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Antifungal activity of actinomycetes against wilt and dry root rot diseases of redgram [*Cajanus cajan* (L.) Millsp.]

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ABSTRACT

Actinomycetes isolates were collected from different redgram growing areas of Tamil Nadu and they were tested for their antagonistic activity against *Fusarium udum* and *Macrophomina phaseolina*. Among the tested isolates of *Actinomycetes* AC (5) reported highest 82.85 per cent reduction of mycelial growth of *Fusarium udum* and 85.13 per cent reduction of mycelial growth of *Macrophomina phaseolina* under *in vitro* condition.

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INTRODUCTION

Redgram [*Cajanus cajan* (L.) Millsp.] is one of the most important pulse crop grown in India which produces 90 per cent of the global production. Redgram is grown in a wide range of agro-ecological situations, its deep rooting and drought tolerant characters make it useful crop in rainfed areas.

The major constraints in the production of red gram are the diseases *viz*., Wilt, root rot, collar rot, damping-off and powdery mildew. Red gram wilt is a very common and worst disease in India, causing severe yield loss wherever the crop is grown especially Bihar, Madhya Pradesh, Maharashtra, Tamil Nadu and Uttar Pradesh. Wilt disease of redgram is caused by *Fusarium udum* and the disease is severe where the redgram grows continuously. The disease incidence is high during flowering and pod formation stages. The second most important disease is dry root rot which is caused by *Macrophomina phaseolina.* The lower leaves become yellow and the infected plants can be pulled out easily.

Management of the diseases through chemicals and the use of resistant varieties are possible to certain extent. Biocontrol is an important component of integrated disease management (IDM) that provides disease control while being relatively harmless to humans, non-polluting and selective in mode of action, difficult for pathogens to develop resistance, unlikely to harm other beneficial microorganisms and generally improves soil health and sustainability of agriculture. Among the several biocontrol agents actinomycetes are very effectively control the plant diseases.

Actinomycetes are known to produce a variety of

antibiotics with diverse chemical structures such as polyketides, β-lactams and peptides in addition to a variety of other secondary metabolites that inhibit the growth of many bacteria, fungi and protozoa. Furthermore several strains of actinomycetes are known to produce chitinase *in vitro* which catalyze the degradation of chitin from fungal cell walls, resulting in inhibition of fungal growth.

Actinomycetes produce 70 to 80 per cent of bioactive secondary metabolites, where approximately 60 per cent of antibiotics developed for agricultural use are isolated from *Streptomyces* spp. (El-Tarabily *et al*., 2000).The antifungal potential of extracellular metabolites from *Streptomyces* against some fungi has been reported. Actinobacteria are a well known source of various secondary metabolites such as antibiotics, enzymes, pesticides, herbicides, immunomodulators, anti-infective and anticancer agents. An investigation was carried out during 2016-2017 to evaluate various actinomycetes isolates against wilt and root rot disease of redgram and develop a suitable eco-friendly management system against major fungal diseases of redgram.

MATERIAL AND METHODS

Survey and disease assessment:

A survey was conducted during 2016-2017 on the incidence wilt and root rot disease in different redgram growing areas of Tamil Nadu. In each village, four fields were selected and four plots in each field having an average area of ten square meters were marked at random. Wilt and root rot affected plants were counted in each plot and expressed as per cent disease incidence.

$$\text{Per cent disease incidence} = \frac{\text{Number of plans affected}}{\text{Total number of plants observed}} \times 100$$

Isolation of major fungal pathogens from redgram:

The pathogens are isolated from the wilt and root rot disease infected portions of redgram by tissue segment method (Rangaswami, 1958). The infected portions of diseased plants were cut into small pieces using sterilized scalpel and these were surface sterilized with 0.1 per cent mercuric chloride for one minute and washed in three changes of sterile distilled water and then placed on previously poured and solidified Petri dish containing potato dextrose agar (PDA) medium. These plates were incubated at room temperature (28 ± 2°C) for five days and observed for the growth of the fungus. The hyphal tips of fungi grown from the pieces were transferred aseptically to PDA slants for maintenance of the culture. The pathogens were identified based on their cultural and morphological characters.

Isolation of actinomycetes:

Actinomycetes were isolated from rhizosphere region of redgram. Samples were suspended in sterile water (10%) and agitated for 30 min at 420 rpm. The supernatant were serially diluted and plated on the Ken Knight's medium (Allen, 1953). The strains were identified based on Bergey's Manual of Systematic Bacteriology (Krieg and Holt, 1984).

Screening of actinomycetes against pathogens *in vitro* condition:

Isolates of actinomycetes were tested for their ability to inhibit mycelial growth of fungal pathogens *in vitro* following the dual culture technique (Dennis and Webster, 1971). Actinomycete is streaked on one side of a Petri dish containing PDA medium at 1 cm from the edge of plate. The mycelial disc (8-mm-dia) to be taken from the margin of 5 - day - old cultures of pathogen on PDA is placed on the opposite side in the Petri dish perpendicular to the actinomycetes.The plates were incubated at room temperature (28± 2°C) for seven days. At the end of incubation period, the zone of inhibition was recorded by measuring the distance between the edges of the fungal mycelium and the actinomycetes.

RESULTS AND DISCUSSION

Root rot and wilt disease infected plants were collected from different redgram growing districts of Tamil Nadu. The incidence of wilt disease was ranged between 25.45 to 56.42 per cent. Maximum incidence of 56.42 per cent wilt disease was recorded at kaveripakkam, Tamil Nadu. The incidence of root rot disease was ranged between 23.52 to 52.75 per cent. Maximum incidence of 52.75 per cent root rot disease was recorded at Melapulam, Tamil Nadu (Table 1).

Fifteen isolates of *Actinomycetes* were collected from different regions of redgram growing areas of Tamil Nadu, tested for their antagonistic activity against *Macrophomina phaseolina* and *Fusarium udum* by dual culture technique. In this experiment, mycelial growth of the *Macrophomina phaseolina* ranged from 1.33 to 6.21 cm. Among the tested isolates,

Actinomycetes (AC5) recorded the maximum (85.13%) inhibition on the mycelial growth of the pathogen followed by *Actinomycetes* (AC 12) recorded 71.62 per cent inhibition on the mycelial growth (Table 2).

In another experiment, fifteen isolates of *Actinomycetes* were tested for their antagonistic activity against *Fusarium udum* by dual culture technique. Mycelial growth of the *Fusarium udum* ranged from 1.54 to 6.57 cm. Among the tested isolates, *Actinomycetes* (AC5) recorded the maximum (82.85%) inhibition on the mycelial growth of the pathogen followed by *Actinomycetes* (AC12) recorded 75.61 per cent inhibition on the mycelial growth (Table 3). Among the fifteen isolates of *Actinomycetes*, AC 5 and AC 12 isolates were found to be superior in inhibiting the mycelial growth of *Macrophomina phaseolina* and *Fusarium udum* under *in vitro* condition.

Actinomycetes are present abundantly in different type of soil and various climatic conditions. (Goodfellow *et al.*,1986). Actinomycetes are an essential group of filamentous, gram-positive bacteria producing various antibiotics.These antibiotics are used in medicine preparation and agricultural purpose as biocontrol agents. Actinomycetes are producing antibiotics which

Table 1: Survey on the incidence of wilt and root rot disease of redgram in Tamil Nadu

Sr. No.	Places	Wilt disease incidence (%)	Root rot disease incidence (%)
1.	Papanari	48.21	36.54
2.	Melapulam	37.64	52.75
3.	Kaveri pakkam	56.42	48.93
4.	Nangamangalam	29.65	45.62
5.	Karikkal thangal	33.12	38.96
6.	North arcot	42.56	32.64
7.	Ocheri	35.72	27.82
8.	Valaja road	30.48	35.45
9.	Annur	25.45	25.76
10.	Coimbatore	28.54	23.52

Table 2 : Antifungal activity of *Actinomycetes* against *Macrophomina phaseolina* under *in vitro* condition

Sr. No.	Isolates	Mycelial growth (cm)*	Per cent reduction over control
1.	*Actinomycetes* (AC1)	2.68	70.05
2.	*Actinomycetes* (AC2)	4.63	48.26
3.	*Actinomycetes* (AC3)	3.52	60.67
4.	*Actinomycetes* (AC4)	3.36	62.45
5.	*Actinomycetes* (AC5)	1.33	85.13
6.	*Actinomycetes* (AC6)	4.28	52.17
7.	*Actinomycetes* (AC7)	6.10	31.84
8.	*Actinomycetes* (AC8)	5.94	33.63
9.	*Actinomycetes* (AC9)	5.40	39.66
10.	*Actinomycetes* (AC10)	4.65	48.04
11.	*Actinomycetes* (AC11)	3.71	58.54
12.	*Actinomycetes* (AC12)	2.54	71.62
13.	*Actinomycetes* (AC13)	6.21	30.61
14.	*Actinomycetes* (AC14)	5.80	37.65
15.	*Actinomycetes* (AC15)	5.62	37.20
16.	Control	8.95	-
	C.D. (P=0.05)	0.41	

* Mean of three replications

Table 3: Antifungal activity of *Actinomycetes* against *Fusarium udum* under *in vitro* condition

Sr. No.	Isolates	Mycelial growth (cm)*	Per cent reduction over control
1.	*Actinomycetes* (AC1)	2.93	67.37
2.	*Actinomycetes* (AC2)	4.85	45.99
3.	*Actinomycetes* (AC3)	3.74	58.35
4.	*Actinomycetes* (AC4)	3.68	59.02
5.	*Actinomycetes* (AC5)	1.54	82.85
6.	*Actinomycetes* (AC6)	4.28	52.33
7.	*Actinomycetes* (AC7)	6.52	27.39
8.	*Actinomycetes* (AC8)	5.56	38.08
9.	*Actinomycetes* (AC9)	5.87	34.63
10.	*Actinomycetes* (AC10)	4.39	51.11
11.	*Actinomycetes* (AC11)	3.84	57.23
12.	*Actinomycetes* (AC12)	2.19	75.61
13.	*Actinomycetes* (AC13)	6.57	26.83
14.	*Actinomycetes* (AC14)	5.54	38.30
15.	*Actinomycetes* (AC15)	5.71	36.41
16.	Control	8.98	-
	C.D. (P=0.05)	0.45	

* Mean of three replications

effectively control the growth of plant pathogenic fungi. Chitinases which release oligo N- acetyl glucosamines that function as elicitors for the activation of defense related responses in plant cells which have the ability to attack the fungal cell wall directly (Zhu *et al.*, 2008).

Actinomycetes comprise a morphologically varied group, distinguished from other Gram-positive bacteria by their filamentous growth and higher GC content in DNA. *Streptomyces griseus* was treated with the roots of tomato before *Fusarium oxysporum* infestation which effectively control the wilt disease (Anitha and Rabeeth, 2009). Adhilakshmi *et al*. (2013) reported that Seed treatment and soil application of *Streptomyces* sp. reduced the incidence of stem rot of groundnut under greenhouse and field conditions.

Shrivastava *et al*. (2017) revealed that the actinobacterial strain had plant growth promoting potential and able to damage cell wall of the fungus *Macrophomina phaseolina* due to chitinase activity. The *Streptomyces* sp. RP1A-12 reduced stem rot disease incidence by 64–67 per cent and 22–49 per cent, respectively in two field trials conducted with higher yield (Simi Jacob *et al*., 2018).

The results proved that actinomycetes reduced the wilt and root rot disease incidence by directly affecting the pathogen activity by antibiosis or reducing the population load of pathogenic propagules or competition towards the pathogen. The present investigation revealed that the potentiality of the actinomycetes as an alternative to the chemicals which controlling the root rot and wilt incidence and enhanced the plant growth and there by increased yield in redgram.

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