil fungi were identified by using wet mount technique [7] Gram's staining and biochemical test were performed for bacteria.

Antagonistic effect

Dual culture method [8]

Colony interaction between the test pathogens and bacteria were studied invitro in dual culture method. The individual test pathogen *Fusarium oxysporum* and *Rhizoctonia solani* were grown separately on potato dextrose agar medium and the individual species of *Pseudomonas fluorescens, Bacillus subtilis* grown separately on nutrient agar medium. Then, the agar blocks (5 mm thickness) cut from the individual just opposed to each other approximately 3 cm apart, on PDA medium in petriplates. Three replicates for each set were maintained and control was set in single. The position of the colony margin on the back of the disc were recorded daily. Assessment were made for the fungi and bacteria achieved an equilibrium after which there was no further alteration in the growth since both of the organisms were naturally inhibited. The assessment was made for both organisms.

$$\text{Inhibition in radial growth} = 100 \times \frac{r^1 - r^2}{r^1}$$

where,

r^1 is the radial mycelia growth in control.

r^2 is the radial mycelia growth in treatment.

Antibiotic interaction(Food poisoning technique) [9]

The individual species of effective fungi like *F.oxysporum* was grown on pure culture on PDA medium in petriplates (90mm diameter). Agar blocks (5mm thickness) cut from the actively growing margin of the young colonies of antagonistic fungi were inoculated separately in 250ml conical flask containing 100ml of sterilized potato dextrose broth. The flasks were incubated at 37^0C for 15 days. After 15 days of incubation, the staling substances were filtered first through (whatman No.40 filter paper and then through membrane filter). The filterate was transferred aseptically into the conical flasks, condensed and stored at 4^0C for further use.

The culture filtrates prepared in such a way were added separately to the cooled PDA medium to give the concentration of 5,10,15 and 20 percent. The amended PDA medium was dispersed in petriplates and allowed to solidify. After solidification, agar blocks (5mm) cut from the actively growing margin of the test fungi like *R.solani* were inoculated at the centre of the plates. The plates were incubated at 37^0C for five days. The radial growth was measured periodically and the mean growth rate was calculated and control was maintained. The percent inhibition of growth was calculated as follows.

$$\% \text{ of inhibition of growth} = \frac{\text{Growth control-Growth in treatment}}{\text{Growth in control}} \times 100$$

Statistical analysis

Random sampling was used for the entire test, the data of all the parameters were statistically analyzed and expressed as mean ± S.D [12]

$$\textbf{Mean} = \overline{X} = \frac{\Sigma X}{N}$$

ΣX = sum of all the values of variable
N = Number of observation

$$\textbf{S.D} = \sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}}$$

Where,

$\sum (x - \bar{x})^2$ = The sum of the square of the deviation of each value from the mean
N = Number of observation.

RESULTS AND DISCUSSION

Antagonistic effect of rhizospheric bacteria were isolated from the rhizosphere soil of tomato were evaluated against black root rot pathogen *Fusarium oxysporum* and collar rot pathogen *Rhizoctonia solani* by dual culture and antibiotic interaction method.

Antagonistic effect

Dual culture method

Phytoroglucinol metabolites are phenolic and are produced by bacteria with broad spectrum antibacterial, antifungal and phototoxic properties [11]. Management of black root rot disease and collar rot disease of tomato has been directed towards the integration of cultural practice with chemical control. However chemical control using effective fungicides has various undesirable effect, such as phototoxic to tomato plant and the requirements for critical limiting of fungicides application hinder its usages [12] cause environmental pollution and decrease diversity of target organisms.

The highest gowth inhibition was measured in *Bacillus subtilis* (36.7%) and *Pseudomonas fluorescens* against *Fusarium oxysporum* (30.0 mm).

Antibiotic interaction

Antagonistic effect of *Bacillus subtilis* and *Pseudomonas fluroscens* were studied against tomato pathogens viz, *Rhizoctonia solani* and *Fusarium oxysporum* in invitro condition. Several strains of *Pseudomonas fluorescens* were isolated from rhizosphere of plants and were identified as bio types C and D. These siderophore (fluorescent

pigment) producing strains showed antagonism in invitro tests, to plant pathogenic fungi, *Fusarium oxysporum*, *Fusarium cubense*, *Rhizoctonia solani* and selected strains of *Pseudomonas fluorescens* usedfor bacterization of cotton seeds enhanced the plant growth by 12-27% in tomato and 8-40% in cotton. These plant growth promoting rhizobacteria could be potentially used as biofertilizer and also for biological control of plant disease in Indian agriculture [13].

Antagonistic microorganisms in the biological control of the disease induced by *Fusarium oxysporum* in tomato although systems have been developed to study other host pathogen biocontrol agent interactions [14]. In agriculture, phytopathogenic fungi can causes plant disease and much loss of crop yields. Pesticide or used to control the plant disease. However, agrochemical treatment causes environmental pollution and diseases. Microorganisms act as biocontrol agents have high potential to control the plant pathogens and no effective on the environmental or other non-targeted organisms. There are numerous report on the potential uses of biocontrol agent as replacement for Agrochemical.Tomato black root rot caused by *Fusarium oxysporum.* It is one of the destrutive tomato disease in worldwide.

In the present investigation, the effect of culture filtrate of soil bacteria was increased and also increased the growth inhibition of plant pathogen. The percentage of filtrate (20µm) in *Pseudomonas fluorescens* on *Rhizoctonia solani.* The growth inhibition was measured as 70.0 mm. and the filtrate of *P.fluorescens* on. *Fusarium oxysporum* was measured as 72.4mm. The culture filtrate of *Bacillus subtilis* and R. solani growth inhibition was measured as 66.6mm and effect of *Bacillus subtilis* on *Fusarium oxysporum* growth was measured as 70.5mm.

The overall percentage of growth inhibition was noticed in *Pseudomonas fluorescence* (70%) when compared with *Bacillus subtilis* (55%).

Through the fungal and bacterial species are in distribution, their population in a particular habitats change, due to the fluctuation in the physico - chemical parameter. It has been studied that the maximum number of fungus and bacteria population occurred during the moisture high [15].

Table – I Antagonistic effect of *Bacillus subtilis* against the test pathogens

Incubation(days)	Average diameter of mycelial growth (mm)			
	Bacillus spp	***R. solani***	***F.oxysporum***	**Percentage of Inhibition**
3	50.0	30.0	45.2	41.7
5	55.0	25.0	40.0	40.0
6	60.0	20.2	30.0	36.7

Table –II Antagonistic effect of *Pseudomonas fluorescens* against test pathogens

Incubation (days)	Average diameter of mycelial growth (mm)			
	P. fluorescens	***F. oxysporum***	***R. solani***	**% of inhibition**
3	40.0	45.2	30.0	38.4
5	50.0	40.0	25.0	38.3
6	56.5	30.0	20.2	35.5

Table - III Effect of filtrate of soil bacteria on the growth of tomato pathogens

S. No.	Culture Filtrate Used	Tomato pathogens					
		Concentration of the culture Filtrate (%)	*Rhizoctonia solani*		*Fusarium oxysporum*		% of growth inhibition
			Growth rate (mm\day)	Growth inhibition	Growth rate (mm/ day)	Growth inhibition	
1.	*P. fluorescens*	5	40.0±0.15	20.0	30±0.25	14.2	25
		10	30.0±0.25	40.0	26±0.35	25.7	35
		15	25.0±0.31	50.0	18±0.36	48.5	55
		20	15.0±0.20	70.0	12±0.29	72.4	70
	Control	-	50.0±0.1	-	35±0.1	-	-
2.	*B. subtilis*	5	40.0±0.16	11.1	35±0.25	41.2	22
		10	35.0±0.24	22.2	30±0.36	47.0	33
		15	25.0±0.36	44.4	26±0.15	55.8	42
		20	15.0±0.25	66.6	20±0.24	70.5	55
	Control	-	45.0±0.1	-	45±0.1	-	-

Values are expressed as Mean ± Standard deviation

The result indicated that there is an untapped native resource of antagonistic microorganisms in the tomato field soil that could be exploited in the biotechnological, medicinal and agricultural industries.

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