I J P S INTERNATIONAL JOURNAL OF PLANT SCIENCES
ISSN-0973-1547 Volume **15** | Issue 2 | July, 2020 | 96-100
DOI: 10.15740/HAS/IJPS/15.2/96-100
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RESEARCH ARTICLE

Management of wilt and dry root rot diseases of redgram [*Cajanus cajan* (L.) Millsp.] by using actinomycetes

■ **S. Malathi**

SUMMARY

Actinomycetes were tested for their antagonistic activity against *Fusarium udum* and *Macrophomina phaseolina* under *in vitro* condition. 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*. In the field experiment, five treatments were tested for the management of wilt and dry root rot disease, T_3- ST+ SA with *Actinomycetes* (AC 5) significantly recorded 71.92 and 70.38 per cent reduction of the wilt and dry root rot diseases, respectively. These biocontrol agents were used an alternative to the chemical fungicide for controlling the wilt and dry root rot incidence and enhanced the plant growth parameters and there by increased yield in redgram.

Key Words : Redgram, *Fusarium udum*, *Macrophomina phaseolina*, Actinomycetes, Management

How to cite this article : Malathi, S. (2020). Management of wilt and dry root rot diseases of redgram [*Cajanus cajan* (L.) Millsp.] by using actinomycetes . *Internat. J. Plant Sci.*, **15** (2): 96-100, **DOI: 10.15740/HAS/IJPS/15.2/96-100,** Copyright@ 2020: Hind Agri-Horticultural Society.

Article chronicle : Received : 24.04.2020; **Revised :** 19.05.2020; **Accepted :** 05.06.2020

Redgram, [*Cajanus cajan* (L.) Millsp.] is one of the most important legume crop grown in a wide range of agro-ecological situations in semiarid tropical regions of Indian subcontinent. India is the largest producer and consumer of red gram in the world. The major constraints in increasing the yield of the crop are its vulnerability to diseases, insects and other abiotic stresses. 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 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. *Fusarium udum* and *Macrophomina phaseolina* are primarily soilborne pathogens. So management of these pathogens are very difficult and application of chemicals is used only for some extent for the control of the disease. Management of plant diseases through biological method by using

AUTHOR FOR CORRESPONDENCE
S. Malathi, Rice Research Station (T.N.A.U.), Tirur, **Tiruvallur (T.N.) India**
Email : malathi_agri@rediffmail.com

antagonistic micro-organisms like rhizobacteria, fungi and actinomycetes. Biological control is a good alternative method, as compared to chemical control which destroys a range of micro and macro-organisms and has a limited impact on the environment (Sigee, 1993).

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 (Behal, 2000). Actinomycetes constitute a morphologically diverse group, distinguished from other Gram-positive bacteria by their filamentous growth and GC-rich DNA. The various formulations of *S. griseus* were assessed for their efficiency in controlling *F. oxysporum* incidence in greenhouse conditions. A significant lowest disease severity on treatment of self fusant (SF*Sg* 5) *S. griseus* suspension (root dipping) and chitin amended *S. griseus* (root dipping) was recorded compared with chitinase enzyme preparation of the same. For effective disease control, *S. griseus* introduced to root system before *Fusarium oxysporum* infestation than that of seed treatment (Anitha and Rabeeth, 2009).

Actinomycetes, particularly *Streptomyces* spp. have been a widely exploited group of micro-organisms in the production of secondary metabolites and enzymes of commercial importance in agricultural applications (Kumar and Gupta, 2006). Chitinase has received attention due to its use as a biocontrol agent (Zhu *et al.*, 2008). Many actinomycetes are known to promote plant growth by production of plant growth-promoting substances such as auxins and gibberellin-like compounds (Bloemberg and Lugtenberg, 2001 and Doumbou *et al.*, 2001).

Manulis *et al.* (1994) reported synthesis of indole-3-acetic acid (IAA) by different *Streptomyces* species. Conn *et al.* (2008) demonstrated that inoculation of *Arabidopsis thaliana* with endophytic actinobacteria induced SAR and JA/ET gene expression. Among the actinomycetes, *Streptomyces* spp. are the most popular and found worldwide in soil and important in soil ecology. The antagonistic actinomycete, *Streptomyces griseoviridis* is commercially available with the trade name Mycostop (Tahvonen and Avikainen, 1987).

Biological control is always better than chemical management practices which suppress the disease without destroy the ecosystem. Keeping this in view, present investigations were considered for the development of suitable ecofriendly management system against wilt and root rot of redgram with the use of actinomycetes.

MATERIAL AND METHODS

Screening of actinomycetes against pathogens *in vitro* condition :

Actinomycetes were isolated from the soil which collected from rhizosphere region of different redgram growing areas of Tamil Nadu. 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 cultures were identified based on Bergey's Manual of Systematic Bacteriology (Krieg and Holt, 1984).

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). Actinomycetes are 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.

Development of bioformulations from antagonistic actinomycetes:

Talc-based powder formulations of the effective actinomycetes (AC5) selected on the basis of their *in vitro* test on dual plate technique and plant growth-promoting activity were developed as described by Vidhyasekaran *et al.* (1997). Ten gram of carboxymethyl cellulose was mixed with one kg of talc (Magnesium silicate) and the pH was adjusted to 7.0 by adding calcium carbonate. The mixture was then autoclaved for 30 min. Actinomycetes was grown in nutrient broth for 48 h at 28 ± 2°C on a rotary shaker. Four hundred milliliters of actinomycetes suspension containing 9 $x10^8$ CFU ml^{-1} was added to one kg of the talc mixture and thoroughly mixed. The formulation was packed with 35 per cent moisture content in polythene bags, sealed and stored at room temperature (28 ± 2°C). The talc formulation of actinomycetes were used for the management of wilt and root rot diseases of redgram in field condition.

Evaluation of actinomycetes against root rot and wilt of redgram in the field condition:

Field experiment was conducted at Anthanur of Vellore district, Tamil Nadu. Randomized Block Design (RBD) was used in the experiments with plot size 5 x 4 m^2 and spacing 45 x 30 cm. The experiment was conducted with five treatments with four replications. Individual application of bioagents and its different combinations were applied with the chemical check carbendazim in the management of root rot and wilt of redgram.

Analysis of the rhizosphere population of actinomycetes under field condition:

The antagonist population in the rhizosphere of redgram was estimated at 0, 30, 60, 90 days after sowing. For this purpose, three plants in each treatment used random. The plants were pulled out gently with roots intact and tapped against the palm to remove the adhering soil. One gram of rhizosphere soil was transferred in to 100 ml sterile water. After thorough shaking, the antagonist population in the suspension was assayed by dilution plate technique (Pramer and Schmidt, 1956). From the dilution, one ml aliquot was pipetted out into sterilized Petri plates and incubated at room temperature (28 ± 2^oC). The number of colony forming units was counted.

RESULTS AND DISCUSSION

Actinomycetes were collected from different regions of redgram growing areas of Tamil Nadu and 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 1).

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. Among the fifteen isolates of *Actinomycetes*, AC 5 and AC 12 isolates were

Table 1: Screening of *Actinomycetes* against *Macrophomina phaseolina* and *Fusarium udum* under *in vitro* condition

Sr. No.	Isolates	*Macrophomina phaseolina*		*Fusarium udum*	
		Mycelial growth (cm)*	Per cent reduction over control	Mycelial growth (cm)*	Per cent reduction over control
1.	*Actinomycetes* (AC1)	2.68	70.05	2.93	67.37
2.	*Actinomycetes* (AC2)	4.63	48.26	4.85	45.99
3.	*Actinomycetes* (AC3)	3.52	60.67	3.74	58.35
4.	*Actinomycetes* (AC4)	3.36	62.45	3.68	59.02
5.	*Actinomycetes* (AC5)	1.33	85.13	1.54	82.85
6.	*Actinomycetes* (AC6)	4.28	52.17	4.28	52.33
7.	*Actinomycetes* (AC7)	6.10	31.84	6.52	27.39
8.	*Actinomycetes* (AC8)	5.94	33.63	5.56	38.08
9.	*Actinomycetes* (AC9)	5.40	39.66	5.87	34.63
10.	*Actinomycetes* (AC10)	4.65	48.04	4.39	51.11
11.	*Actinomycetes* (AC11)	3.71	58.54	3.84	57.23
12.	*Actinomycetes* (AC12)	2.54	71.62	2.19	75.61
13.	*Actinomycetes* (AC13)	6.21	30.61	6.57	26.83
14.	*Actinomycetes* (AC14)	5.80	37.65	5.54	38.30
15.	*Actinomycetes* (AC15)	5.62	37.20	5.71	36.41
16.	Control	8.95	-	8.98	-
	C.D. (P=0.05)	0.41		0.45	

* Mean of three replications

found to be superior in inhibiting the mycelial growth of *Macrophomina phaseolina* and *Fusarium udum* under *in vitro* condition.

Among the five treatments tested for the management of wilt disease under field condition, T_3- ST+ SA with *Actinomycetes* (AC 5) significantly recorded 71.92 per cent disease reduction followed by T_2 - SA with *Actinomycetes* (AC 5) which accounted 65.49 per cent reduction of the disease. In the root rot disease management, among the five treatments tested T_3- ST+ SA with *Actinomycetes* (AC 5) significantly recorded 70.38 per cent disease reduction followed by T_2- SA with *Actinomycetes* (AC 5) which accounted 64.47 per cent reduction of the disease. In this experiment, T_3- ST+ SA with *Actinomycetes* (AC 5) significantly recorded highest yield 1780 kg/ha followed by T_2 SA with *Actinomycetes* (AC 5) treatment recorded 1540 kg/ha (Table 2).

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).

Gopalakrishnan and Srinivas (2019) reported that the actinobacterial strains of *Streptomyces*, *Amycolaptpsis*, *Micromonosporo* and *Frankia* were reported to exert effective control on soil borne pathogens and help the host plant to mobilize the nutrients. The increase in groundnut pod yield due to application of *Streptomyces* sp. may be associated with the decrease in disease incidence and increase in plant growth because of the plant growth promoting characteristics of actinomycetes (Doumbou *et al.,* 2001).

The amount of antagonists present in the redgram rhizosphere was determined. The results revealed that the mean population of *Actinomycetes* present in redgram rhizosphere was high $30.59x10^5$cfu/g in the plot treated (T_5) with ST+ SA with *Actinomycetes* (AC 5) (Table 3). On thirty days after sowing, *Actinomycetes* population increased in all the treatments compared to that of 0 DAS. The above results showed that the increased population of actinomycetes in rhizosphere region of redgram which decrease the soil borne pathogen infection. Reduced disease incidence by application of biocontrol agents can be attributed to protection of infection court by rhizosphere colonization of antagonistic bacteria in addition to that which increase the rhizosphere population of antagonistic micro-

Table 2: Effect of actinomycetes (AC 5) on wilt and root rot disease of red gram under field condition

Sr. No.	Treatments	Wilt disease		Root rot disease		Yield kg/ha
		Disease incidence * (%)	Per cent reduction over control	Disease incidence* (%)	Per cent reduction over control	
1.	T_1- ST with *Actinomycetes* (AC 5)	20.42	57.79	21.65	57.83	1455
2.	T_2- SA with *Actinomycetes* (AC 5)	16.74	65.49	18.24	64.47	1540
3.	T_3- ST+ SA with *Actinomycetes* (AC 5)	13.65	71.92	15.65	70.38	1780
4.	T_4-Carbendazim (0.1%)	14.36	70.46	17.42	66.06	1695
5.	T_5-Control	48.62		51.34		1340
	C.D. (P=0.05)	1.52		1.75		

*Mean of three replications

Table 3 : Population of actinomycetes (AC 5) on redgram rhizosphere in field condition

Sr. No.	Treatments	Actinomycetes (AC 5) $x10^5$ CFU/g soil				
		0 DAS*	30 DAS*	60 DAS*	90 DAS*	Mean
1.	T_1- ST with *Actinomycetes* (AC 5)	2.48	18.24	42.94	45.62	27.32
2.	T_2- SA with *Actinomycetes* (AC 5)	2.36	20.65	45.58	48.76	29.33
3.	T_3- ST+ SA with *Actinomycetes* (AC 5)	2.64	21.48	47.92	50.34	30.59
4.	T_4-Carbendazim (0.1%)	2.32	2.48	2.85	2.94	2.64
5.	T_5-Control	2.15	2.37	2.45	2.61	2.39

DAS - Days after sowing* Seed treatment (ST)- 4g/kg of seed Soil application (SA) - @ 2.5 kg/ha

organisms (Ahamed and Baker, 1987).

The results showed that actinomycetes reduced the wilt and root rot disease incidence by directly deactivate the propagules of soilborne pathogens before its emergence. 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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