Biocontrol potential of endophytic bacteria on *Meloidogyne incognita* and its effect on plant growth in bhendi

P. Vetrivelkalai[1*], M. Sivakumar[2] and E. I. Jonathan[1]

ABSTRACT

A random survey was conducted to obtain endophytic bacterial isolates in different agroecosystems comprising of ten districts in Tamil Nadu. Nineteen endophytic bacterial isolates were obtained from surface-sterilized roots of different crops. Study on the morphological, phenotypic and biochemical characterization of endophytic bacteria revealed that eight isolates *viz.*, EB1 to EB8 belong to the group of *Pseudomonas* sp., ten isolates *viz.*, EB9 to EB18 belong to the group of *Bacillus* sp. and isolate EB19 belongs to *Methylobacterium* sp. On seed bacterization with nineteen endophytic bacterial isolates, four isolates *viz.*, EB3, EB16, EB18 and EB19 significantly enhanced the germination percentage, shoot and root length and vigour index of bhendi seedlings by roll towel technique and pot culture studies. Eight endophytic bacterial isolates were screened for their nematicidal action against *Meloidogye incognita* under pot culture conditions. The study revealed that the culture filtrates of endophytic bacterial isolates *viz.,* EB3, EB16, EB18 and EB19 significantly reduced the number of adult females, egg masses, eggs/ eggmass, root and soil infestation of *M. incognita.* The lowest root gall index (1.00) was registered both in EB16 and EB18 isolates and it was followed by EB19 and EB3 (1.33) compared to untreated control (4.67).

Keywords: Endophytic bacterial isolates, Plant Growth Promotion, Biocontrol, Bhendi *Meloidogyne incognita* and Vigour index.

INTRODUCTION

Bhendi (*Abelmoschus esculentus* L.) is important vegetable grown throughout the tropical and warm temperate regions of the world for its green tender fruits. The southern root knot nematode, *Meloidogyne incognita* is one of the major constraints in the production of bhendi in tropical and subtropical regions. In India, the annual losses caused by root knot nematode, *M. incognita* is 14.1 per cent in bhendi (Jain *et al*., 2007). Present strategies for nematode management largely depend on cultural practices such as crop rotations are widely used but not effective when adopted individually. The use of resistant varieties for commercial purpose has limited scope due to lack of resistance genes in cultivable crops. Nematicides are neurotoxic further, the residual effects are reflected in the food chain which is hazardous to environment and health. Bacteria associated with roots and the rhizosphere of many plant species known to benefit the plants through growth promotion and biological protection against diseases and pests. In mutualistic associations endophytes colonized plants are protected from nematode attack and host plant in turn provides shelter and nutrition to the endophytes. Hence, an attempt was made to study the plant growth promotion and biocontrol potential of endophytes on *M. incognita* in bhendi.

MATERIALS AND METHODS

Isolation of endophytic bacteria

The field survey was conducted during July 2007 to February 2008 in different ecosystem of ten districts *viz*., Coimbatore, Dindigul, Madurai, Tirunelveli, Theni, Dharmapuri, Krishnagiri, Erode, Salem and Namakkal. Endophytic bacteria were isolated by Mc Inroy and Kloepper (1995) method. Roots samples were made sections (2-3cm long) by using a sterile scalpel and surface sterilized with 1% Sodium hypochlorite (NaOCl) in 0.05% Triton X-100 for 10 min and rinsed four times in 0.02M sterile Potassium phosphate buffer pH 7. An aliquot of 0.1ml was taken from the final buffer wash and transferred to 9.9 ml Tryptic Soya Broth (TSB) to serve as sterile check. Each sample was macerated with a sterile pestle and mortar in 9ml of the final buffer wash. Serial dilution upto 10^7 were made in phosphate buffer. One ml of 10^6 and 10^7 dilution of each sample was plated on three different media *viz*., Tryptic Soya Agar (TSA), Nutrient Agar (NA) and King's B medium (KB). The plates were incubated at 28 ± 2°C for 48-72h.

Identification and characterization of endophytic bacterial isolates

The isolates were initially categorized into two broad groups based on Gram staining by Hucker's modified method (Rangaswami and Bagyaraj, 1993). Morphological and cultural characters of the isolates were used for further grouping. Based on the results of various biochemical tests *viz*., starch hydrolysis, KOH test, citrate utilization, catalase, methyl red, gelatin hydrolysis, growth at 4°C, the organisms were identified upto generic level by Bergey's manual.

Testing for plant growth promotion

To test the germination and vigour index, seed bacterization was done for the isolated endophytic bacterial strains and a standard Pf1 strain which was obtained from Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, India. The bacterial treated bhendi seeds were assessed by modified roll towel method (ISTA, 1993) as well as pot culture conditions. The germination percentage, shoot length and root length were recorded at 18 and 25 days after germination of bhendi seeds by roll towel and pot culture studies respectively. The Vigour index (VI) was calculated using following formula (Abdul Baki and Anderson, 1973).

VI = Germination percentage X Seedling length (shoot length + root length)

Nematicidal efficacy of endophytic bacterial culture filtrate

The endophtic bacterial isolates were grown in their respective medium. *Peudomonas* on King's B broth and *Bacillus* on Nutrient broth were separately cultured for 3-4 days. The liquid culture was filtered through Whatman No.1 filter paper and passed through bacterial filter (Aalten and Gowen, 1998). Filtrates were centrifuged at 6000 rpm for 15 min. The supernatant was taken and the suspended residue was discarded. Bhendi seeds were surface sterilized with 0.1 per cent mercuric chloride for two minutes and washed with distilled water, then seeds were soaked with culture filtrate (100 %) for 3hr. Treated seeds were sown in 5 kg earthern pots filled with sterilized pot mixture. Five days after germination, seedlings were thinned to one per pot and 5000 J_2 of *M. incognita* was inoculated per pot. After 45 days of inoculation, final nematode population in soil, number of adult females, number of egg masses, number of eggs/eggmass and root population were observed. The collected soil samples were processed by Cobb's sieving and decanting method (Cobb, 1918) and Modified Baermann funnel technique (Schindler, 1961) to assess the population of root knot nematode infesting tomato. The representative 5g root samples of each pot were washed free of soil and stained with 0.1% acid fuchsin in lactophenol solution to examine the gall index, number of females, egg masses and eggs/ eggmass per 5g root. The gall index was graded with 1 to 5 scale rating (Headle *et al*., 1989). All the data were statically analyzed and critical difference determined (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Isolation and identification of endophytic bacteria

In the present study, nineteen endophytic bacterial strains were isolated from the root of cereals, pulses, oilseeds, fruits, vegetables and flowers. Among 10 districts of Tamil Nadu, three isolates each from Theni and Coimbatore districts, two isolates each from Dindigul, Dharmapuri, Krishnagiri, Salem, Tirunelveli and Namakkal districts and one isolate from Erode district were obtained and used for the study (Table 1). Isolation of endophytic bacterial strains from various monocots and woody plants has been reported by several authors (Bent and Chanway, 1998; Shishido *et al*., 1999). Hallmann *et al*. (1997) observed that the endophytic bacterial population in roots ranged from 10^3 to 10^5 cells per gram fresh weight. Based on the Gram reaction and cell morphology studies indicated that out of nineteen isolates, nine isolates EB1 to EB8 and EB19 belonged to Gram negative and rod shaped non spore forming bacteria and ten isolates *viz*., EB9 to EB18 belonged to Gram positive and rod shaped, spore forming bacteria. Hung and Annapurna (2004) isolated equal proportion of Gram positive (49%) and Gram negative (51%) bacterial endophytes in soyabean.

The results obtained by analyzing primary character and nutrient source utilization of different endophytic bacteria revealed that eight endophytic bacterial isolates *viz*., EB1 to EB8 belonged to *Pseudomonas* spp (Table 2). Samuthiravalli, (2006) identified three isolates of *Pseudomonas* spp. by utilization of nutrient source and production of metabolites. Based on the positive and negative results of different biochemical test, production of metabolites and utilization of nutrient source, ten endophytic bacterial isolates *viz*., EB9 to EB18 were identified as *Bacillus* spp. result is in confirmation with the findings of Mathiyazhagan (2003). Based on the cells stained, Gram negative, pink to red coloured colonies, catalase and oxidase positive and carbon source of utilization the isolate EB19 was identified as *Methyloba cterium* spp. This was also supported by Madhaiyan (2003) and Aken *et al*. (2004).

Table 1. Endophytic bacterial isolates and source of isolation

Name of the isolates	Source	Place
EB 1	Banana	Chinnamanur (Theni Dt.)
EB 2	Paddy	Attur (Salem Dt.)
EB 3	Banana	Pappireddypatty (Dhamapuri Dt.)
EB 4	Paddy	TNAU wetland (Coimbatore Dt.)
EB 5	Cotton	Theni (Theni Dt.)
EB 6	Sugarcane	Bhavani (Erode Dt)
EB 7	Chilli	Palacode (Dhamapuri Dt.)
EB 8	Maize	Rasipuram (Namakkal Dt.)
EB 9	Groundnut	Tirunelveli (Tirunelveli Dt.)
EB 10	Ragi	Sankarankovil (Tirunelveli Dt.)
EB 11	Maize	Sempatti (Dindigul Dt.)
EB 12	Brinjal	Royakottai (Krishnagiri Dt.)
EB 13	Jasmine	Sankagiri (Salem Dt.)
EB 14	Brinjal	Saravanampatti, (Coimbatore Dt.)
EB15	Banana	Thenkanikottai (Krishnagiri Dt.)
EB 16	Chilli	Oddanchatram (Dindigul Dt.)
EB 17	Maize	Thiruchengodu (Namakkal Dt.)
EB 18	Papaya	Theni (Theni Dt.)
EB 19	Paddy	TNAU Paddy Breeding Station (Coimbatore Dt.)

Plant growth promotion activity

The results revealed that seed bacterization with endophytic bacterial isolates *viz*., EB19, EB16, EB18 and EB3 recorded highest vigour index in bhendi with 3383.07, 3175.63, 2967.37, 2719.73 and 5127.90 4856.43, 4565.47, 4293.63 by roll towel and pot culture studies respectively (Table 3).

Similarly, several reports have indicated that bacterial endophytes promote the growth and health of crop plants (Sturz *et al*., 2000). The endophytic bacteria may promote plant growth and suppress plant diseases probably by means similar to plant growth promoting rhizobacteria (Feng *et al*., 2006). The mechanisms by which plant growth is improved may be similar to those exhibited by rhizosphere microorganisms and include the production of phytohormones, promotion through enhanced availability of nutrients, reduction of ethylene levels, production of antibiotics and induced systemic resistance (Holland, 1997). Adhikari *et al*. (2001) reported potentiality of endophytic bacterial strains for controlling the seedling disease of rice and promoting the plant growth. Harish (2005) found that application of endophytic bacterial strains significantly increased the growth parameters *viz*., pseudostem height, girth, number of leaves and physiological parameters *viz*., chlorophyll stability index, stomatal resistance and transpiration in banana plants both under greenhouse and field conditions. The present results were also in conformity with the earlier reports.

Effect of culture filtrate on *M. incognita*

Eight bacterial isolates were screened for their nematicidal action against *M. incognita*. Bhendi seeds treated with culture filtrates of EB19, EB18, EB16 and EB3 significantly reduced the number of adult females, egg masses, eggs/ eggmass, root and soil population of *M. incognita* under

Table 2. Biochemical characterization of endophytic bacterial isolates

Isolates	Biochemical test									
	Gram staining	Cell morphology	Starch hydrolysis	KOH	Citrate utilization	Catalase test	Gelatin hydrolysis	Methyl red	Growth at 4°C	Pigment production
EB1	- ve	rod	-	+	+	+	+	+	+	-
EB2	- ve	rod	-	+	+	+	+	+	+	-
EB3	- ve	rod	-	+	+	+	+	+	+	-
EB4	- ve	rod	-	+	+	+	+	+	+	-
EB5	- ve	rod	-	+	+	+	+	+	+	-
EB6	- ve	rod	-	+	+	+	+	+	+	-
EB7	- ve	rod	-	+	+	+	+	+	+	-
EB8	- ve	rod	-	+	+	+	+	+	+	-
EB9	+ ve	rod			+	+		+	-	-
EB10	+ ve	rod			+	+		+	-	-
EB11	+ ve	rod			+	+		+	-	-
EB12	+ ve	rod			+	+		+	-	-
EB13	+ ve	rod			+	+		+	-	-
EB14	+ ve	rod			+	+		+	-	-
EB15	+ ve	rod			+	+		+	-	-
EB16	+ ve	rod			+	+		+	-	-
EB17	+ ve	rod			+	+		+	-	-
EB18	+ ve	rod			+	+		+	-	Red
EB19	- ve	rod	-	+	+	+	+	+	+	Pink

Table 3. Plant growth promotion activity of endophytic bacterial isolates in bhendi

Isolates	Germination (%)		Shoot length (cm)		Root length (cm)		Vigour index	
	Roll	Pot	Roll	Pot	Roll	Pot	Roll	Pot
EB 1	41.33^{jk}	55.33^{j}	7.67^{ij}	17.53^{jk}	15.07^{ij}	12.63^{k}	939.40^{mn}	1673.13^{mn}
EB 2	68.00^{d}	76.67^{cd}	12.90^{e}	26.80^{d}	17.97^{ef}	22.13^{de}	2098.80^{f}	3749.70^{f}
EB 3	75.67^{bc}	78.67^{bc}	15.77^{c}	28.97^{c}	20.20^{cd}	25.60^{c}	2719.73^{d}	4293.63^{d}
EB 4	54.00^{fg}	69.67^{g}	9.20^{h}	20.57^{gh}	17.17^{gh}	17.63^{gh}	1423.40^{j}	2659.77^{j}
EB 5	52.33^{gh}	66.67^{hi}	9.20^{fh}	19.63^{hi}	16.17^{hi}	16.30^{hi}	1325.87^{jk}	2395.33^{k}
EB 6	62.33^{e}	74.00^{ef}	10.60^{g}	23.70^{ef}	17.57^{fg}	19.63^{f}	1757.73^{h}	3207.87^{h}
EB 7	49.33^{h}	65.00^{i}	9.07^{h}	18.07^{ij}	16.67^{gh}	14.73^{ij}	1269.43^{k}	2131.40^{l}
EB 8	40.33^{k}	51.67^{kl}	7.40^{j}	16.13^{kl}	14.33^{jk}	11.13^{kl}	876.57^{no}	1409.00^{o}
EB 9	34.33^{lm}	49.67^{l}	5.90^{k}	15.90^{kl}	11.93^{l}	9.07^{mn}	610.23^{pq}	1240.43^{op}
EB 10	66.33^{d}	75.33^{de}	12.47^{e}	24.53^{e}	16.53^{gh}	21.67^{e}	1921.37^{g}	3477.80^{g}
EB 11	74.00^{c}	77.67^{cd}	14.43^{d}	28.07^{d}	18.90^{de}	23.77^{d}	2466.73^{e}	4028.93^{e}
EB 12	44.00^{ij}	57.00^{j}	8.57^{hi}	18.27^{ij}	16.53^{gh}	14.40^{j}	1103.87^{l}	1863.80^{m}
EB 13	31.00^{m}	41.33^{n}	5.90^{k}	14.97^{lm}	8.43^{m}	7.93^{n}	444.73^{r}	948.00^{q}
EB 14	36.67^{l}	41.33^{n}	5.67^{k}	13.87^{m}	9.80^{m}	8.30^{n}	567.73^{qr}	916.13^{q}
EB 15	34.67^{l}	45.00^{m}	5.67^{k}	15.53^{lm}	8.77^{m}	8.93^{mn}	497.77^{qr}	1103.80^{pq}
EB 16	79.00^{ab}	81.67^{ab}	18.23^{ab}	32.13^{a}	21.93^{ab}	27.33^{ab}	3175.63^{b}	4856.43^{b}
EB 17	40.33^{k}	51.33^{kl}	6.07^{k}	16.50^{kl}	12.40^{l}	10.33^{lm}	745.47^{op}	1375.93^{o}
EB 18	77.33^{bc}	79.67^{abc}	17.13^{b}	31.10^{b}	21.23^{bc}	26.20^{bc}	2967.37^{c}	4565.47^{c}
EB 19	80.33^{a}	82.67^{a}	19.27^{a}	33.37^{a}	22.87^{a}	28.70^{a}	3383.07^{a}	5127.90^{a}
Pf 1	56.67^{f}	72.33^{fg}	10.83^{f}	22.07^{fg}	17.23^{gh}	18.43^{fg}	1589.60^{i}	2929.07^{i}
Control	45.33^{i}	54.33^{jk}	9.67^{h}	15.60^{lm}	13.27^{l}	11.47^{kl}	1041.03^{lm}	1469.10^{no}
S Ed	1.71	1.80	0.55	0.91	0.68	0.83	67.03	115.57
CD (P=0.01)	4.56	4.62	1.31	2.22	1.68	2.16	162.23	286.29

In column means followed by a common letter are not significant at 1 per cent level by DMRT

pot culture conditions (Table 4). The lowest root gall index (1.00) was registered both in EB16 and EB18 isolates and it was followed by EB19 and EB3 (1.33) compared to untreated control (4.67). Cannayane and Rajendran (2001) who reported that gall index, egg mass production, eggs/egg mass and soil nematode

Table 4. Effect of culture filtrate of endophytic bacterial isolates on *M. incognita* reproductive potential in bhendi (Mean of three replications)

Isolates	No. of females (5g root)	No. egg masses (5g root)	No. of eggs / egg mass	Root knot index	Soil population (250cc soil)	Root population (5g root)
EB 2	37.67^{f}(61.30)	29.67^{g}(44.72)	179.00^{f}(34.75)	2.67	115.33^{g}(55.87)	72.67^{g}(44.95)
EB 3	24.67^{d}(74.66)	17.67^{d}(67.08)	124.67^{c}(54.56)	1.33	92.67^{d}(64.54)	50.00^{d}(62.12)
EB 6	50.67^{h}(47.95)	38.67^{h}(27.95)	222.33^{h}(18.96)	3.00	137.00^{i}(47.58)	86.67^{i}(34.34)
EB 10	44.33^{g}(54.45)	33.33^{g}(37.89)	196.33^{g}(28.43)	3.00	121.67^{h}(53.44)	79.67^{h}(39.65)
EB 11	35.33^{f}(63.70)	25.67^{f}(52.17)	147.67^{e}(46.17)	2.33	108.67^{f}(58.42)	66.33^{f}(49.75)
EB 16	10.33^{a}(89.38)	5.33^{a}(90.06)	97.67^{a}(64.40)	1.00	65.67^{a}(74.87)	32.67^{a}(75.25)
EB 18	14.67^{b}(84.93)	9.33^{b}(82.61)	106.33^{a}(61.24)	1.00	76.33^{b}(70.79)	38.67^{b}(70.71)
EB 19	19.33^{c}(80.14)	13.67^{c}(74.53)	115.67^{b}(57.84)	1.33	84.33^{c}(67.73)	44.33^{c}(66.41)
Pf 1	30.67^{e}(68.49)	21.67^{e}(59.63)	136.00^{d}(50.43)	1.67	101.00^{e}(61.35)	60.33^{e}(54.29)
Control	97.33^{i}	53.67^{i}	274.33^{i}	4.67	261.33^{j}	132.00^{j}
S Ed	1.87	1.86	4.18	-	2.57	2.41
CD (P = 0.05)	3.89	3.90	8.72	-	5.37	5.04

In column means followed by a different letters are significantly from each other at 5 per cent level by DMRT
Figures in parentheses are per cent reduction over control

population were significantly reduced in plants treated with culture filtrates of *B. subtilis, B. cereus* and *Arthrobotrys cladodes*. Siddiqui and Shaukat (2003) also found that aqueous cell suspension of *P. fluorescens* strains CHA0 or CHA0/pME3424 at various inoculum levels 10^7, 10^8, 10^9 cfu/g significantly reduced root knot development in tomato under glasshouse conditions. Sikora (1988) reported that *B. subtilis* reduced *Meloidogyne* spp. and *R. reniformis* reproduction and galling on cotton, tomato and peanut. Endophytic *B. subtilis* strains EPb5, 22, 31 and EPC 16 were effective against *M. incognita*, *Radopholus similis, Pratylenchus coffeae* and *Helicotylenchus multicintus* in banana (Jonathan and Umamaheswari 2006).

The mechanisms by which reduction on nematode population might be due to premature egg hatching and reduction in viability and mortality of juveniles induced by secondary metabolites such as 2,4 Diacetyl phloroglucinol and lytic enzymes (Dunne *et al*., 1998) antibiotics and hydrogen cyanide (Ahl *et al*., 1986) produced by *Pseudomonas* spp. and non cellular extract and toxic metabolites like bacillopeptidase, subtillin E and â lactamase from *Bacillus* spp. *Methylobacterium* spp. produced indole acetic acid able to utilize ACC deaminase as sole carbon source, which regulates ethylene production by metabolizing ACC into Ü ketobutyrate and ammonia (Glick *et al*., 1998) and this ammonia is toxic to nematodes. The bacteria also cause nematode suppr ession and growth promotion of secondary metabolites and competition for space and nutrients (Kloepper *et al*., 1991). Hence, the promising endophytic bacterial isolates obtained from the present study may be commercially formulated as effective biocontrol agents for the management of *M. incognita* in bhendi.

REFERENCES

Aalten, P. M. and Gowen, S. R. 1998. Entomopathogenic nematodes and fluorescent Pseudomonas rhizosphere bacteria inhibited *Radopholus similis* invasion in banana roots. *Pest and Diseases*, **2:** 14-25.

Abdul Baki, A. A. and Anderson, J. D. 1973. Vigour determination in soybean seed by multiple criteria. *Crop Science*, **31:** 630-633.

Adhikari, T. B., Joseph, C. M., Yang, G., Phillips, D. A. and Nelson, L. M. 2001. Evaluation of bacteria isolated from rice for plant growth promotion and biological control of seedling disease of rice. *Canadian Journal Microbiology*, **47:** 916-924.

Ahl, P., Voisarad, C. and Defago, G. 1986. Iron siderophores, cyanic acid and antibiotics involved in suppression of *Thielaviopsis basicola* by *Pseudomonas fluorescens* strain. *Journal of Phytopathology*, **116:** 121-134.

Aken, B.V., Peres, C. M., Doty, S. L., Yoon, J. M. and Schnoor, J. L. 2004. *Methylobacterium populi* sp. nov., a novel aerobic, pink-pigmented, facultatively methylotrophic, methane-utilizing bacterium isolated from poplar trees (*Populus deltoids* x *nigra* DN34). *International Journal of Systematic and Evolutionary Microbiology*, **54:**1191-1196.

Bent, E. and Chanway, C. P. 1998. The growth-promoting effects of a bacterial endophyte on loegepole pine are partially inhibited by the presence of other Rhizobacteria. *Canadian Journal Microbiology*, **44:** 980-988.

Cannayane, C. and Rajendran, G. 2001. Management of *Meloidogyne incognita* by bacterial and fungal culture filtrates on bhendi, *Abelmoschus esculentus* L. *Current Nematology*, **12:** 85-89.

Cobb, N. A. 1918. Estimating the nematode population of soil. *United States Department of Agriculture, Circular No*. 1, 48 **PP**.

Dunne, C., Moenne-Loccoz, Y., McCarthy, J., Higgins, P., Powell, J., Dowing, D. N. and Gara, F. O. 1998. Combining proteolytic and phloroglucinol-producing bacteria for improved biocontrol of *Pythium*-mediated damping off of sugarbeet. *Plant Pathology*, **47:** 299-307.

Feng, Y., Shen, D. and Song, W. 2006. Rice endophyte Pantoea agglomerans YS19 promotes host plant growth and affects allocations of host photosynthates. *Journal of Applied Microbiology*, **100:** 938-945.

Glick, B. R., Penrose, D. M and Li, J. 1998. A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. *Journal Theoretical Biology*, **190:** 63-68.

Gomez, K. A. and Gomez, A. A.1984. Statistical Procedure for Agricultural Research John Wiley and Sons, New York.

Hallmann, J., Quadt- Hallmannn, A., Mahafee, W. F. and Kloepper, J. W. 1997. Bacterial endophytes in agriculture crops. *Canadian Journal of Microbiology*, **43:** 895-914.

Harish, S. 2005. Molecular biology and diagnosis of Banana buncy top virus and its management through induced systemic resistance. *Ph. D., Thesis*, TNAU, Coimbatore, India. 12 **P.**

Headle, C. M., Briton, B. D. and Davis, R. M. 1989. Influence of *Glomus intradices* and soil phosphorus on *Meloidogyne incognita* infecting *Cucumis melo*. *Journal of Nematology*, **21:** 69-73.

Holland, M. A. 1997. CAM's razor applied to hormonology: Are cytokinnins produced by plants. *Plant Physiology*, **115:** 865-868.

Hung, P. Q. and Annapurna, K. 2004. Isolation and characterization of Endophytic bacteria in soybean (*Glycine* sp.) *Omon Rice*, **12:** 92-101.

ISTA, 1993. Proceedings of the International seed testing Association, International Rules for seed testing. *Seed Science and Technology*, **21:** 25-30.

Jain, R. K., Mathur, K. N. and Singh, R. V. 2007. Estimation of losses due to plant parasitic nematodes on different crops in India. *Indian Journal Nematology*, **37:** 219-221.

Jonathan, E. I. and Umamaheswari, R. 2006. Biomanage ment of nematodes infesting banana by bacterial endophytes (*Bacillus subtilis*). *Indian Journal Nematology*, **36:** 30-233.

Kloepper. J. W., Zablotowiz, R. M., Tippimg, E. M. and Lifshitz, R. 1991. Plant growth promotion mediated by bacterial rhizosphere colonizers. In: The rhizosphere and plant growth. D.L. Keister and P.B. Cregan. (eds.). Kluwer Academic Publishers, Dordrecht, the Netherlands. 315-326 **PP**.

Madhaiyan, M. 2003. Molecular aspects, diversity and plant interaction of facultative methylotrophs ocuuring in tropics plants. *Ph.D.*, Thesis, TNAU, Coimbatore, India

Mathiyazhagan, S. 2003. Studies on the soil borne diseases of *Phyllanthus amarus* (Schum and Thonn) and their management. M.Sc., (Ag.) Thesis, TNAU, Coimbatore, India.129 **P**.

McInroy, J. A. and Kloepper, J. W. 1995. Survey of indigenous bacterial endophytes from cotton and sweet corn. *Plant Soil*, **173:** 337-342.

Rangaswami, G. and Bagayaraj, D. J. 1993. Microbial Biotechnology. *In: Agricultural Microbiology*. Prentice Hall of India Pvt. Ltd., New Delhi. 389-405 **PP**.

Samuthiravalli, M. 2006. Management of root knot nematode, *Meloidogyne incognita* Kofoid and White, 1919) Chitwood, 1949 and wilt disease *Fusarium oxysporum* f.sp. *lycopersici* (Sacc.) Snyder and Hansen complex on tomato by endophytic bacteria. M.Sc., (Ag.) Thesis, TNAU, Coimbatore, India. 103 **P.**

Schindler, A. F. 1961. A simple substitute for a Baermann funnel. *Plant Disease Reporter,* **45:** 747-748.

Shishido, M., Breuil, C. and Chenway, C. P.1999. Endophytic colonization of spruce by plant growth-promoting rhizobacteria. *FEMS Microbiology and Ecology,* **29:** 184-188.

Siddiqui, I. A. and Shaukat, S. S. 2003. Supprpession of root knot disease by *Pseudomonas fluorescens* CHA0 in tomato: importance of bacterial secondary metabolite, 2,4-diacetylphologlucinol. *Soil Biology and Biochemistry*, **35:** 1615-1623.

Sikora, R. A. 1988. Interrelationship between plant health promoting bacteria, plant parasitic nematodes and soil microorganisms. Med. Fac. Landbouww. Rijks Univ. Gent 53 (2b), 867-878.

Sturz, A. V., Christie, H. R. and Nowak, J. 2000. Bacterial endophytes: Potential role in developing sustainable systems of crop production. *Critical Reviews in Plant Sciences*, **19:** 1-30.

P. Vetrivelkalai[1*], M. Sivakumar[2] and E. I. Jonathan[1]

[1]Centre for Plant Protection Studies, [2]Department of Nematology, Tamil Nadu Agricultural University, Coimbatore, 641 003, Tamil Nadu, India. *E-mail: vetrinem@gmail.com

Received: October 17, 2009; Revised: February 12, 2010; Accepted: March 10, 2010