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EFFECT OF SEED AND SOIL APPLICATION WITH DIFFERENT DOSES OF *SERRATIA MARCESCENS* ON PLANT GROWTH AND INCIDENCE OF DAMPING-OFF (*PYTHIUM APHANIDERMATUM* (EDSON) FITZ.) OF BRINJAL UNDER POT CULTURE

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Abstract

The present study was undertaken to investigate the effect of *Serratia marcescens* for the successful biological management of damping-off of brinjal. The results revealed that seed treatment with *S. marcescens* @ 14.0 ml/Kg of seeds recorded the minimum pre-emergence damping-off (06.98%) and post-emergence damping-off (07.97%) and maximum growth parameters which was on par with *S. marcescens* @ 12.0 ml/ kg of seeds. This was followed by Metalaxyl @ 0.1 %. Also, among the soil application dosages *S. marcescens* @ 3.0 ml/pot recorded a reduction of 81.56 per cent pre-emergence and 83.49 per cent post-emergence damping-off disease incidence over control and was on par with *S. marcescens* @ 2.5 ml/pot (80.45 per cent pre-emergence and 83.10 per cent post-emergence). *S. marcescens* @ 3.0 ml/pot as soil application recorded minimum damping-off incidence (Pre and post-emergence), maximum seed germination, growth parameters and minimum population of *P. aphanidermatum*.

Key words : *Serratia marcescens,* damping-off, *Pythium aphanidermatum,* incidence.

Introduction

The brinjal (*Solanum melongena* L.) also known as the *eggplant* is one of the most popular and vegetable crop grown in India and other parts of the world. The crop belongs the family Solanaceae and genus *Solanum.* The fruit contain approximately 92 per cent moisture, six per cent carbohydrate, One per cent proteins, 0.3 per cent fats and some minerals (Das *et al.*, 2000). Brinjal are fairly good source of calcium, phosphorus, iron and vitamin B. Such a potential crop is known to suffer from several fungal, bacterial and phytoplasmal diseases. Among these damping-off of brinjal seedlings caused by several species of *Pythium* is very common all over the world. Besides, it occurs both in tropical and temperate climates and in almost every glass house and green house conditions (Klean *et al.,* 2003).

Pythium spp. are essentially soil borne and their role in causing damping-off disease in nurseries is well established (Agrios, 2005). Being a facultative parasite, the population of pathogen is always present in the nursery soil and causes severe disease problems. The management of *Pythium* is very difficult due to its wide host range, soil borne nature and prolonged survival of propagules in the soil (Hendrix and Campbell, 1973). Use of chemical fungicides are known to exert their inherent ill effects like accumulation of residual toxicity, causing environmental pollution and upsetting the biological balance in the soil by over killing the non-targeted microorganisms. It is therefore essential to develop an effective, cheap and environmentally safe non-chemical method for the management of damping-off disease. For this the use of biological methods seems to be the promising alternative method (Harman, 1991).

Many of the introduced antagonists failed to survive in the soil due to lack of favourable conditions like food

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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 damping-off of brinjal caused by *Pythium aphanidermatum* (Edson) Fitz. *S. marcescens* a gram negative soil bacterium produces chitinolytic enzymes and certain antifungal low molecular weight molecules which cause degradation of the fungal cell walls (Someya *et al.,* 2000). Therefore in the present study we investigated the Effect of seed and soil application with different doses of *Serratia marcescens* on plant growth and incidence of damping-off of brinjal under pot culture

Materials and Methods

Preparation of liquid formulation of *S. marcescens*

For the preparation of liquid formulations the method suggested by Manikandan *et al*., (2010) 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 ($1x10^8$) following serial dilution plating technique and the formulation thus prepared was sealed in plastic containers and used for further studies.

Pot culture experiments

All the pot trial was conducted in pot culture yard at Department of Plant Pathology, Faculty of Agriculture, Annamalai University. The experiments were conducted in a randomized block design (RBD) with three replications for each treatment.

Efficacy of seed treatment with *S. marcescens* on plant growth and disease incidence of brinjal

Sterilized soil (5.0kg) was filled in 30 cm earthen pots. Surface sterilized sand maize medium was mixed with soil @ 5% level. Seed treatment with Metalaxyl @ 2g kg^{-1} was used for comparison and the pathogen alone inoculated pots served as control. The experiment was conducted with three replications in a randomized block design (RBD). The per cent disease incidence, germination percentage, shoot and root length (cm) of the plant were recorded at harvest.

Treatment schedule

T_1 - Seed treatment of *S. marcescens* @ 4.0ml. / Kg of seeds

T_2 - Seed treatment of *S. marcescens* @ 6.0 ml. / kg of seeds

T_3 - Seed treatment of *S. marcescens* @ 8.0 ml. / kg of seeds

T_4 - Seed treatmen of *S. marcescens* t@ 10.0 ml. / kg of seeds

T_5 - Seed treatment of *S. marcescens* @ 12.0 ml. / kg of seeds

T_6 - Seed treatment of *S. marcescens* @ 14.0 ml. / kg of seeds

T_7– Metalaxyl as seed treatment @ 2.0 g /kg of seeds

T_8- Control

Effect of soil application with different doses of *S. marcescens* on the incidence of damping-off and growth of brinjal seedlings

Sterilized soil (1.0kg) was mixed with the pathogen inoculum @ 100g (multiplied on sand maize medium) and filled in 15 x 30 cm dia. earthen pots. Liquid formulation of the antagonist was applied to the soil 10 days before sowing. The treatment schedule followed is mentioned below.

Treatment schedule

T_1- Soil application of *S. marcescens* @ 1.5 ml/pot.

T_2- Soil application of *S. marcescens* @ 2.0 ml/pot.

T_3- Soil application of *S. marcescens* @ 2.5 ml/pot.

T_4- Soil application of *S. marcescens* @ 3.0 ml/pot.

T_5- Soil drenching of Metalaxyl @ 0.1%

T_6- Control

Soil drenching with metalaxyl @ 0.1% was used for comparison and pathogen alone inoculated pots served as control. The experiment was conducted in a randomized block design and replicated thrice. The seeds were sown in pathogen inoculated soil @ 25-50seeds per pot and irrigated daily. The observations on the incidence of pre-emergence damping -off was recorded on seventh day of sowing and the incidence of post-emergence damping- off was recorded on 14^{th} day after sowing. The shoot length and root length (cm) of the plants were recorded at 25 days after sowing. In order to find out the population of pathogen, the soil samples were collected at 0, 10, 20, and 30 days after sowing.

Enumeration of *P. aphanidermatum* population in soil

The population of *Pythium* in soil was estimated following the method of Stanghellini and Hancock (1970). The soil sample collected (0, 10, 20, and 30 days) was sieved and one gram of soil sample was suspended in 10ml of sterile water and shaken well. Then one ml of this suspension was drawn immediately and transferred into 9 ml of sterile water by means of a sterile pipette to get 10^{-2} dilution. One ml of this dilution was immediately dispensed as small drops on the surface of 3 days old 2% plain agar in Petri plates and these plates were incubated at 28 ± 2°C and observed after 24 h.The number of hyphal strands emerging from the perimeter of each drop was counted under low power of the microscope. Hyphae were readily distinguished from other fungal hyphae by their rapid growth and tendency to grow in straight line away from the drop.

Results and Discussion

Effect of seed treatment with different doses of *S. marcescens* on plant growth and incidence of damping-off of brinjal (Pot culture)

Among the various treatments, *S. marcescens* @ 14.0 ml/ kg of seeds recorded the minimum pre-emergence damping-off (06.98 %) and post-emergence damping-off (07.97 %) which was at par with that of *S. marcescens* @ 12.0 ml/ kg of seeds. The same treatment also recorded the maximum growth parameters with 16.7cm and 9.4 cm of shoot and root length, germination (90.88%), vigour index (2400.67). This was followed by metalaxyl @ 0.1 % recorded pre-emergence damping-off (9.45 %) and post-emergence damping-off (10.43 %). The maximum pre-emergence damping-off (34.50 %) and post-emergence damping-off of (40.33 %) was recorded in control (table 1&2). This corresponds with the study done by Okamato *et al.* (1998) who reported that red pigment of *S. marcescens viz*., prodigiosin as a biocontrol agent against many damping-off diseases of vegetable crops. Roberts *et al.,* (2005) reported that *S. marcescens* N1-14 provided significant suppression of *R. solani* and *P. ultimum* causing damping-off disease of cucumber. Seed treatment with bacterial antagonist has been widely used for the manajement of plant disease (O'Sullivan and O'gava, 1992; Vidhyasekaran *et al.,* 1997). An increase in grain yield due to the application of *S. marcescens* may be associated with increased plant growth because of the plant growth promoting characters and decreased disease incidence (El-Tarability *et al.,* 2000). Seed application of *S. marcescens* increased the various growth parameters of black gram at the same time reduced the root rot disease incidence (Ezhilarasi, (2006). These reports are in line and add support to the present findings.

Effect of soil application of *S. marcescens* on plant growth and incidence of brinjal damping-off

The results regarding the effect of soil application of *S. marcescens* on the damping-off incidence, seed germination and growth parameters of brinjal are given in the (Tables 3, 4). Soil application of *S. marcescens* @ 3.0 ml/pot recorded a reduction of 81.56 per cent pre-emergence and 83.49 per cent post-emergence damping-off disease incidence over control and was on par with *S. marcescens* @ 2.5 ml/pot (80.45 per cent pre-emergence and 83.10 per cent post-emergence). The maximum damping off incidence was observed in control (Table 3). The results depicted in table 4 revealed the highest seed germination (92.92), shoot length (20.5 cm), root length (10.1cm), and vigour index (2843.35) in the treatment with soil application of *S. marcescens* @ 3.0 ml/pot and also on par with *S. marcescens* @ 2.5 ml/pot. The minimum growth was recorded in soil application of S. *marcescens* @ 1.0 ml/pot. Similar such efficacy of the soil application method of delivery system against damping-off disease was reported by earlier workers (Ramesh, 2004). Ezhilarasi, (2006) reported that soil application of *S. marcescens* reduced the root rot disease incidence of blackgram. Stefen Kurze *et al.,* (2001) plant growth promoting *S. plymuthica* strain HRO-C48 was found to control *Verticillium* wilt and *Phytophthora* root rot in strawberry. *S. marcescens* also produces prodigiosin (PGN), which has antifungal, antibacterial and immune suppressive effects (Okamoto *et al.,* 1998). De Queiroz and De Melo (2006) used *S. marcescens* strain R-35 isolated from washed root surface of healthy citrus plants for controlling root rot disease in citrus. In greenhouse trials, the strain suppressed more than 50% of the root rot caused by *P. parasitica*.

The antagonistic bioinoculants (*S. marcescens*) applied either through seed or soil application has resulted in quicker proliferation leading to faster establishment of the antagonistic bio inoculants in the rhizosphere. This might be the reason for the increased activity of these antagonists at post emergency of crop growth. In addition to this by their high rhizosphere competence leading to improved root health might have protected brinjal seedlings against pre and post-emergence damping-off the observation made in the present study has revealed the significant effect of delivery system on the seedling vigour index (Muthukumar, 2008).

Effect of application of *S. marcescens* on the population of dynamics of *P. aphanidermatum*

The population of *P. aphanidermatum* in soil was gradually reduced in all the treatments. The maximum reduction was noticed in soil application of *S. marcescens* @ 3.0 ml/pot and was on par with *S. marcescens* @ 2.5 ml/pot (Table 5). These results are in accordance with the findings of Manoranjitham *et al*., (2000) and Ramesh (2004). Additions of antagonists to soil have suppressed the growth and competitive saprophytic ability of *P. aphanidermatum* resulting in the reduction of inoculum

Table 1: Effect of Seed treatment with different doses of *S. marcescens* on the incidence of brinjal damping-off under pot culture.

Tr. No	Treatments	% incidence of damping – off			
		Pre -emergence (%)	Per cent decrease over control	Post -emergence (%)	Per cent decrease over control
1	Seed treatment @ 4.0 ml / Kg of seeds	27.32 (31.51)	20.81	37.45 (37.73)	07.14
2	Seed treatment @ 6.0 ml / Kg of seeds	21.45 (27.59)	37.82	32.33 (34.65)	19.83
3	Seed treatment @ 8.0 ml / Kg of seeds	15.38 (23.08)	55.42	24.54 (29.69)	39.15
4	Seed treatment @ 10.0 ml / Kg of seeds	07.61 (16.01)	77.94	08.98 (17.43)	77.73
5	Seed treatment @ 12.0 ml / Kg of seeds	07.05 (15.39)	79.56	08.45 (16.89)	79.04
6	Seed treatment @ 14.0 ml / Kg of seeds	06.98 (15.31)	79.76	07.97 (16.39)	80.23
7	Seed treatment with Metalaxyl @ 2.0g/Kg. of seed	9.45(17.90)	72.60	10.43 (18.84)	74.13
8	Control	34.50 (35.97)	-	40.33 (39.42)	-
	SEd	0.12	-	0.24	-
	CD (p=0.05)	0.24	-	0.49	-

Data in parentheses indicate angular transformed values.

Table 2: Effect of seed treatment with of *S. marcescens* on the plant growth parameters of brinjal under pot culture.

Tr.No.	Treatments	Germination Percentage (%)	Shoot Length (cm)	Root Length (cm)	Vigour index
1	Seed treatment @ 4.0 ml / Kg of seeds	80.21 (63.58)	7.4	3.3	858.24
2	Seed treatment @ 6.0 ml / Kg of seeds	84.32 (66.67)	11.9	4.9	1416.57
3	Seed treatment @ 8.0 ml / Kg of seeds	86.15 (68.15)	13.8	5.9	1697.15
4	Seed treatment @ 10.0 ml / Kg of seeds	87.59(69.37)	15.6	7.7	2040.84
5	Seed treatment @ 12.0 ml / Kg of seeds	91.92 (73.48)	16.4	9.3	2362.34
6	Seed treatment @ 14.0 ml / Kg of seeds	91.98(73.54)	16.7	9.4	2400.67
7	Seed treatment with Metalaxyl @ 2.0g/Kg. of seed	88.52 (70.19)	16.5	9.0	2257.26
8	Control	68.21 (55.67)	9.9	3.5	914.01
	S.Ed	0.12	0.20	0.05	-
	CD (p=0.05)	0.25	0.43	0.15	-

Data in parentheses indicate angular transformed values.

Table 3: Effect of soil application with different doses of *S. marcescens* on the incidence of brinjal damping-off under pot culture.

Tr. No	Treatments	% incidence of damping – off			
		Pre -emergence (%)	Per cent decrease over control	Post -emergence (%)	Per cent decrease over contro
1	Soil application @ 1.0 ml/pot	31.21 (33.96)	15.64	39.42 (38.89)	18.26
2	Soil application @ 1.5 ml/pot	25.78 (30.51)	30.32	31.54 (34.16)	34.60
3	Soil application @ 2.0 ml/pot	15.25 (22.98)	58.78	22.42 (28.26)	53.51
4	Soil application @ 2.5 ml/pot	07.23(15.59)	80.45	08.15 (16.58)	83.10
5	Soil application @ 3.0 ml/pot	06.82(15.13)	81.56	07.96 (16.38)	83.49
6	Soil application with metalaxyl @ 0.1 %	09.52 (17.97)	74.27	10.12 (18.54)	79.01
7	Control	37.00 (37.46)	-	48.23 (43.98)	-
	SEd	0.21	-	0.12	-
	CD (p=0.05)	0.44	-	0.25	-

Data in parentheses indicate angular transformed values.

levels in soil (Bhuvaneshwari, 2008).

Addition of antagonists to soil has suppressed the growth and competitive saprophytic ability of *P. aphanidermatum* resulting in the reduction of inoculums levels in soil (Someya *et al.,* 2002). The combined application of antagonist resulted in lesser disease incidence besides increasing seedling vigour and reduced the population of *P. aphanidermatum.* This may be due to the competition and hyperparasitisation and increased seedling vigour may be due to the synergistic effect of native microflora and simulative effect of bioinoculants

Table 4: Effect of soil application with **of** *S. marcescens* on the plant growth parameters of brinjal under Pot culture.

Tr.No.	Treatments	Germination Percentage (%)	Shoot Length (cm)	Root Length (cm)	Vigour index
1	Soil application @ 1.0 ml/pot	80.98(64.14)	12.5	5.9	1490.03
2	Soil application @ 1.5 ml/pot	85.43 (67.56)	15.9	7.1	1922.17
3	Soil application @ 2.0 ml/pot	89.13 (70.74)	17.8	8.3	2326.29
4	Soil application @ 2.5 ml/pot	92.92 (74.56)	20.5	10.1	2843.35
5	Soil application @ 3.0 ml/pot	93.89 (75.68)	20.9	10.4	2938.76
6	Soil application with metalaxyl @ 0.1 %	89.98 (71.54)	19.7	9.8	2654.41
7	Control	68.21 (55.67)	8.9	3.7	859.44
	S.Ed	0.51	0.21	0.15	-
	CD (p=0.05)	1.03	0.45	0.32	-

Data in parentheses indicate angular transformed values.

Table 5: Effect of soil application of *S. marcescens* on the population dynamics of *P. aphanidermatum* in brinjal under pot culture.

Tr. No.	Different doses of *S. marcescens*	Population of *P. aphanidermatum* (× 10^3 cfu / g of soil)				
		0 days	10 days	20 days	30 days	Mean
1	Soil application @ 1.0 ml/ha	3.42	13.67	17.64	20.76	13.79
2	Soil application @ 1.5 ml/ha	3.40	11.45	15.30	17.23	11.84
3	Soil application @ 2.0 ml/ha	3.39	09.87	12.43	14.33	10.00
4	Soil application @ 2.5 ml/ha	3.37	6.11	7.12	8.44	6.26
5	Soil application @ 3.0 ml/ha	3.36	5.99	7.13	8.41	6.22
6	Soil application with metalaxyl @ 0.1 %	3.34	6.17	8.14	9.33	6.74
7	Control	3.40	19.92	23.33	26.18	18.20
	SEd	0.01	0.05	0.01	0.05	-
	CD (p=0.05)	0.03	0.13	0.03	0.12	-

(Muthukumar, 2008).

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