The Bioscan 11(1): 205-209, 2016
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

FIELD EVALUATION OF PROMISING BACTERIAL ANTAGONIST *BACILLUS* SPP. AGAINST *MELOIDOGYNE INCOGNITA* IN *GERBERA JAMESONII*

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KEYWORDS
Bacterial Antagonist
Bacillus Subtilis
Meloidogyne Incognita
Gerbera Jamesonii
Bacillus
Amyloliquefaciens

Received on :
07.10.2015

Accepted on :
17.02.2016

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ABSTRACT
The present research deals with the study of antagonistic effect of *Bacillus* spp. against *Meloidogyne incognita* infesting *Gerbera jamesonii*. Two bacterial strains of *Bacillus Subtilis* were isolated from rhizosphere region of gerbera and the antagonistic potential against nematodes was observed by hatching and mortality studies. Field studies were conducted to assess the bioefficacy of liquid and talc formulations of *Bacillus* spp. *viz*., *B. subtilis* strain BG42, BG37 and B. *amyloliquefaciens* strain B4. Application of these three strains at 1%/m^2 significantly increased the gerbera growth and quality parameters and decreased the nematode population compared to control. Among the treatments, Soil drenching of liquid formulation of *B. subtilis* strain BG42 gave maximum reduction of juveniles per 250g soil (71.92%), number of adult females/5g root (66.68%), number of eggmasses/ 5g root (76.35%), gall index (1.50) and increased flower yield/m^2 (129.89%). Soil drenching of liquid formulation of *B. subtilis* strain BG37 was next line in efficacy. Further liquid formulation of *B. subtilis* strain BG42 had a positive influence on gerbera quality parameters *viz*., flower diameter (9.77 cm), colour of the flower, length of flower stalk (58.48 cm) and vase life (11.17 days). This study suggests that *B. subtilis* might be potential biological control agents of gerbera plants.

INTRODUCTION

Gerbera (*Gerbera jamesonii* Hook) is an important commercial cut flower grown throughout the world in a wide range of climatic conditions. It stands sixth in the International market and second in domestic market. According to the global trends in floriculture, gerbera occupies the fourth place among cut flowers (Choudhary and Prasad, 2000). Cultivation of gerbera on commercial scale for domestic and export purpose is relatively recent in India. These crops are being grown under hi-tech and controlled environmental conditions, mainly in and around cities like Bangalore, Callicut, Coimbatore, The Nilgiris, Hosur, Delhi, Nasik, Pune, Srinagar, besides few other places in Karnataka, Tamil Nadu, Maharshtra, Uttar Pradesh, Punjab, *etc*.

Although a multitude of plant parasitic nematodes are found associated with gerbera elsewhere in the world (Lamberti *et al*., 1987). Plant parasitic nematodes, *viz*. *Meloidogyne incognita*, *Helicotylenchus multicinctus, Pratylenchus coffeae, Tylenchorhynchus* spp. and *Rotylenchulus reniformis* have found to be associated with gerbera growing areas of Tamil Nadu (Manju and Subramanian, 2015). But root knot nematode, *M. incognita* is one of the serious limiting factors in commercial cultivation of gerbera grown under polyhouse conditions. In India, yield losses due to *M. incognita* in gerbera were estimated to the tune of 31.1 per cent (Nagesh and Parvatha Reddy, 2000). The commercial floricultural industry is growing almost daily. Cut flowers have proved to be high value cash crops grown commercially where increase in production has become a necessity.

No doubt, chemical control of root knot nematode is the most efficient method. However, due to the negative effects associated with pesticides on human health and environment, biological based strategies are increasingly becoming popular alternatives (Kyalo *et al*., 2007). The use of antagonistic rhizobacteria has been shown to offer an eco-friendly solution to management of plant diseases (Anu Rajan *et al*., 2013; Karanja *et al*., 2007). One such organism is *Bacillus subtilis* Cohn., a biological agent, that has been used in the control of diseases caused by *Pythium, Rhizoctonia, Gaeumannomyces, Sclerotinia, Fusarium* and nematodes (Oyekanmi *et al*., 2007) and it also promotes plant growth and health (Karanja *et al*., 2007; Siddiqui *et al*., 2007). Plant growth promoting rhizobacteria (PGPR) including *Bacillus subtilis*, enhances seed emergence, root colonization and stimulation of plant growth, mineral nutrient uptake, water utilization and disease suppression. There exists an enormous potential in the biocontrol of plant pathogens through manipulation of crop rhizosphere using PGPR (Siddiqui *et al*., 2001). The potential of this biocontrol bacterium has been reported to be effective against plant pathogenic nematodes (Siddiqui and Mahmood, 1999; Siddiqui and Ehteshamul, 2001) and other soil borne pathogens (Asaka and Shoda, 1996; Edgecomb and Manker, 2006; Muduli *et al*., 2013).

Hayder Munshid *et al*. (2013) reported that soil application of

both *P. fluorescens* and *B. subtilis* alone or in combination was able to reduce the nematode population and improve the onion growth parameters in terms of shoot length, root length, shoot fresh and dry weight, root fresh weight. *B. subtilis* 1% W.P. significantly reduced the disease complex of bell pepper (*Capsicum annum* L.) caused by *M. incognita* and *Ralstonia solanacearum*. This bio-pesticide also significantly increased plant growth parameters (shoot and root length) and also bell pepper yield to the tune of 66% under field conditions (Manoj Kumar *et al.*, 2013). The objective of this study is to evaluate the bioefficacy of liquid and talc formulations of *Bacillus* spp. on *M. incognita* infesting gerbera under field conditions.

MATERIALS AND METHODS

Isolation of *Bacillus* spp.

Ten *Bacillus* spp. isolates were obtained from rhizosphere region of gerbera grown in different districts of Tamil Nadu comprising of Coimbatore, The Nilgiris, Salem and Krishnagiri. Bioefficacy of *Bacillus* spp. isolates was assayed against root knot nematode by hatching and mortality tests (Shahnaz Dawar *et al.*, 2008). Among the ten isolates screened, the highest inhibition in egg hatching and highest per cent mortality of *M. incognita* juveniles was observed in *Bacillus* isolate BG42 followed by BG37. The partial 16SrDNA sequences of the isolated strains BG37 and BG42 showed 99% per cent similarity to *B. subtilis* isolate and were deposited in the GenBank under accession numbers of KM454178 and KM588210 respectively (Manju and Subramanian, 2015). Existing strain *B. amyloliquefaciens* B4 reported to be effective against plant pathogens was obtained from the Center for Plant Protection studies, Department of plant pathology, Tamil Nadu Agricultural University, Coimbatore.

Development of formulations of the antagonistic *Bacillus* spp. for field application

Bacillus spp. liquid formulation

The *Bacillus* strains were grown in the nutrient broth with constant shaking at 150 rpm for 48 h at room temperature (28 ± 2^{o}C). The bacterial cells were harvested and centrifuged at 6000 rpm for 15 min and the cells were resuspended in phosphate buffer (0.01M, pH 7.0). The concentration was adjusted using a spectrophotometer to approximately 10^{8} cfu/ml (OD_{595} = 0.3) and used as bacterial inoculums (Thompson, 1996). These strains were kept at -80^{o}C in 44 per cent glycerol and cells from stock were first grown in NA broth. Inoculum was prepared by transferring one loopful from each culture to 100 ml of NA broth in a 250 ml conical flask and incubated at room temperature (28 ± 2^{o}C) on a shaker at 150 rpm for 48 hr.

Bacillus spp. talc formulation

A loopful of *Bacillus* isolates were inoculated into nutrient broth separately and incubated in a rotary shaker at 150 rpm for 48 h at room temperature (28 + 2^{o} C). After 48 hr of incubation, the broth containing 9 x 10^{8} cfu/ml was used for the preparation of talc based formulations. To 400 ml of bacterial suspension, one kg of the purified talc powder (sterilized at 105^{o} C for 12 h), 15 g of calcium carbonate (to adjust the pH to neutral) and 10 g of carboxy methyl cellulose (CMC) as an adhesive were mixed under aseptic conditions following the method described by Nandhakumar *et al.* (2001). The product was shade dried over night to reduce the moisture content to less than 20 per cent and then packed in polypropylene bags and sealed. At the time of application, the population of bacteria in talc formulation was assessed as 2.5 to 3 x 10^{8} cfu/g.

Field experiment

The experiment was conducted at Tamil Nadu during September 2014 to February 2015 at two sites (The Nilgiris and Krishnagiri district) in gerbera polyhouses with severe infestated with root knot nematode. The spacing adopted for gerbera was 40 cm X 30 cm, accommodating 8 plants/ m^{2}. The crop was maintained by applying recommended dosages of fertilizers and plant protection chemicals. The experiment was designed in a randomized block, three replications and eight treatments. The treatments were, T1- Soil drenching of liquid formulation of *B. subtilis* BG42 @ 1%/m^{2} ; T2: Soil application of talc formulation of *B. subtilis* BG42 @ 1%/m^{2}; T3 : Soil drenching of liquid formulation of *B. subtilis* BG37 @ 1%/m^{2}; T4 : Soil application of talc formulation of *B. subtilis* BG37 @ 1%/m^{2}; T5 : Soil drenching of liquid formulation of *B. amyloliquefaciens* B4 @ 1%/m^{2}; T6 : Soil application of talc formulation of *B. amyloliquefaciens* B4 @ 1%/m^{2}; T7- Soil application of Carbofuran (3.3g/m^{2}) and T8-Untreated control.

Assessment quality parameters of gerbera

Observations on quality parameters *viz.*, flower diameter, colour of the flower, length of flower stalk and vase life were recorded. Diameter of flower was recorded at full bloom stage. The readings were taken from each flower and then average was worked out and expressed in centimeters. The colour of the disc floret was noted by visual observation and rated 1-3 scale as 1 - Very good; 2 - Good; 3 - Satisfactory. The length of flower stalk was measured from the point just below the flower head up to point of origin of stem and then average of stem in each treatment was worked out and expressed in centimeters. The vase life was expressed in terms of days from the date of harvest to final observation. To determine the gerbera flower vase life, the flowers soon after harvesting were kept in distilled water. Later these flowers stalks were cut to have uniform stalk length. After that flowers were kept individually in flask containing 250 ml of distilled water. Flowers were observed daily and discarded when they were found to be unfit for containing in vase. Number of flowers per m^{2} also recorded (Nagesh and Parvatha Reddy, 2005).

Assessment of nematode parameters

Nematode incidence in terms of number of adult females per 5 g root, egg masses per 5 g root, final soil nematode population per 250 g of soil and gall index were recorded. Five gram of roots randomly taken from each plant was stained using acid fuchsin-lactophenol and number of females per gram of root observed. To count the egg masses, it were stained by dipping the roots for 15 minutes in an aqueous solution of phloxine B (0.15 gm /L water) and then washed with running tap water to remove excess stain (Holbrook *et al.*, 1983). Soil from pots were thoroughly mixed and J2 population density was assessed from 250 g of sub samples by Cobb's decanting and sieving technique followed by modified Baermann's funnel technique (Southey, 1986). Root knot index was recorded on 1-5 scale

on the basis of number of galls per root system (Taylor and Sasser, 1978) and graded from 0 to 5 (0 = no galls, 1 = 1–2 galls; 2 = 3–10 galls, 3 = 11–30 galls, 4 = 31–100 galls, and 5 = >100 galls).

Statistical analysis

The pooled data of both the experiments were statistically analyzed and critical differences determined (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

In the present study, there was a significant increase in gerbera quality parameters and decrease in nematode population observed in all the treatments compared to control. Amongst treatments, Soil drenching of liquid formulation of *B. subtilis* strain BG42 @ 1%/m^2 recorded the maximum flower diameter (9.77 cm), stalk length (58.48 cm), flower colour and vase life (11.17 days) followed by Soil drenching of liquid formulation of *B. subtilis* BG37 @ 1%/m^2 which showed the stalk length of 56.48 cm, diameter of 9.3 cm and vase life of 10.33 days. Untreated control recorded the flower stalk length of 37.55 cm, diameter of 6.35 cm and vase life of 6.33 days (Table 1). The present findings are in accordance with the findings of Ardhanareeswaran (2012) who has observed increase in plant growth parameters *viz*., shoot length, root length, shoot weight, root weight and number of leaves, quality parameters such as flower stalk length, flower diameter, colour, vase life and the reduction in the population of *M. incognita* in gerbera and carnation due to combined application of liquid formulation of *P. fluorescens* strain Pfbv 22 + *B. subtilis* Bbv 57 at 1000ml/ha.

Significant reduction in number of adult females (12.83 5g $^{-1}$ root) was observed in gerbera plants treated with liquid formulation of *B. subtilis* strain BG42 (Table 2). It showed 66.68 per cent decrease over control. This was followed by liquid formulation of *B. subtilis* strain BG37 (14.17 5g $^{-1}$ root), which attributed for 63.19 per cent decrease over control. The plants treated with carbofuran resulted in 27.17 5g $^{-1}$ root which showed 29.43 per cent decrease over control. The highest number of adult females (38.50) was recorded with the control. Bacterial antibiotics and other toxic compound present in metabolites as well as direct interaction might be responsible for the *M. incognita* juvenile immobility, production of metabolites by rhizosphere bacteria causes lysis of nematode eggs and affects vitally of second stage juveniles of root knot nematode (Becker, 1988). *B. subtilis* have been shown to effectively control plants pathogens (Sivasakthi *et al*., 2014). There are few reports on the biocontrol of *B. subtilis* on root knot nematodes (Siddiqui and Mahmood, 1999; Siddiqui and Ehteshamul-Haque, 2001; Ali *et al*., 2002; Xia *et al*., 2011).

The number of egg masses (7.33 5g $^{-1}$ root) was found to be significantly reduced in liquid formulation of *B. subtilis* strain BG42 treated gerbera plants, which accounted for 76.35 per cent decrease over control. This was followed by liquid formulation of *B. subtilis* strain BG37 and carbofuran which recorded 9.17 5g $^{-1}$ root and 21.83 5g $^{-1}$ root which resulted in 68.71 and 29.58 per cent reduction respectively. The highest number of egg masses was recorded in the untreated control (31.00 g $^{-1}$ root). The highest reduction of *M. incognita* juveniles in soil was observed with liquid formulation of *B. subtilis* strain BG42 treated plants which recorded 71.92 per cent decrease over control. This was followed by liquid formulation of *B. subtilis* strain BG37 which recorded 67.54 per cent decrease over control. The untreated control plants recorded the highest population of 616.67. Similar results were reported by (Getha *et al*., 2005) who observed that *B. subtilis* were effective antagonists against *F. oxysporum*.

Soil drenching of liquid formulation of *B. subtilis* strain BG42 resulted in a significant increase in the flower yield which recorded 100nos/m^2 with 129.83 per cent increase over the control. This was followed by a yield of 93nos/m^2 in the liquid formulation of *B. subtilis* strain BG37 treated plants. Flower yield recorded in control plots were 43.5nos/m^2. Similar result was recorded in the studies conducted by Tamalika Sarangi (2014) who has reported that talc formulation of *B. weihenstephanensis* at 5kg/ha enhanced the plant growth and yield of tomato and reduced nematode fungal disease complex

Table 1: Efficacy of *Bacillus* spp. formulations on quality parameters of gerbera infested with root knot nematode under polyhouse conditions (Pooled data from two experiments)

Treatment	Stalk length (cm)	Per cent increase over control	Flower diameter (cm)	Per cent increase over control	Flower Colour and Visual grade «	Vase life(No. of days)	Per cent increase over control
Soil drenching of liquid formulation of *B. subtilis* strain BG42 @ 1%/m^2	58.48	55.74	9.77	53.86	1	11.17	76.46
Soil application of talc formulation of *B. subtilis* strain BG42 @ 1%/m^2	56.64	50.84	8.88	39.84	2	9.67	52.76
Soil drenching of liquid formulation of *B. subtilis* strain BG37 @ 1%/m^2	56.48	50.41	9.30	46.46	1.5	10.33	63.19
Soil application of talc formulation of *B. subtilis* strain BG37 @ 1%/m^2	54.50	45.14	8.41	32.44	2	8.67	36.97
Soil drenching of liquid formulation of *B. amyloliquefaciens* strain B4 @ 1%/m^2	49.31	31.32	7.72	21.57	2	8.00	26.38
Soil application of talc formulation of *B. amyloliquefaciens* strain B4 @ 1%/m^2	49.32	31.34	7.23	13.86	2	7.50	18.48
Soil application of Carbofuran @ 3.3g/m^2	43.43	15.66	7.24	14.02	2	7.83	23.70
Untreated control	37.55		6.35		3	6.33	
CD(p = 0.05)	4.92		0.48		-	1.45	

★ Colour and visual grade: 1 – Very good; 2 – Good and 3 – Satisfactory; Mean of 3 replications

Table 2: Efficacy of *Bacillus* spp. formulations on root knot nematode population in gerbera under polyhouse conditions (Pooled data from two experiments)

Treatment	No. of females/g root	Per cent decrease over control	No. of egg masses/ 5g root	Per cent decrease over control	Nematode population /250g of soil	Per cent decrease over control	Root knot index	Flower Yield/m^2	Per cent increase over control
Soil drenching of liquid formulation of *B. subtilis* strain BG42 @ 1%/m^2	12.83	66.68	7.33	76.35	173.17	71.92	1.50	100.00	129.89
Soil application of talc formulation of *B. subtilis* strain BG42 @ 1%/m^2	15.33	60.18	10.50	66.13	187.00	69.68	2.17	87.50	101.15
Soil drenching of liquid formulation of *B. subtilis* strain BG37 @ 1%/m^2	14.17	63.19	9.17	68.71	200.17	67.54	1.83	93.00	113.79
Soil application of talc formulation of *B. subtilis* strain BG37 @ 1%/m^2	17.50	54.55	12.50	70.42	222.67	63.89	2.67	81.50	87.36
Soil drenching of liquid formulation of *B. amyloliquefaciens* strain B4 @ 1%/m^2	24.67	35.92	19.83	36.03	329.83	46.51	3.00	73.00	67.82
Soil application of talc formulation of *B. amyloliquefaciens* strain B4 @ 1%/m^2	27.00	29.87	21.50	30.65	343.83	44.24	3.33	66.50	52.87
Soil application of Carbofuran @ 3.3g/m^2	27.17	29.43	21.83	29.58	363.33	41.08	3.33	58.00	33.33
Untreated control	38.5		31.00		616.67		4.50	43.50	
CD(p = 0.05)	3.61		3.38		7.95		-	2.53	

Mean of 3 replications

of tomato. The yield increase in the field experiment treated with liquid formulation of *B. subtilis* strain BG42 was significantly higher than the untreated control.

The lowest gall index (1.5) was observed in the liquid formulation of *B. subtilis* strain BG42 treated plants whereas the highest gall index of 4.5 was recorded in the untreated control. The liquid formulation of *B. subtilis* strain BG37, talc formulation of *B. subtilis* strain BG42, talc formulation of *B. subtilis* strain BG37, liquid formulation of *B. amyloliquefaciens* strain B4, talc formulation of *B. amyloliquefaciens* strain B4 and carbofuran recorded the next best in reducing the gall index.

Siddiqui (2000) suggested that rhizobacteria and *B. subtilis* not only enhance plant growth but also suppress root knot infection and nematode density in the soil. The reduction of plant parasitic nematodes associated with *B. subtilis* may be attributed to diverse mechanisms which involve phytohormones production, mineral solubilisation, reduction of the activity of egg hatching factors, alteration of root exudates and inhibition of nematode penetration into the roots as well as reducing galling (Karanja *et al.,* 2007). The results of these field experiments clearly demonstrate the potential use of *B. subtilis* BG42 in management of the root knot nematode.

ACKNOWLEDGEMENT

The financial assistance provided by Department of Science and Technology (DST), Government of India, New Delhi, in the form of INSPIRE Fellowship to carry out this research work is greatly acknowledged.

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