



## Mycoparasitic effect of combined application of *Serratia marcescens* and *Allium sativum* on the anthracnose (*Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cavara) incidence of dolichos bean under *In vivo* conditions

K Sanjeev Kumar<sup>1</sup>, K Papitha<sup>1</sup>, P Balabaskar<sup>1</sup>, S Kumar<sup>2</sup>, S Sudhasha<sup>1</sup>

<sup>1</sup>Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Tamil Nadu, India

<sup>2</sup>Department of Horticulture, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Tamil Nadu, India

### Abstract

Anthraco-nose disease caused by *Colletotrichum lindemuthianum* major constraints to dolichos bean production. The studies on the integrated management of anthracnose disease revealed that the integrated treatment consisting the combined application of *S. marcescens* (ST) @10.0 ml/Kg of seeds + *S. marcescens* as 2% foliar spray + *A. sativum* 30% as foliar spray @ 40, 50 & 60 DAS (T<sub>7</sub>) recorded the minimum disease incidence and increased plant growth and yield parameters of dolichos bean in pot and field conditions when compared to all other treatments and control.

**Keywords:** dolichos bean, *Colletotrichum lindemuthianum*, *Serratia marcescens*, anthracnose, combined application

### Introduction

Dolichos bean (*Lablab purpureus* L. Sweet) is an annual herbaceous vegetable cum pulse in the tropics commonly called as field bean belongs to the family leguminosae, sub family Papilionaceae. It is formerly known as *Dolichos lablab* L. (Murphy and Colucci, 1999) [10]. It is a native of tropical Asia, India or Africa and has spread to tropical and subtropical countries of the world like China, Egypt Sudan and other countries. In India, field bean is mostly confined to the peninsular zone (Shivashankar *et al.*, 1993) [17]. It is one of the oldest vegetable crops grown for green pods which are cooked as vegetables like other beans in India. It is grown as a field crop in Tamil Nadu, Andhra Pradesh, Karnataka, Madhya Pradesh and Maharashtra. It is good source of calcium, vitamin C, zinc, carotene, iron, and vitamin B1, amino acid, lysine and it contains 20-28% crude protein (Khalequzzaman, 2015) [7].

Dolichos bean production is influenced by both biotic and abiotic stresses; biotic factors are responsible for major losses. Six major diseases (anthracnose, rust, angular leaf spot, common bacterial blight, *Bean Golden Mosaic virus* and *Bean common mosaic virus*) are known to hamper dolichos bean production. Among the diseases, anthracnose disease caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cavara, a hemibiotrophic fungus is found in almost every bean growing region of the world (Kelly *et al.*, 1994) [6]. It is considered as one of the most destructive diseases in subtropical and temperate regions (Sharma and Sugha, 1995) [16].

Management of anthracnose disease of beans may be controlled by chemicals, but chemicals create health hazard and environment pollution. To overcome these problems the present study was mainly focused on the use of bio-control agents and some botanicals which are eco-friendly, safer and good alternative for chemical fungicide. Several bio control agents such as *Pseudomonas* sp, *Trichoderma* sp, and *Bacillus* spp. have been used to reduce the severity of diseases caused by different pathogens (Raajmakers *et al.*,

1995; Zaidi *et al.*, 2004; Gowdar and Kulkarni, 2003) [3]. Many of the introduced antagonists failed to survive in the soil due to lack of favourable conditions like food base, moisture conditions *etc.*, Therefore, the search for newer antagonist has become imminent for exploiting these organisms to the advantage. Hence, a new bio control agent *Serratia marcescens* was tried for the management of dolichos bean anthracnose caused by *C. lindemuthianum*.

### Materials and Methods

#### Preparation of liquid formulation of *S. marcescens*

For the preparation of liquid formulations the method suggested by Manikandan *et al.* (2010) [9] was followed. The log phase culture of *S. marcescens* was inoculated into chitin luria broth and incubated at room temperature (28 ± 2°C) for three days. Further, the broth was added with glycerol at 2 per cent level. After the incubation period, the formulation was assessed for adequate CFU (1x10<sup>8</sup>) following serial dilution plating technique and the formulation thus prepared was sealed in plastic containers and used for further studies.

#### Preparation of Plant leaf extracts

For the preparation of Plant leaf extracts, the method described by Gerald Ezhilan *et al.* (1994) [2] was followed. The plant parts were freshly collected and washed in running tap water and sterile distilled water, processed with sterile distilled water @ 1ml per 1g of plant parts (1:1 V/W) with pestle and mortar and filtered through a double layered sterile cheese cloth and was centrifuged at 5000 rpm for 10 min and the clear supernatant was saved. This formed the standard plant extract solution (100 %).

#### Effect of combined application with *S. marcescens* and *A. sativum* leaf extract on plant growth and disease incidence of dolichos bean (Pot culture)

Using the best treatments identified in the earlier my studies a new treatment schedule was formulated by integrating the

use of bio control agents and plant extract for conducting pot culture and field studies. A pot culture experiment was conducted in our department pot culture yard in a completely randomized block design with three replications and two plants per pot were maintained. The treatments were given as per the schedule to the pathogen inoculated plant in order to assess their efficacy on the management of fruit rot incidence and growth parameter. The per cent disease incidence was recorded at 60 DAS, 75 DAS and 90 DAS. At the time of harvest, biometric parameters viz., Plant height (cm), No. of pods/ plant, biomass (g), pod yield (g/pot) and biomass were recorded using standard procedures.

#### Treatment schedule

- T<sub>1</sub>-Seed treatment with *S. marcescens* @ 10.0 ml/kg of seeds  
 T<sub>2</sub>-Foliar spray with *S. marcescens* @ 2 % in 40, 50 and 60 DAS  
 T<sub>3</sub>-Foliar spray with *A. sativum* leaf extract @ 30 % in 40, 50 and 60 DAS  
 T<sub>4</sub>-T<sub>1</sub>+ T<sub>2</sub>  
 T<sub>5</sub>-T<sub>1</sub>+ T<sub>3</sub>  
 T<sub>6</sub>-T<sub>2</sub>+ T<sub>3</sub>  
 T<sub>7</sub>-T<sub>1</sub>+ T<sub>2</sub>+ T<sub>3</sub>  
 T<sub>8</sub>-Carbendazim 50 % WP (ST @ 2g/kg of seeds & FS @ 0.1%)  
 T<sub>9</sub>- Control.

#### Field Studies

Based on the results obtained from the pot culture experiments, the field trial was conducted in anthracnose incidence prone farmer's field at Puthur in Krishnagiri district of Tamil Nadu by integrating the best treatments identified in the pot culture experiments. The blanket fertilizer schedule of 30:80:80 NPK/ha recommended by the state horticultural department was followed. A plot size of 5 × 4m was used for each treatment. Each treatment was replicated thrice and a suitable control was also maintained. The local variety was used for study. Carbendazim 50 % WP @ 0.1 % was used for comparison. All the agronomic practices were followed as per the standard procedure as recommended by the State horticultural department. Treatment schedule and all the observations as indicated in pot culture studies.

## Results and Discussion

### Effect of combined application of *S. marcescens* and *A. sativum* on the incidence of anthracnose and biometrics of Dolichos bean (Pot and field trial)

The combined application of seed treatment of *S. marcescens* (ST) @ 10.0 ml/Kg of seeds + *S. marcescens* as 2% foliar spray + *A. sativum* extract 30% as foliar spray @ 40, 50 &

60 DAS (T<sub>7</sub>) showed significant reduction in anthracnose incidence in pot (06.45, 07.34 and 08.15% of anthracnose incidence at 60, 75 DAS and 90 DAS respectively) and field (04.34, 08.87 and 09.54% of anthracnose incidence at 60, 75 DAS and 90 DAS respectively) conditions when compared with control. This was followed by T<sub>8</sub> and T<sub>4</sub> in the decreasing order of merit. The maximum disease incidence was recorded in control (T<sub>9</sub>) (Table 1 & 3). The results revealed that different plant colonization pattern and different mechanism of disease suppression elicited by *S. marcescens* might have offered greater protection to the plants against the anthracnose disease. *S. marcescens* effectively controlled the late leaf spot in groundnut (Krishna Kishore *et al.*, 1999) [8]. Someya *et al.* (2000) [19] recorded reduced damping-off incidence with *S. marcescens* under greenhouse conditions. The disease suppression observed in the present study might be due to the activity of the antibiotic production (Okamoto *et al.*, 1998) [11]. Berg *et al.* (2005) [1] found *S. plymuthica* 3Re4-18 to be the most effective isolate among 2648 bacterial strains isolated from potato rhizosphere in controlling soil borne pathogens *Verticillium dahliae* and *Rhizoctonia solani*. Shen *et al.* (2002) [18] observed 100% control of *Phytophthora* blight incidence in pepper by *S. plymuthica* strain A21-4 in pot trials and substantial disease suppression in green house studies. Integration of *A. sativum* 30% extract along with *S. marcescens* further reduced the dolichos bean anthracnose incidence. The present result is supported by Sanjeevkumar (2008) [13] who reported that application of *S. marcescens* along with pungam oil cake extract significantly reduced the wilt incidence in banana. Integrated treatment of combination consisting of application of FYM (SA) plus *S. marcescens* (SA+ST) plus micronutrient mixture (SA+ST) significantly reduced the panama wilt incidence (Sanjeevkumar *et al.*, 2018) [14]. Combination effect of antagonist and botanicals might be attributed as the reason for the enhanced disease suppression.

**Table 1:** Effect of combined application of *S. marcescens* and *A. sativum* on the anthracnose incidence of dolichos bean under pot culture

Tr. No.	Treatments	Anthracnose incidence (%)			Percent decrease over control		
		60 DAS	75 DAS	90 DAS	60 DAS	75 DAS	90 DAS
1	<i>S. marcescens</i> as ST @ 10 ml / kg of seeds	19.43 (26.15)	21.67 (27.74)	22.53 (28.33)	48.25	60.53	63.18
2	<i>S. marcescens</i> as foliar spray @ 2% in 40,50 and 60 DAS	20.99 (27.26)	22.12 (28.05)	23.41 (28.93)	43.72	59.71	61.74
3	<i>A. sativum</i> 30% as foliar spray @ 40,50 and 60 DAS	21.34 (27.51)	23.33 (28.88)	24.28 (29.52)	42.78	57.51	60.32
4	T <sub>1</sub> +T <sub>2</sub>	09.76 (18.20)	10.32 (18.73)	11.22 (19.57)	73.83	81.20	81.66
5	T <sub>1</sub> +T <sub>3</sub>	10.54 (18.94)	11.87 (20.15)	12.33 (20.55)	71.74	78.38	79.85
6	T <sub>2</sub> +T <sub>3</sub>	17.67 (24.85)	19.63 (26.29)	21.73 (27.78)	52.62	64.25	64.49
7	T <sub>1</sub> +T <sub>2</sub> +T <sub>3</sub>	06.45 (14.71)	07.34 (15.71)	08.15 (16.58)	82.70	86.63	86.68
8	Carbendazim 50 % WP 0.1% (ST 2g/kg of seeds + FS @ 40, 50 DAS)	08.13 (16.56)	09.12 (17.57)	10.65 (19.04)	78.20	83.39	82.59
9	Control	37.30(37.64)	54.91(47.81)	61.20 (51.47)	-	-	-
	S. Ed	0.12	0.11	0.35	--	--	--
	CD (p=0.05)	0.26	0.28	0.81			

Data in parentheses indicate angular transformed values

### Effect of combined application of *S. marcescens* and *A. Sativum* on growth and yield of dolichos bean under pot and field trail

The results obtained on the efficacy of the *S. marcescens* and *A. sativum* extract in a pot culture experiment are furnished in (Table 2). Among the various levels of *S. marcescens* and *A. sativum* tested, the treatment with *S. marcescens* (ST) @12.0 ml/Kg of seeds + *S. marcescens* as

2% foliar spray at 40, 50 and 60 DAS + *A. sativum* 30% as foliar spray @ 40, 50 & 60 DAS (T<sub>7</sub>) recorded the maximum plant height (160.53 cm), biomass (110.21 g), number of pods per plant (88.67) and pod yield/pot (619.95 g/ plant). This was followed by treatment T<sub>4</sub> and T<sub>5</sub>. The least biometric values were observed in control. The same trend was also recorded in field trail (Plant height (161.30

cm), number of pods per plant (93.23) and pod yield (10.91 t/ha) (Table 4).

The combined application of *S. marcescens* (ST) @10.0 ml/Kg of seeds + *S. marcescens* as 2% foliar spray + *A. sativum* 30% as foliar spray @ 40, 50 & 60 DAS (T<sub>7</sub>) proved as an alternative to chemical fungicide by offering environmentally safer disease management in dolichos bean. The results have clearly revealed that an integration of several strategies like application of bio control agents along with botanicals showed a synergistic action and enhanced greater disease suppression and improved the consistency of biological control with improved plant growth and crop yield. Similar such enhanced plant growth and yield due to the application of antagonists and botanicals in the field was reported by several workers in various crops (Sanjeevkumar, 2008; Santhoshkumar, 2017) [13]. Treatment

with *S. plymuthica* resulted in an increase of the number of buds, blossoms, and fruit in addition intense root branching for plants treated with HRO- C48 was observed in greenhouse and field trial also in the field condition, the disease incidence was high and *S. plymuthica* was able to reduce disease symptoms and to enhance the yield Stefan kurze *et al.*, (2001) [20]. Jadon *et al.* (2016) [5] who reported that the combined application of botanicals and bio-control agents reduced the seedling mortality and enhanced plant biomass of bell pepper. Seeds of mungbean with bioagents and botanicals increased the seed germination, seedling vigour index and field emergence of mungbean (Jadhav and Ambadkar, 2007) [4]. The combined application of *S. marcescens* with botanicals increased the plant growth and yield of brinjal (Subharathinam, 2018) [21].

**Table 2:** Effect of combined application of *S. marcescens* and *A. sativum* on the growth and yield of dolichos bean under pot culture.

Tr. No.	Treatments	Plant height (cm)	No. of pods/plant	Pod yield (g/pot)	Biomass (g)
1	<i>S. marcescens</i> as ST @ 10 ml / kg of seeds	106.67	66.87	389.43	88.43
2	<i>S. marcescens</i> as foliar spray @ 2% in 40,50 and 60 DAS	119.25	68.98	402.74	90.56
3	<i>A. sativum</i> 30% as foliar spray @ 40,50 and 60 DAS	97.50	64.26	351.25	87.54
4	T <sub>1</sub> +T <sub>2</sub>	154.10	78.96	590.32	102.12
5	T <sub>1</sub> +T <sub>3</sub>	148.75	72.65	576.54	101.98
6	T <sub>2</sub> +T <sub>3</sub>	128.96	70.23	540.65	92.75
7	T <sub>1</sub> +T <sub>2</sub> +T <sub>3</sub>	160.53	88.67	619.95	110.21
8	Carbendazim 50 % WP 0.1% (ST 2g/kg of seeds + FS @ 40, 50 DAS)	136.31	70.33	568.98	101.12
9	Control	65.89	35.12	299.75	20.48
	S. Ed	0.32	0.25	0.56	0.12
	CD (p=0.05)	0.71	0.59	1.45	0.24

**Table 3:** Effect of combined application of *S. marcescens* and *A. sativum* on the anthracnose incidence of dolichos bean under field trail

Tr. No.	Treatments	Anthracnose incidence (%)			Percent decrease over control		
		60 DAS	75 DAS	90 DAS	60 DAS	75 DAS	90 DAS
1	<i>S. marcescens</i> as ST @ 10 ml / kg of seeds	15.24 (22.78)	18.27(25.30)	20.64(27.02)	48.82	46.72	48.55
2	<i>S. marcescens</i> as foliar spray @ 2% in 40,50 and 60 DAS	16.01 (23.58)	20.64(27.02)	22.76(28.49)	46.23	39.81	43.27
3	<i>A. sativum</i> 30% as foliar spray @ 40,50 and 60 DAS	17.55 (24.76)	21.29(27.47)	23.82(29.21)	41.08	37.91	40.62
4	T <sub>1</sub> +T <sub>2</sub>	05.66 (13.76)	07.70(16.11)	09.61(18.05)	80.99	77.54	76.04
5	T <sub>1</sub> +T <sub>3</sub>	08.65 (17.10)	10.85(19.23)	11.87(20.15)	70.95	68.36	70.41
6	T <sub>2</sub> +T <sub>3</sub>	11.12 (19.47)	12.21(20.45)	13.04(21.16)	62.65	64.39	67.49
7	T <sub>1</sub> +T <sub>2</sub> +T <sub>3</sub>	04.34 (12.02)	05.87(14.02)	07.54(15.93)	85.42	82.88	81.21
8	Carbendazim 50% WP 0.1% (ST 2g/kg of seeds + FS @ 40, 50 DAS)	05.14 (13.10)	07.34(15.71)	09.11(17.56)	82.74	78.59	77.29
9	Control	29.78 (33.07)	34.29(35.84)	40.12(39.30)	-	-	-
	S. Ed	0.01	0.02	0.12			
	CD (p=0.05)	0.03	0.05	0.26	--	--	--

Data in parentheses indicate angular transformed values

**Table 4:** Effect of combined application of *S. marcescens* and *A. sativum* on the growth and yield of dolichos bean under field trail

Tr. No.	Treatments	Plant height (cm)	No. of pods/plant	pod yield (t/ha)
1	<i>S. marcescens</i> as ST @ 10 ml / kg of seeds	107.25	68.91	08.96
2	<i>S. marcescens</i> as foliar spray @ 2% in 40,50 and 60 DAS	120.34	71.62	08.99
3	<i>A. sativum</i> 30% as foliar spray @ 40,50 and 60 DAS	99.99	67.43	08.93
4	T <sub>1</sub> +T <sub>2</sub>	155.20	81.75	09.90
5	T <sub>1</sub> +T <sub>3</sub>	148.97	75.67	09.71
6	T <sub>2</sub> +T <sub>3</sub>	126.49	73.32	09.22
7	T <sub>1</sub> +T <sub>2</sub> +T <sub>3</sub>	161.30	93.23	10.91
8	Carbendazim 50 % WP 0.1% (ST 2g/kg of seeds + FS @ 40, 50 DAS)	137.19	76.34	09.43
9	Control	65.25	35.87	06.25
	S.Ed	0.42	0.46	0.04
	CD (p=0.05)	0.89	0.97	0.05

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