



Effect of *Bacillus* spp. on Gerbera plant growth and control of *Meloidogyne incognita*

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Abstract: A greenhouse experiment was conducted to evaluate the efficacy of *Bacillus* spp. against *Meloidogyne incognita* (Kofoid and White) Chitwood infesting gerbera plants (*Var.* Valletta). Investigations were undertaken in pots filled with 5 kg of sterilized potting mixture consisting of red soil:sand:FYM (2:1:1 v/v) to assess the effect of liquid and talc formulations of *Bacillus* spp. viz., *B. subtilis* strain BG42, *B. subtilis* strain BG37 and *B. amyloliquefaciens* strain B4. The results indicated that Soil drenching of liquid formulation of *B. subtilis* strain BG42 @ 1%/m² (1x10⁸ colony forming units/g) gave maximum reduction of juveniles per 250g soil (67.40%), number of adult females/5g root (73.46%), number of eggmass/5g root (69.44%), gall index (1.67) and increased flower yield/m² (127.03%). Soil drenching of liquid formulation of *B. subtilis* strain BG 37 were next in line in efficacy. Further liquid formulation of *B. subtilis* strain BG42 had a positive influence on growth parameters viz., shoot length, root length, shoot and root weight, number of leaves / plant and flower yield/m² and quality parameters viz., flower diameter, colour of the flower, length of flower stalk and vase life . The endophytic colonization potential of the *Bacillus* spp. introduced into the soil was confirmed by re-isolating them from gerbera roots.

Keywords: *Bacillus amyloliquefaciens*, *Bacillus subtilis*, Biocontrol, *Gerbera jamesonii*, *Meloidogyne incognita*

INTRODUCTION

Gerbera (*Gerbera jamesonii* Hook) belongs to the Asteraceae family, which is a perennial Mediterranean plant. Gerbera popularly known as Transvaal daisy, is one of the ten most popular commercial cut flowers in the world and according to the global trends in floriculture, it occupies the fourth place among cut flowers (Choudhary and Prasad, 2000). The tremendous variability in gerbera with reference to flower colour, shape and size makes it more useful for cut flowers, bouquet, decoration in marriages and landscaping in gardening (Aswath and Surway, 2004). It is inconsiderable demand in both domestic and export markets.

Of the several limiting factors that cause serious concern to commercial production, plant parasitic nematodes are most important. Although a multitude of plant parasitic nematodes are found associated with carnation and gerbera elsewhere in the world (Lamberti *et al.*, 1987), root knot nematodes belonging to *Meloidogyne* spp. are predominant in India (Nagesh and Parvath Reddy, 2001). Exotic varieties of these plants from Europe suffered 40-60% mortality due to Root knot nematode infection and its interaction with other soil-borne pathogens (Nagesh and Parvath Reddy, 1996). In India, yield losses due to *M. incognita* in carnation and gerbera were estimated to the tune of 26.6 and 31.1 per cent, respectively (Nagesh and Parvath Reddy, 2000).

Several control strategies, such as host plant resistance,

rotation with non-hosts, sanitation and avoidance, destruction of residual crop roots, and judicious use of nematicides, have been reported to effectively control root knot nematodes (Whitehead, 1998). Due to environmental concerns and increased regulations on use of chemical fumigants, more management strategies for management of root knot nematodes investigated (Nico *et al.*, 2004). Biological control using microbial antagonists is one potential alternative to chemical nematicides.

Rhizosphere microorganism includes some bacteria and fungi may provide defense against pathogen attack (Weller 1988). The rhizoplane and rhizosphere are colonized or otherwise occupied by many microorganisms. Plant growth promoting bacteria produce plant growth promoting substances and antibiotics. They are capable of providing substantial protection against nematode diseases (Siddiqui and Mahmood 1999).

Among the plant growth-promoting rhizobacteria (PGPR), *Pseudomonas* and *Bacillus* are the genera most commonly described as having PGPR but many other taxa also contain PGPR (Barea *et al.*, 2005).

Several *Bacillus* species such as *B. cereus*, *B. subtilis*, *B. nematocida*, *B. firmus*, *B. thuringiensis* and *B. megaterium* (Oka *et al.*, 1993; Krebs *et al.*, 1998; Siddiqui, 2002; Wei *et al.*, 2003; Niu *et al.*, 2007; Karanja *et al.*, 2007; Mendoza *et al.*, 2008; Salehi jousani *et al.*, 2008; Terefe *et al.*, 2009; Huang *et al.*, 2009; Xia *et al.*, 2011; Khalil *et al.*, 2012) have been shown to effectively control *Meloidogyne* species.

Now-a-days the use of bioagents (*Bacillus spp.*) are considered as one of the alternative approaches for the management of nematodes as it is safe to environment, no health hazards to human beings, economic and easily available to farmers in comparison to chemicals. Therefore, an attempt was made to evaluate the talc and liquid formation of *Bacillus spp.* as soil application and soil drenching against *M. incognita* in gerbera.

MATERIALS AND METHODS

Greenhouse experiment was conducted at the Department of Nematology, Tamil Nadu Agricultural University, Coimbatore during 2014-2015 with the following treatments: T1- Soil drenching of liquid formulation of *B. subtilis* BG42 @ 1%/m²; T2: Soil application of talc formulation of *B. subtilis* BG42 @ 1%/m²; T3 : Soil drenching of liquid formulation of *B. subtilis* BG37 @ 1%/m²; T4 : Soil application of talc formulation of *B. subtilis* BG37 @ 1%/m²; T5 : Soil drenching of liquid formulation of *B. amyloliquefaciens* B4 @ 1%/m²; T6 : Soil application of talc formulation of *B. amyloliquefaciens* B4 @ 1%/m²; T7- Soil application of Carbofuran (3.3g/m²) and T8-Untreated control.

Isolation of *Bacillus spp.* from gerbera Rhizosphere: Ten isolates of *Bacillus spp.* were isolated from gerbera rhizosphere from Tamil Nadu districts namely Coimbatore, The Nilgiris, Salem and Krishnagiri. Bio efficacy of *Bacillus* isolates was assayed against root knot nematode by hatching and mortality tests. Among the ten isolates screened, highest inhibition in egg hatching and highest per cent mortality of *M. incognita* juveniles was observed at *Bacillus* isolate BG42 followed by BG37. The partial 16S rDNA sequences of the isolated strains BG37 and BG42 showed 99% identity with *Bacillus subtilis* and were deposited in GenBank under accession numbers KM454178 and KM588210 respectively. Existing strain *B. amyloliquefaciens* B4 reported to be effective against plant pathogens was obtained from the Centre for Plant Protection studies, Department of plant

pathology, Tamil Nadu Agricultural University, Coimbatore.

Development of formulations of the antagonistic *Bacillus spp.* for greenhouse 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°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 10⁸ cfu/ml (OD₅₉₅ = 0.3) and used as bacterial inoculum. These strains were kept at -80°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°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°C). The broth containing 9 x 10⁸ 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°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 Vidhyasekaran and Muthamilan (1999). The product was shade dried 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 1 x 10⁸ cfu/g.

Greenhouse experiment: Earthen pots of 30×20×18 cm size filled with 5 kg sterilized pot mixture containing red soil:sand:FYM, 2:1:1 (v/v). The tissue culture derived 35 days old gerbera *var.* Valletta seedlings were planted in the above pots at the rate of one plant / pot. After establishment of the seedlings the *Bacillus spp.* were applied as scheduled. Simultaneously, second stage juveniles of *M. incognita* were inoculated into the pots at the root zone of gerbera (one juvenile per g soil) by making three holes around the plant and covered with sterilized soil. Each treatment was replicated three times and pots arranged in a completely randomized block design. Regular watering was done with tap water passed through 325 mesh sieve.

The crop was maintained by applying recommended dosages of fertilizers and plant protection chemicals. No pest or disease was recorded during the study. Emerging flowers were picked at regular intervals and flower yield recorded. The experiment was terminated 180 days after planting (DAP). The plants were carefully depotted and observation on plant growth parameters viz., shoot length, root length, shoot and root weight and number of leaves / plant quality parameters viz.,



Fig. 1. Variation in root growth of gerbera treated with different *Bacillus spp.* under controlled conditions (T1 – Liquid formulation of *B. subtilis* strain BG42; T3 - Liquid formulation of *B. subtilis* strain BG37; T7 – Untreated control).

flower diameter, colour of the flower, length of flower stalk and vase life and nematode incidence in terms of number of egg masses per 5 g root, eggs per egg mass, adult females per 5 g root, final soil nematode population and gall index 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 colours 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.

Regarding nematode incidence five gram of roots randomly taken from each plant and it 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 Cobbs decanting and sieving technique followed by modified Baermann's

funnel technique (Southey, 1986). Root knot index were 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).

To assess the root colonization of introduced *Bacillus* spp., one gram of root was taken at random from each plant, crushed in a sterile pestle and mortar, serial dilutions were made in sterile distilled water and plating was done on Nutrient agar medium. After incubation at 28±2°C for 3 days the *Bacillus* spp. colonies (*Bacillus* colonies that are opaque, wrinkled and cream in colour) were counted. Data of the present study were subjected to the analysis of variance test (ANOVA) as complete randomized design (CRD), for greenhouse experiment.

RESULTS AND DISCUSSION

Biological control through the use of antagonistic microorganisms plays a key role in modern agriculture as it is economical and ecofriendly. Such management strategy would help in preventing the pollution and also health hazards. So in the present investigation an attempt has been made to evaluate the effect of bio-agents on gerbera root knot nematode management.

Results of the study revealed that among all the treatments the liquid formulation of *B. subtilis* strain BG42 showed a significant increase in plant growth parameters expressed in terms of shoot length (44.72cm), root length (34.98cm) and shoot weight (287.45 g) (Fig 1). On the other hand a decrease in root weight (86.35g) in this treatment followed by other treatments over control (187.85g) was recorded which might be due to

Table 1. Efficacy of talc and liquid formulations of *Bacillus* isolates on *M. incognita* infestation in gerbera var. Valletta in pot culture studies.

Treatment	No. of females/5g root	No. of egg masses/5g root	Nematode population/ 250 g of soil	Root knot index	Colony forming units(per g soil) 1×10^8	Flower yield/m ²
Soil drenching of liquid formulation of <i>B. subtilis</i> strain BG42 @ 1%/m ²	14.33 (73.46)	11.00 (69.44)	186.67 (67.40)	1.67	82.32 x 10 ⁸	84.00 (127.03)
Soil application of talc formulation of <i>B. subtilis</i> strain BG42 @ 1%/m ²	16.67 (69.13)	13.67 (62.03)	199.33 (65.19)	3.00	70.64 x 10 ⁸	75.00 (102.70)
Soil drenching of liquid formulation of <i>B. subtilis</i> strain BG37 @ 1%/m ²	14.67 (72.83)	13.00 (63.89)	194.33 (66.06)	2.33	78.43 x 10 ⁸	79.00 (102.70)
Soil application of talc formulation of <i>B. subtilis</i> strain BG37 @ 1%/m ²	17.33 (67.90)	15.67 (56.47)	206.00 (64.03)	3.33	66.98 x 10 ⁸	68.50 (83.78)
Soil drenching of liquid formulation of <i>B. amyloliquefaciens</i> strain B4 @ 1%/m ²	34.00 (37.04)	28.67 (20.36)	271.67 (52.56)	3.33	62.41 x 10 ⁸	64.00 (72.97)
Soil application of talc formulation of <i>B. amyloliquefaciens</i> strain B4 @ 1%/m ²	39.33 (27.17)	29.67 (17.58)	292.00 (49.01)	4.33	57.10 x 10 ⁸	53.00 (43.24)
Soil application of Carbofuran @ 3.3g/m ²	39.67 (26.54)	29.33 (18.53)	282.00 (50.76)	3.67	--	49.50 (32.43)
Untreated control	54.00	36.00	572.67	5.00	--	37
SEd	1.32	1.03	4.07	--	--	1.51
CD(p=0.05)	2.80	2.18	8.63	--	--	3.28

Figures in parentheses are per cent decrease over control.

reduction in formation of galls which contribute for the root weight. The second best treatment was application of liquid formulation of *B. subtilis* strain BG37 followed by talc formulation of *B. subtilis* strain BG42 (Table 1).

The mechanism involved in the PGPR mediated plant growth promotion are directly related to the production of plant growth regulators viz., auxin, gibberellin and cytokinin or indirectly by stimulating nutrient uptake (Dangar and Basu, 1987; Dobbelaere *et al.*, 2003 and Dey *et al.*, 2004). Broadbent *et al.* (1977) reported the use of *B. subtilis* may also improve plant growth by suppressing non parasitic root pathogen or by the

production of biologically active substances or by unavailable mineral and organic compounds into forms available to plants. Jonathan *et al.* (2000 and 2004) observed that *P. fluorescens* and *B. subtilis* were reported to induce profuse root development in banana, tomato and betelvine and reduce the infestation of *M. incognita*. Siddiqui and Mahmood (1993) also reported that *B. subtilis* improved plant growth system of chickpea.

In respect of quality parameters significant differences were observed among the talc and liquid formulations of *Bacillus* isolates. Flower stalk length was more in the application of liquid formulation of *B. subtilis*

Table 2. Efficacy of talc and liquid formulations of *Bacillus* isolates on growth of gerbera var. Valletta infested with *M. incognita* juveniles in pot culture studies.

Treatment	Shoot length (cm)	Root length (cm)	Shoot weight (Fresh)g	Root weight (Fresh) g	Shoot weight (Dry)g	Root weight (Dry) g	No. of leaves / plant
Soil drenching of liquid formulation of <i>B. subtilis</i> strain BG42 @ 1%/m ²	44.72 (41.74)	34.48 (89.14)	287.45 (25.50)	86.35 (-54.03)	4.43 (203.42)	1.71 (-48.80)	14.67 (83.38)
Soil application of talc formulation of <i>B. subtilis</i> strain BG42 @ 1%/m ²	42.77 (35.56)	32.34 (77.40)	279.89 (22.20)	91.12 (-51.49)	3.25 (122.60)	1.93 (-42.22)	12.67 (58.38)
Soil drenching of liquid formulation of <i>B. subtilis</i> strain BG37 @ 1%/m ²	43.25 (37.08)	31.77 (74.27)	284.40 (24.17)	88.22 (-53.04)	3.78 (158.90)	1.85 (-44.61)	13.33 (66.63)
Soil application of talc formulation of <i>B. subtilis</i> strain BG37 @ 1%/m ²	41.49 (31.51)	28.28 (55.13)	275.05 (20.08)	95.42 (-49.20)	2.94 (101.37)	2.12 (-36.53)	12.00 (50)
Soil drenching of liquid formulation of <i>B. amyloliquefaciens</i> strain B4 @ 1%/m ²	38.56 (22.22)	24.30 (33.30)	265.16 (15.77)	145.78 (-22.39)	2.45 (67.81)	2.34 (-29.94)	11.33 (41.63)
Soil application of talc formulation of <i>B. amyloliquefaciens</i> strain B4 @ 1%/m ²	36.53 (15.78)	22.03 (20.84)	251.54 (9.82)	169.63 (-9.70)	2.04 (39.73)	2.72 (-18.56)	10.67 (33.38)
Soil application of Carbofuran @ 3.3g/m ²	36.26 (14.93)	23.77 (30.39)	242.81 (6.01)	175.06 (-6.81)	1.72 (17.81)	2.98 (-10.78)	10.33 (29.13)
Untreated control	31.55	18.23	229.05	187.85	1.46	3.34	8.00
SEd	3.20	2.71	3.57	5.05	0.12	0.16	0.91
CD(p=0.05)	6.79	5.75	7.60	10.72	0.34	0.30	1.93

Figures in parentheses are per cent decrease or increase over control.

Table 3. Efficacy of talc and liquid formulations of *Bacillus* isolates on quality parameters of gerbera var. Valletta infested with *M. incognita* juveniles under greenhouse conditions.

Treatment	Stalk length (cm)	Flower diameter (cm)	Colour and Visual grade*	Shelf life of flower (No. of days)
Soil drenching of liquid formulation of <i>B. subtilis</i> strain BG42 @ 1%/m ²	58.21 (48.84)	11.03 (75.08)	1	11.67 (40.10)
Soil application of talc formulation of <i>B. subtilis</i> strain BG42 @ 1%/m ²	56.63 (44.80)	9.17 (45.56)	1	10.33 (24.01)
Soil drenching of liquid formulation of <i>B. subtilis</i> strain BG37 @ 1%/m ²	57.79 (47.76)	10.03 (59.21)	1	11.00 (32.05)
Soil application of talc formulation of <i>B. subtilis</i> strain BG37 @ 1%/m ²	54.59 (39.58)	8.57 (36.03)	1	10.00 (20.05)
Soil drenching of liquid formulation of <i>B. amyloliquefaciens</i> strain B4 @ 1%/m ²	50.83 (29.97)	8.17 (29.68)	2	9.67 (16.09)
Soil application of talc formulation of <i>B. amyloliquefaciens</i> strain B4 @ 1%/m ²	47.26 (20.84)	7.80 (23.81)	2	9.33 (12.00)
Soil application of Carbofuran @ 3.3g/m ²	47.50 (21.45)	7.10 (12.70)	2	9.67 (16.09)
Untreated control	39.11	6.30	3	8.33
SEd	3.54	0.37	-	0.76
CD(p=0.05)	7.56	0.78	-	1.62

Figures in parentheses are per cent increase over control, *Colour and visual grade:1–Very good; 2–Good and 3–Satisfactory

strain BG42 which recorded the stalk length of 58.21 cm which was 48.84 per cent increase over control followed by the application of liquid formulation of *B. subtilis* strain BG37 which showed the stalk length of 57.79cm. There was a significant variation in flower size and diameter in liquid formulation of *B. subtilis* strain BG42. The flower diameter was 11.03cm which recorded 75.03 per cent increase over control, followed by the application of liquid formulation of *B. subtilis* strain BG37 which showed the flower diameter of 10.03 cm (Table 2).

Vase life parameter varied significantly among the treatments. The application of liquid formulation of *B. subtilis* strain BG42 extended its vase life upto 11.67 days. This recorded 40.10 per cent increase over control, followed by the application of liquid formulation of *B. subtilis* strain BG37 with the vase shelf life of 11 days. Untreated control flowers recorded the shelf life of 8.33 days. Manual grading on gerbera was rated as 1-3 by visual grading. The grade I was given to the flowers treated with liquid and talc formulations of *B. subtilis* strain BG42 and *B. subtilis* strain BG37. Least grade of 3 was given to control. These results are in agreement with the findings Ardhanareeswaran (2012) who has observed the reduction in the population of *M. incognita* and increase in plant growth parameters viz., shoot length, root length, shoot weight, root weight and number of leaves, quality parameters such as stalk length, flower diameter, colour and shelf life in gerbera due to combined application of talc formulation of *P. fluorescens* strain Pfbv 22 + *B. subtilis* Bbv 57 at 2.5 kg/ha.

The data present in Table 3 indicated that application of all the treatments reduced the *M. incognita* population density in soil, infestation in roots and root gall development compared to untreated control. Liquid formulation of *Bacillus subtilis* BG42 @ 1%/m² was most effective in controlling *M. incognita*. It reduced nematode population in soil by 67.40 % and was significantly superior to other treatments. This might be due to the production of toxic metabolites like lipopeptides, iturins, surfactins, bacillomycin D and fengycins, which are secondary metabolites mainly with antifungal activity (Chen *et al.*, 2006). Surfactin is capable of inhibiting the growth of plant pathogens (Singh and Cameotra, 2004). Joshi and Gardener (2006) observed that genes encoding surfactin and iturin synthesis are common to numerous antagonistic *Bacillus* spp. used as commercial biopesticide and have a pronounced capability to suppress soil borne pathogens. It was followed by Liquid formulation of *Bacillus subtilis* BG37, Talc formulation of *Bacillus subtilis* BG42, talc formulation of *Bacillus subtilis* BG37, Liquid formulation of *Bacillus subtilis* B4, talc formulation of *Bacillus subtilis* B4, carbofuran and control respectively.

A significant reduction in number of adult females was

observed in the liquid formulation of BG42 (14.33/ 5 g root). The number of egg masses is also found to be significantly reduced (11 / 5 g root) in the application of liquid formulation of *B. subtilis* strain BG42 which showed in 69.44 per cent reduction of *M. incognita* egg masses over the untreated control. Soil drenching of liquid formulation of *B. subtilis* strain BG 37 is the next best treatment in reducing the number of egg masses which accounted for 63.89 per cent reduction over untreated control.

A significant reduction of gall index was observed in liquid formulation of *B. subtilis* strain BG42 (1.67), compared to other treatments. The liquid formulation of *B. subtilis* strain BG37 treated plants is the next best treatment in reducing the gall index which recorded a value of 2.33. 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).

All the biocontrol agents applied to the soil were reisolated from gerbera roots on 180 DAP. The results revealed that among the talc and liquid formulations of *Bacillus* isolates evaluated, the root colonization was higher (82.32 x 10⁸cfu/g) in liquid formulation of *Bacillus subtilis* strain BG42 followed by Liquid formulation of *Bacillus subtilis* strain BG37 (78.43 x 10⁸cfu/g) and talc formulation of *Bacillus subtilis* strain BG42 (70.64 x 10⁸cfu/g) treated gerbera plants. Root colonization depends on the environmental factors, bacterial characteristics and plant exudates (Benizri *et al.*, 2001).

Soil drenching of liquid formulation of *B. subtilis* strain BG42 resulted in a significant increase in the flower yield which recorded 84nos/m² with 127.03 per cent increase over the control. This was followed by a yield of 79nos/m² in the liquid formulation of *B. subtilis* strain BG37 treated plants. Flower yield recorded in control plots were 37nos/m². The results revealed that among the talc and liquid formulations of *Bacillus* isolates evaluated, Liquid formulation of *Bacillus subtilis* BG42 @1%/m² was the most effective in controlling *M. incognita* on gerbera followed by Liquid formulation of *Bacillus subtilis* BG37 @1%/m² and carbofuran were least effective against *M. incognita* on gerbera.

Soil drenching of liquid formulation of *B. subtilis* strain BG42 not only controlled root knot nematode, but also promoted plant growth and flower yield. This gives them an advantage over the use of chemical nematicides against root knot nematode management.

Effective root colonization is a pre-requisite attribute for the success of liquid formulation of *B. subtilis* strain BG42 in gerbera plant growth and flower yield.

Conclusion

The results presented through this manuscript suggested that the utilization of liquid formulation of *B. subtilis* strain BG42 for the control of *Meloidogyne incognita* is a promising strategy for the management of the nematodes. This affirmation is supported by the fact that all tested *Bacillus* spp. isolates, reduced the nematode severity on the plants. Such reduction was evident due to the decrease in the number of root gall formation and de abolishment of the presence of severe symptoms on the gerbera plant. Under the green house conditions, liquid formulation of *B. subtilis* strain BG42 can be considered for a next level of evaluation as a promising isolate due to its good performance in terms of colonization, plant growth promotion, flower yield and the reduction of the *M. incognita* population.

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