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Isolation and Evaluation of Indigenous Isolates of *Bacillus* Spp Against *Meloidogyne Incognita*

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ABSTRACT: *In an attempt to isolate the most effective indigenous isolates of Bacillus spp, twelve isolates were collected from different crops in Coimbatore district of Tamil Nadu. All the isolates were subjected to bioassay for their nematicidal activity against Meloidogyne incognita in vitro. Among the twelve isolates, five isolates viz.B.subtilis (BS2 and BSD3), B.amyloliquefaciens (BSC7), B.cereus (BSC11) and B.pumilus (BSC4) were found to be most effective to inhibit cent percent egg hatching and to cause mortality of juveniles of M.incognita.*

Key words: *Bacillus, culture filtrate, Meloidogyne incognita , root-knot nematode*

INTRODUCTION

Plant parasitic nematodes are causing an economic yield loss of 12.3% per cent world wide (Sasser, 1988). Among them the root knot nematodes considered as key nematode pest of vegetables are causing economic yield loss to the extent of 5 to 43% (Sasser,1980 ; Sasser and Carter, 1982). For example, (Sikora and Fernandez, 2005) reported yield losses of over 30% in three highly susceptible vegetable crops (egg-plant, tomato and melon). In the current scenario the management of phytonematodes using suitable microbial agents is considered as suitable alternative to the existing methods of nematode management owing to several advantages. (Kerry, 2000; Mankau, 1980; Jatala, 1986).

The plant growth promoting rhizobacteria *Bacillus* spp is regarded as promising biocontrol agent of phytonematodes by many earlier workers (Mankau, 1980; Stirling, 1991; Siddiqui and Mahmood, 1999). However the earlier work on this line is inadequate and needs to be intensified. Hence it is programmed to identify the most effective indigenous isolates of *Bacillus* spp and to study their effectiveness in order to develop the same as suitable and viable formulation for the management of nematodes. (Tian *et al*., 2007 and Anwar-ul-Haq *et al*., 2011).

MATERIALS AND METHODS

Isolation of *Bacillus* spp from Rhizosphere Soil

Samples (250g) were collected from the rhizosphere region of different crops *viz.* carnation, cotton and noni. One gram sample of rhizosphere soil was transferred to 250 ml. conical flask containing 10 ml. sterile distilled water. After thorough shaking for 15 min in a shaker the suspension was serially diluted in sterile distilled water. One ml. of each 10^{-5} and 10^{-6} dilutions was pipetted out and poured into Petri plates. Later, 15ml. of melted Nutrient Agar (NA) medium poured into the same Petri plates, rotated gently in clockwise and anticlockwise direction and incubated at room temperature (28 ± 2°C) for 24 to 48h for the development of colonies of*Bacillus* spp. After incubation, the colonies of *Bacillus* spp., from the selective medium were transferred to nutrient agar slants as well as nutrient agar Petri plates.The colonies formed were identified as *Bacillus* spp based on description given by Bergey's manual of Systematic Bacteriology (Krieg and Holt, 1984). Further through different bio-chemical as well as molecular characterisation tests, all the species of *Bacillus* isolates collected in the present study were identified and confirmed (Table 1).

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Table 1
Identification of *Bacillus* spp through Molecular Characterization based on Comparison of3′ end 16S rDNA and 5′ end 16S-23S ITS Nucleotide Sequences
(Goto *et al*., 2000)

Identified Bacillus spp	*Isolates*
B. amyloliquefaciens	BSC6, BSC7
B. cereus	BSC5,BSC11
B. licheniformis	BSD1
B. methylotrophicus	BSD2
B. pumilus	BSC4
B.subtilis	BSC1,BSC3,BSD3,BS2
B.tequilensis	BSC2

All the identified 12 species of *Bacillus* were subjected for their nematicidal activity as follows.

Preparation of Culture Filtrate of *Bacillus*spp

The isolated *Bacillus* spp. culture filtrate was prepared using 24-48 h old *Bacillus* culture and nutrient agar broth. Through inoculation needle a loop full *Bacillus* culture inoculated in to broth taken in conical flask. Then the inoculated broth was kept in a shaker under 150rpm for 2 days at 35 °C. After two days, the culture filtrate was centrifuged for 30 min at 10,000rpm. to get cell free culture filtrate. Then through Mili pore filter (0.2µm. PVDF Media) the culture filtrate was filtered to get clear suspension.

Influence of Culture Filtrate of *Bacillus* spp Against Eggs and Juveniles of *M. incognita*

Root knot nematode egg masses collected from pure culture maintained on tomato under glasshouse conditions were taken at the rate of one egg mass/ Petri plate (5cm) containing five ml. culture filtrate of the above *Bacillus* spp to study the influence of *Bacillus* spp on egg hatching of *M. incognita* at 24h interval for three days. Similarly 100 juveniles of *M. incognita* were transferred to Petri plate (5cm) containing different concentrations of culture filtrate of *Bacillus* spp. Observations on mortality of juveniles

Table 2
Influence of *Bacillus* spp on per cent egg hatching of *M. incognita*

Bacillus spp and their isolates		*Concentration (%) and Time of exposure (h)*								
		25%			*50%*			*100 %*		
		24h	*48h*	*72h*	*24h*	*48h*	*72h*	*24h*	*48h*	*72h*
B. subtilis	**BS2**	98.95	98.99	99.14	100.00	98.41	99.48	100.00	100.00	**100.00**
		(84.90)	(83.15)	(84.71)	(89.20)	(83.98)	(85.86)	(89.20)	(89.20)	**(89.20)**
	BSD3	98.41	98.48	98.79	98.95	98.90	99.14	100.00	100.00	**100.00**
		(83.89)	(83.15)	(83.70)	(84.90)	(83.98)	(84.71)	(89.20)	(89.20)	**(89.20)**
	BSC3	95.23	97.45	98.10	97.89	97.80	98.62	98.95	98.48	98.62
		(77.50)	(80.84)	(82.09)	(81.74)	(81.49)	(83.26)	(84.90)	(83.15)	(81.32)
	BSC1	91.01	90.18	94.82	95.77	93.09	96.20	97.88	97.45	98.10
		(72.55)	(71.80)	(70.77)	(78.16)	(74.82)	(78.79)	(81.74)	(80.84)	(82.14)
B.amyloliquefaciens	**BSC7**	95.77	96.72	98.10	100.00	99.27	99.48	100.00	100.00	**99.48**
		(78.16)	(79.50)	(82.11)	(89.20)	(85.72)	(85.86)	(89.20)	(89.20)	**(86.40)**
	BSC6	94.75	94.54	96.55	97.36	95.63	97.58	98.95	96.36	97.93
		(76.72)	(76.52)	(79.33)	(80.73)	(76.88)	(81.07)	(84.90)	(79.01)	(81.75)
B. cereus	**BSC11**	95.23	96.72	97.93	98.41	98.41	98.62	100.00	100.00	**99.00**
		(77.50)	(79.66)	(81.76)	(83.89)	(84.98)	(83.26)	(89.20)	(89.20)	**(86.97)**
	BSC5	96.30	93.65	93.96	96.30	96.00	97.24	97.36	97.45	98.10
		(77.53)	(77.99)	(75.85)	(78.94)	(78.47)	(80.44)	(80.73)	(81.03)	(82.09)
B. pumilus	**BSC4**	96.30	96.00	97.76	97.36	98.18	98.79	100.00	100.00	**98.85**
		(78.28)	(80.20)	(78.80)	(80.73)	(82.32)	(83.70)	(89.20)	(85.72)	**(86.97)**
B. licheniformis	BSD1	90.47	92.36	95.34	93.65	94.91	96.89	100.00	98.18	98.62
		(72.05)	(73.97)	(77.54)	(75.47)	(76.96)	(79.86)	(89.20)	(82.32)	(83.26)
B. tequilensis	BSC2	93.65	94.91	96.89	96.82	97.03	98.10	100.00	94.18	96.03
		(76.72)	(78.09)	(81.23)	(79.83)	(80.20)	(82.09)	(89.20)	(82.32)	(84.24)
B. methylotrophicus	BSD2	94.71	94.18	96.03	96.30	95.63	96.55	98.98	98.12	98.62
		(76.72)	(77.31)	(78.61)	(78.94)	(78.09)	(79.42)	(84.90)	(82.32)	(83.36)
	Broth	49.74	57.82	79.65	55.03	60.72	76.55	72.49	76.55	84.14
		(44.83)	(49.20)	(59.78)	(47.89)	(51.19)	(61.03)	(58.38)	(58.28)	(68.50)
	Control	63.00	91.66	193.33	63.00	91.66	193.33	63.00	91.66	193.33
		(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
	SEd	1.17	1.46	0.87	1.32	1.28	0.50	0.88	0.78	0.79
	CD (P = 0.05)	2.40	3.02	1.80	2.73	2.63	1.03	1.81	1.61	1.53

Figures in parentheses are arc sine transformed values

were taken at 24h interval for three days. In this study the broth and distilled water taken as untreated control and all the treatments were replicated three times in Complete Randomized Design (CRD).

RESULTS AND DISCUSSION

The culture filtrate of *B. subtilis* effectively inhibited egg hatching and caused the mortality of juveniles of root knot nematode by cent per cent. The effectiveness of *Bacillus* spp in inhibiting egg hatching and causing mortality of juveniles of *M. incognita* was found to be directly related to the concentration of culture filtrate and time of exposure to *Bacillus* spp. Among the four indigenous isolates of *B. subtilis* collected from Tamil Nadu, the isolates BS2 and BSD3 isolated from carnation rhizosphere registered the highest per cent inhibition in egg hatching of *M. incognita* and it was cent per cent in both cases respectively. Similarly the isolates collected from cotton rhizosphere *viz.B. amyloliquefaciens*(BSC7) (99.48%), *B. cereus* (BSC11) (99.00%) and *B. pumilus* (BSC4) (98.85%) also had effect to inhibit egg hatching of *M. incognita* in the present study. (Table 2).

With regard to influence of *Bacillus* spp on juveniles of *M .incognita* it is observed that all the four species of *Bacillus* had effect to cause mortality of juveniles at all the concentrations of culture filtrate and time of exposure used in the present study and it was ranged from 25 to100 per cent. Among them, *Bacillussubtilis* (BS2) recorded the highest per cent (99.50%) mortality of juveniles of *M. incognita* and it was followed by same *Bacillussubtilis* isolate BSD3 (99.30%).The same trend was noticed with other species of *Bacillus viz. B. amyloliquefaciens* isolate BSC7 (99 %), *B. cereus* isolate BSC11 (98.30%) and *B. pumilus* isolate BSC4 (97.00%). (Table 3).

Table 3
Influence of *Bacillus* spp on percent juveniles mortality of *M. incognita*

Bacillus spp and their isolates		*Concentration (%) and Time of exposure (h)*								
		25 %			*50 %*			*100 %*		
		24h	*48h*	*72h*	*24h*	*48h*	*72h*	*24h*	*48h*	*72h*
B.subtilis	**BS2**	71.00 (57.72)	78.00 (62.15)	89.00 (71.60)	77.00 (61.54)	79.00 (62.96)	91.00 (73.44)	82.00 (66.00)	83.00 (65.87)	**99.50 (82.43)**
	BSD3	68.66 (56.19)	72.66 (58.52)	87.00 (69.33)	75.33 (60.37)	54.33 (59.40)	88.66 (73.44)	89.00 (70.95)	91.66 (73.53)	**99.30 (77.96)**
	BSC1	61.66 (51.80)	71.66 (57.84)	72.66 (58.55)	65.33 (54.04)	75.66 (62.26)	39.00 (62.06)	67.66 (55.50)	71.66 (58.01)	85.00 (67.24)
	BSC3	43.66 (41.32)	46.66 (43.07)	50.3 (45.19)	48.30 (40.36)	54.33 (44.16)	61.66 (51.82)	55.66 (44.97)	62.00 (52.07)	71.66 (43.16)
	BSC7	53.33 (46.91)	55.66 (48.26)	67.00 (55.10)	57.66 (49.44)	68.00 (55.61)	73.66 (54.22)	86.00 (68.27)	89.66 (71.45)	**99.00 (77.25)**
B.amyloliquefaciens	BSC6	46.66 (43.06)	53.00 (46.72)	56.66 (48.83)	49.00 (44.42)	51.66 (45.95)	52.33 (46.34)	53.66 (46.10)	55.66 (48.45)	59.00 (25.09)
B. cereus	**BSC11**	49.33 (44.62)	51.33 (45.66)	53.00 (77.10)	54.00 (47.29)	53.00 (48.64)	67.33 (55.22)	77.66 (61.92)	86.00 (68.19)	**98.30 (70.67)**
	BSC5	46.00 (42.68)	49.00 (44.21)	51.00 (48.90)	47.33 (43.45)	48.66 (74.21)	54.33 (47.46)	49.66 (44.80)	54.66 (47.68)	61.00 (51.41)
B. pumilus	**BSC4**	44.66 (41.93)	47.66 (43.65)	49.66 (44.80)	51.66 (45.95)	52.33 (46.23)	62.66 (52.38)	73.00 (58.99)	79.66 (63.42)	**97.00 (70.64)**
B. licheniformis	BSD1	44.66 (41.92)	47.66 (46.35)	51.00 (45.87)	46.33 (42.88)	50.33 (45.19)	56.66 (49.61)	51.66 (45.95)	60.00 (50.84)	63.33 (52.80)
B. tequilensis	BSC2	30.00 (33.66)	38.33 (38.02)	39.33 (39.75)	36.00 (36.64)	40.66 (39.43)	51.33 (45.75)	45.66 (42.50)	51.00 (46.24)	61.00 (51.38)
B. methylotrophicus	BSD2	33.66 (34.45)	35.66 (36.55)	37.66 (37.78)	34.33 (35.69)	37.66 (37.74)	40.33 (39.36)	41.66 (40.11)	47.00 (43.25)	52.33 (46.34)
	Broth	7.00 (15.23)	10.00 (18.30)	12.33 (20.45)	10.66 (18.05)	16.33 (23.07)	20.66 (26.88)	14.00 (21.71)	19.00 (25.65)	21.33 (28.10)
	Control	0.00 (0.28)	0.00 (0.28)	0.00 (0.28)	0.00 (0.28)	0.00 (0.28)	0.00 (0.28)	0.00 (0.28)	0.00 (0.28)	0.00 (0.28)
	SEd	6.30	5.88	6.35	60.22	6.24	6.07	5.96	5.83	4.94
	CD (P = 0.05)	12.95	12.10	13.6	12.79	12.83	12.47	12.25	11.99	10.16

Figures in parentheses are arc sine transformed values

The present findings fall in line with the report of (Kloepper *et al*., 2004),who observed that *Bacillus* spp inhibited egg hatching and causing mortality of juveniles of *M. incognita*. Further as observed in the present study it is reported that the influence of *Bacillus* spp in inhibiting egg hatching and causing mortality of juveniles of *M.incognita* is related to per cent concentration and time of exposure to the culture filtrate of *Bacillus* spp by earlier workers.(Yap Chin Ann, 2012 and Xiao *et al.,* 2012).

Therefore the present study inferred that the local and indigenous isolates of *Bacillus subtilis* collected from carnation rhizosphere ofCoimbatore district of Tamil Nadu are effective against *M. incognita*. Hence it is programmed to advance the effective local isolates of different species of *Bacillus* spp to study for their effectiveness under field conditions in order to develop the same as biopesticide in suitable formulations. (Broadbents *et al*., 1977) (Backman *et al*. 1997).

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