



MYCOPARASITIC EFFECT OF *SERRETIA MARCESCENS* AND *ALLIUM SATIVUM* ON THE ANTHRACNOSE INCIDENCE, PLANT GROWTH AND INDUCED SYSTEMIC RESISTANCE OF DOLICHOS BEAN.

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Abstract

Dolichos bean (*Lablab purpureus*) is an important vegetable cum pulse crop. Dolichos bean production is influenced by fungal, bacterial and viral diseases, fungal diseases are responsible for major losses. It is considered as one of the most destructive diseases in subtropical and temperate regions. In the present investigation we study the role of combined application *Serratia marcescens* and *Allium sativum* in the incidence of systemic resistance against anthracnose (*Colletotrichum lindemuthianum*) in dolichos bean. Among the treatment, 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 and 60 DAS (T₇) recorded the minimum disease incidence and increased plant growth and yield parameters of dolichos bean in field conditions. Further, the same treatment (T₇) with challenge inoculated with *C. lindemuthianum* showed induction of earlier and increased levels of defense enzymes viz., PO, PPO, PAL and Phenol content.

Key words: Dolichos bean, *Colletotrichum lindemuthianum*, *Serratia marcescens*, *Allium sativum*, anthracnose, induced systemic resistance.

Introduction

Dolichos bean (*Lablab purpureus*) is an annual herbaceous vegetable cum pulse crop. It is formerly known as *Dolichos lablab* L. (Murphy and Colucci, 1999). 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). 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 (Khalequzzaman, 2015).

Dolichos bean anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. and Magnus) Briosi and Cavara is one of the most important seed borne diseases of dolichos bean in the world (Amin *et al.*, 2014). The disease is serious in areas that had cool and wet weather conditions, causing up to 100% yield loss (Pastor-Corrales

and Tu, 1989). It is difficult to control *C. lindemuthianum* due to its wide host range, seed borne, air borne and prolonged survival in the seed and plant debris. Despite wide spread use of synthetic chemical for the management of plant diseases, recent advances about their environmental hazards warrants eco-friendly alternative method for disease control.

Application of biocontrol agents in combination with leaf products, in plant disease control is emerging as an acceptable method. In the present investigation, the biocontrol agents like *Serratia marcescens* and plant extracts like *Allium sativum* were taken up and their efficacy was studied against *C. lindemuthianum* under *in vivo* conditions.

Materials and Methods

Preparation of Plant leaf extracts

For the preparation of Plant leaf extracts, the method described by Gerald Ezhilan *et al.*, (1994) was followed.

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Preparation of liquid formulation of *S. marcescens*

For the preparation of liquid formulations the method suggested by Manikandan *et al.*, (2010) was followed.

Effect of combined application with *S. marcescens* and *A. sativum* extract on disease incidence and plant growth, yield attributes of dolichos bean (Field trial)

Based on the results obtained from the previous pot culture experiments, the field trial was conducted in anthracnose incidence prone farmer's field at Puthur in Krishnagiri district of Tamil Nadu during October to February-2019 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:

T₁ - Seed treatment with *S. marcescens* @ 10.0 ml/kg of seeds.

T₂ - Foliar spray with *S. marcescens* @ 2 % in 40, 50 and 60 DAS.

T₃ - Foliar spray with *A. sativum* leaf extract @ 30% in 40, 50 and 60 DAS.

T₄ - T₁ + T₂

T₅ - T₁ + T₃

T₆ - T₂ + T₃

T₇ - T₁ + T₂ + T₃

T₈ - Carbendazim 50% WP (ST @ 2kg/seeds and FS @ 0.1%).

T₉ - Control.

The treatments were given as per the schedule. The percent 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 and pod yield (qn/ha) were recorded using standard procedures.

Analysis of defense related proteins and chemicals against anthracnose infection of dolichos bean.

- Collection of plant samples: Samples were collected from individual treatments to study the induced systemic resistance in response to pathogen inoculation in dolichos bean plants under glass house conditions. Leaf tissues

from treated plants inoculated with pathogen were collected at 0, 3, 5, 7 and 9 DAI.

- Peroxidase (PO): Peroxidase activity was assayed as per the procedure described by Hammerschmidt *et al.*, (1982).

- Polyphenol Oxidase (PPO): One gram of sample was homogenized in 2 ml of 0.1M sodium phosphate buffer (pH 6.5) at 4°C. The supernatant served as enzyme source and PPO activity was determined as per the procedure given by Mayer *et al.*, (1965).

- Phenylalanine Ammonia Lyase (PAL): The PAL activity was assayed as per the method described by Ross and Sederoff, (1992).

- Phenol content: Phenol content was estimated as per the procedure given by Zieslin and Ben-Zaken, (1993).

Results and Discussions

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

The results obtained in the field studies are furnished in (Table 1). In general the anthracnose incidence showed an increasing pattern with an increase in the age of the crop in all the treatments and also control plots. Observations taken at 60, 75 DAS and 90 DAS revealed that the treatment, *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 and 60 DAS (T₇) recorded 05.36, 7.90 and 9.67% of anthracnose incidence at 60, 75 DAS and 90 DAS respectively, which was followed by the treatment T₈ and T₄. The untreated control recorded 29.89, 35.29 and 39.80 percent anthracnose incidence at 60, 75 and 90 DAS of dolichos bean. Shen *et al.*, (2002) 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) who reported that application of *S. marcescens* along with pungam oil cake extract significantly reduced the wilt incidence in banana. Nnullie *et al.*, (2010) reported that spraying with *P. fluorescens* and *A. sativum* (10%) showed the maximum reduction in fruit rot incidence. 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). Combination effect of antagonist and botanicals might

Table 1: 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	16.25(23.77)	19.28(26.04)	22.67(28.43)	42.29	45.36	43.04
2	<i>S. marcescens</i> as foliar spray @ 2% in 40, 50 and 60 DAS	17.02(24.36)	21.65(27.72)	24.81(29.87)	43.05	38.65	37.66
3	<i>A. sativum</i> 30% as foliar spray @ 40, 50 and 60 DAS	18.56(25.51)	22.30(28.17)	25.84(30.55)	37.90	36.80	35.07
4	T ₁ +T ₂	06.67(14.96)	08.72(17.17)	11.72(20.01)	77.68	75.29	70.55
5	T ₁ +T ₃	09.67(18.11)	11.87(20.15)	14.56(22.43)	67.64	66.40	63.41
6	T ₂ +T ₃	12.72(20.89)	14.04(22.00)	16.02(23.59)	57.44	60.15	59.74
7	T ₁ +T ₂ +T ₃	05.36(13.38)	07.90(16.32)	09.67(18.11)	82.06	77.61	75.70
8	Carbendazim 50% WP 0.1% (ST 2g/kg of seeds + FS @ 40, 50 DAS)	06.14(14.34)	08.36(16.80)	11.14(19.49)	79.45	76.31	72.01
9	Control	29.89(33.14)	35.29(36.44)	39.80(39.11)	-	-	-
	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.

be attributed as the reason for the enhanced disease suppression.

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

The data depicted in (Table 2) revealed that the application of *S. marcescens* and *A. Sativum* extract increased the biometrics of dolichos bean when compared to control. Among the treatment T₇ recorded maximum plant height (159.99 cm), number of pod/plant (90.46) and pod yield (10.12 t/ha.), followed by T₄ (153.18 cm). The treatment control recorded the minimum parameters.

Jadon *et al.*, (2016) who reported that the combined application of botanicals and bio-control agents reduced the seedling mortality and enhanced plant biomass of bell

pepper. Muthukumar *et al.*, (2010) reported that the seed treatment with combined application of *T. viride* + *P. fluorescens* + Zimmu leaf extract increased the plant growth (shoot length and root length) and yield of chilli. The combined application of *S. marcescens* with botanicals increased the plant growth and yield of brinjal (Subharathinam, 2018). Basal application of FYM plus sucker treatment plus soil application of *S. marcescens* and micronutrient mixture significantly reduced the panama wilt incidence of banana (7.95%) to the minimum and increased the plant growth and yield parameters to the maximum in both plant and ratoon crops of banana *CV. Monthan* (Sanjeevkumar *et al.*, 2019). The results of the present study clearly revealed that an integration of several strategies like application of *S. marcescens* agents along with plant extract exerted a synergism which enhanced the plant growth and yield

Table 2: 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	105.23	67.88	08.91
2	<i>S. marcescens</i> as foliar spray @ 2% in 40, 50 and 60 DAS	118.34	69.56	08.98
3	<i>A. sativum</i> 30% as foliar spray @ 40, 50 and 60 DAS	97.98	65.35	08.78
4	T ₁ +T ₂	153.18	79.75	09.86
5	T ₁ +T ₃	146.97	73.64	09.67
6	T ₂ +T ₃	125.35	71.32	09.11
7	T ₁ +T ₂ +T ₃	159.99	90.46	10.12
8	Carbendazim 50% WP 0.1% (ST 2g/kg of seeds + FS @ 40, 50 DAS)	135.23	71.36	09.41
9	Control	64.67	35.85	06.45
	S.Ed	0.42	0.46	0.01
	CD (p=0.05)	0.89	0.97	0.03

Table 3: Changes in peroxidase (PO) activity in dolichos bean crop treated with *S. marcescens*, *A. sativum* extract and challenge inoculated with *C. lindemuthianum*.

Tr. No.	Treatments	Changes in absorbance/min/g of Units				
		No. of Days				
		1	3	5	7	9
1	<i>S. marcescens</i> as ST @ 10 ml / kg of seeds	0.95	1.06	1.19	1.08	0.64
2	<i>S. marcescens</i> as foliar spray @ 2% in 40, 50 and 60 DAS	1.17	1.28	1.39	1.13	1.03
3	<i>A. sativum</i> 30% as foliar spray @ 40, 50 and 60 DAS	0.96	1.07	1.20	0.84	0.73
4	T ₁ +T ₂	1.54	1.69	1.83	1.49	1.34
5	T ₁ +T ₃	1.37	1.44	1.59	1.28	1.12
6	T ₂ +T ₃	1.20	1.28	1.36	1.12	1.01
7	T ₁ +T ₂ +T ₃	2.12	2.35	2.84	1.99	1.12
8	Carbendazim 50% WP 0.1% (ST 2g/kg of seeds + FS @ 40, 50 DAS)	1.29	1.38	1.45	1.17	1.11
9	Inoculated Control	0.68	0.73	0.78	0.57	0.46
10	Healthy Control	0.58	0.62	0.66	0.46	0.32
	S.Ed	0.01	0.03	0.01	0.03	0.02
	CD(p=0.05)	0.03	0.07	0.02	0.07	0.04

attributes of dolichos bean to the maximum.

Enzyme studies

• Changes in peroxidase (PO) activity: In the present study, it was observed that the peroxidase activity was maximum in the treatment with *S. marcescens* (ST) @ 10.0 ml/Kg of seeds + *S. marcescens* as 2% foliar spray + *A. sativum* 30% as foliar spray @ 40, 50 and 60 DAS and challenge inoculation of the pathogen (Table 3). Accumulation of peroxidase has been correlated with induced systemic resistance in several crops (Krishnaveni, 2006; Balabaskar, 2006; Muthukumar et al., 2011; Boominathan and Sivakumar, 2012; Rubini, 2013; Manikandan, 2017).

Suppression in the wilt incidence of cucumber and higher levels of defense enzyme peroxidase and catalase were observed in plants treated with *S. marcescens*

indicating that the production of phytoalexin or lignin might be involved in disease suppression (Okamoto et al., 1998). Increased activity of peroxidase was observed in the combination of *S. marcescens* plus FYM plus micronutrient mixture treated banana plants (Sanjeevkumar, 2008). Subharathinam, (2018) reported that seed treatment of *S. marcescens* @ 12 ml/kg of seeds + soil application of *S. marcescens* @ 2.5 l/ha + soil application of mahua oilcake @ 450 kg/ha and challenge inoculated with pathogen increased peroxidase activity of upto fifth days inoculation and there after the activity declined drastically. These earlier reports lend support to the present findings.

• Changes in polyphenol oxidase (PPO) activity: Application on bioagents and plant extracts viz., *S. marcescens* (ST) @ 10.0 ml/Kg of seeds + *S. marcescens* as 2% foliar spray + *A. sativum* 30% as

Table 4: Changes in peroxidase (PO) activity in dolichos bean crop treated with *S. marcescens*, *A. sativum* extract and challenge inoculated with *C. lindemuthianum*.

Tr. No.	Treatments	Changes in absorbance/min/g of Units				
		No. of Days				
		1	3	5	7	9
1	<i>S. marcescens</i> as ST @ 10 ml / kg of seeds	0.72	0.79	0.88	0.94	0.62
2	<i>S. marcescens</i> as foliar spray @ 2% in 40, 50 and 60 DAS	0.85	0.91	0.96	0.76	0.68
3	<i>A. sativum</i> 30% as foliar spray @ 40, 50 and 60 DAS	0.78	0.87	0.92	0.67	0.58
4	T ₁ +T ₂	1.37	1.44	1.66	1.32	1.29
5	T ₁ +T ₃	1.24	1.36	1.42	1.17	1.05
6	T ₂ +T ₃	1.11	1.21	1.29	1.11	0.99
7	T ₁ +T ₂ +T ₃	1.74	1.95	2.47	1.79	1.43
8	Carbendazim 50% WP 0.1% (ST 2g/kg of seeds + FS @ 40, 50 DAS)	1.15	1.27	1.33	1.13	1.09
9	Inoculated Control	0.47	0.52	0.59	0.51	0.41
10	Healthy Control	0.37	0.46	0.52	0.49	0.38
	S.Ed	0.02	0.02	0.01	0.03	0.02
	CD(p=0.05)	0.04	0.05	0.03	0.06	0.05

Table 5: Changes in phenylalanine ammonia-lyase (PAL) activity in dolichos bean crop treated with *S. marcescens*, *A. sativum* extract and challenge inoculated with *C. lindemuthianum*.

Tr. No.	Treatments	n mol transciennamic acid/min/g of Units				
		No. of Days				
		1	3	5	7	9
1	<i>S. marcescens</i> as ST @ 10 ml / kg of seeds	57.39	61.53	65.31	44.82	39.23
2	<i>S. marcescens</i> as foliar spray @ 2% in 40, 50 and 60 DAS	65.28	68.58	72.44	63.34	61.80
3	<i>A. sativum</i> 30% as foliar spray @ 40, 50 and 60 DAS	61.92	63.42	67.51	54.67	48.34
4	T ₁ +T ₂	83.67	90.12	97.89	74.56	52.47
5	T ₁ +T ₃	81.65	83.85	85.47	80.83	76.40
6	T ₂ +T ₃	70.50	72.63	76.24	68.47	66.85
7	T ₁ +T ₂ +T ₃	90.23	94.89	107.46	80.56	71.36
8	Carbendazim 50% WP 0.1% (ST 2g/kg of seeds + FS @ 40, 50 DAS)	73.85	75.36	78.74	68.53	65.45
9	Inoculated Control	48.80	49.78	52.45	46.67	42.21
10	Healthy Control	44.27	46.75	48.67	45.26	40.54
	S.Ed	0.46	0.37	0.47	0.25	0.43
	CD(p=0.05)	0.98	0.85	0.99	0.56	0.92

foliar spray @ 40, 50 and 60 DAS and challenge inoculation with *C. lindemuthianum* led to increased PPO activity up to 5th day (Table 4). Murugan, (2015) observed that PPO activity in *Coleus* plants were induced by the soil application of *P. liancinus* @ 10 g/kg of soils + soil application of *T. viride* @ 2.5 kg / ha + Soil application of *P. fluorescens* @ 2.5 kg/ha against *F. chlamydosporum*. Such induction of higher level of PPO's could be responsible for the enhanced disease suppression observed in the present study.

The present results also results were confirmed by the results of Senthilraja *et al.*, (2013), who reported that the application of *P. fluorescens* (Pf1 and TDK1) and *Beauveria bassiana* (B2) strain combination significantly increased the PPO activity and also the expression of more polyphenol oxidase, against *S. rolfsii* and *Aproaerema modicella*. Parthasarathy, (2016) observed

that PPO activity in tomato plants were induced by the *P. fluorescens* as (ST) @10.0 ml/Kg of seeds + *P. fluorescens* as 2% foliar spray at 45 and 60 DAS + neem extract @ 10 ml/ lit against *A. solani*. Likewise, Seed treatment of *S. marcescens* @ 12 ml/kg of seeds + soil application of *S. marcescens* @ 2.5 l/ha + soil application of mahua oilcake @ 450 kg/ha and challenge inoculated with pathogen increased PPO activity (Subharathinam, 2018). These earlier reports lend support to the present observations.

• Changes in phenylalanine ammonia lyase (PAL) activity: In the present study, *S. marcescens* (ST) @ 10.0 ml/Kg of seeds + *S. marcescens* as 2% foliar spray + *A. sativum* 30% as foliar spray @ 40, 50 and 60 DAS treated plants challenge inoculated with *C. lindemuthianum* recorded maximum PAL activity on the 5th day (Table 5). Perusal of literature showed that numbers of publications

Table 6: Changes in total phenol activity in dolichos bean crop treated with *S. marcescens*, *A. sativum* extract and challenge inoculated with *C. lindemuthianum*.

Tr. No.	Treatments	n mol transciennamic acid/min/g of Units				
		No. of Days				
		1	3	5	7	9
1	<i>S. marcescens</i> as ST @ 10 ml / kg of seeds	88.45	110.65	128.30	92.60	89.45
2	<i>S. marcescens</i> as foliar spray @ 2% in 40, 50 and 60 DAS	91.70	118.74	132.28	97.22	92.70
3	<i>A. sativum</i> 30% as foliar spray @ 40, 50 and 60 DAS	91.45	116.54	130.25	95.34	91.12
4	T ₁ +T ₂	96.20	135.65	150.92	105.80	103.49
5	T ₁ +T ₃	95.28	134.85	148.67	101.67	99.45
6	T ₂ +T ₃	95.20	129.65	145.54	100.75	96.37
7	T ₁ +T ₂ +T ₃	100.40	150.25	175.70	158.69	120.60
8	Carbendazim 50% WP 0.1% (ST 2g/kg of seeds + FS @ 40, 50 DAS)	94.96	120.20	140.54	99.25	94.30
9	Inoculated Control	75.40	89.75	91.28	88.76	70.25
10	Healthy Control	61.65	79.60	84.25	75.30	65.78
	S.Ed	0.46	0.37	0.47	0.25	0.43
	CD(p=0.05)	0.98	0.85	0.99	0.56	0.92

are available on the increased activity of PAL in bio-control inoculated plants challenge inoculated with the pathogen.

Phenylalanine ammonia-lyase (PAL) plays a vital role in the biosynthesis of phenols and phytoalexin (Daayf *et al.*, 1997). The product of PAL is *trans-cinnamic* acid, which is an immediate precursor for the biosynthesis of salicylic acid, a single molecule in systemic acquired resistance (SAR) (Klessig and Malamy, 1994). PAL activity could be induced in plant-pathogen interaction and fungal elicitor treatment (Ramanathan *et al.*, 2000). Rhizosphere colonization of *P. aeruginosa* activated PAL in bean roots and increased the salicylic acid levels in leaves (De Meyer *et al.*, 1999). Induction of PAL by fluorescent pseudomonas was reported in sugarcane against *C. falcatum* (Viswanathan and Samiyappan, 1999). Early and increased synthesis of PAL was observed in the *S. marcescens* pre treated blackgram plants challenged with *M. phaseolina* (Ezhilarasi, 2006). Increased activity of PAL was observed in the combination of *S. marcescens* plus FYM plus micronutrient mixture treated banana plants (Sanjeevkumar, 2008).

Phenylalanine ammonia lyase is the enzyme of phenyl propanoid metabolism in higher plants and it played an important role in the accumulation of phenolics, phytoalexin and lignin which is responsible for disease resistance (Vidyasekaran, 1988). Boominathan and Sivakumar, (2012) reported that turmeric plants treated with *P. fluorescens* and *T. viride* induced the plants to synthesize PAL when the plants were challenge inoculated with *P. aphanidermatum*. Seed treatment with *S. marcescens* and combination of mahua oil cake extract induced the accumulation of high level of PAL enzyme activity in brinjal damping-off (Subharathinam, 2018).

- Changes in total phenol content: In general, there was increase in total phenols of dolichos bean, than the uninoculated control. Increase in total phenol content was significantly very high in the treatment with *S. marcescens* (ST) @ 10.0 ml/Kg of seeds + *S. marcescens* as 2% foliar spray + *A. sativum* 30% as foliar spray @ 40, 50 and 60 DAS treated plants challenge inoculated with *C. lindemuthianum* (Table 6). Phenolics seem to inhibit disease development through different mechanisms involving the inhibition of extracellular fungal enzymes (Cellulases, Pectinases, Laccase and Xylanase) (Ashry and Mohamed, 2011). Similar increase in the phenolics activity in banana plants due to treatment with *S. marcescens* and challenge inoculation with *F. oxysporum* f.sp. *cubense* (Sanjeevkumar, 2008) in rice against *P. oryza* (Jaiganesh, 2005). Meeta Lavania *et al.*, (2006) indicated that increase in phenol content of betelvine seedling was due to the treatment with endophytic bacteria

S. marcescens maximum phenol content was observed at sixth day after treatment. Several earlier workers have reported about enhanced phenolics due to treatment with biocontrol agent in combination with plant extracts which led to conferring resistance to the plants against pathogens (Ezhilarasi, 2006; Muthukumar *et al.*, 2011; Boominathan and Sivakumar, 2012; Murugan, 2015; Manikandan, 2017). Santhoshkumar, (2017) observed that phenol activity in chilli plants were induced by the *T. viride* as (ST) @ 6.0 ml/Kg of seeds + *T. viride* as (SA) 3.0 lit/ha + *A. marmelos* (FS) leaf extract 40% @ 35 and 75 DAT against *C. capsici*. These earlier reports lend support to the present findings.

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