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

P. MANJU* AND S. SUBRAMANIAN
Department of Nematology,
Tamil Nadu Agricultural University, Coimbatore - 641 003, Tamil Nadu, INDIA
e-mail: agrimanju@gmail.com

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, Calicut, Coimbatore, The Nilgiris, Hosur, Delhi, Nasik, Pune, Srinagar, besides few other places in Karnataka, Tamil Nadu, Maharashtra, 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, Tylenchorynchus 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 plant 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°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 10⁶ cfu/ml (OD₅₉₅ = 0.3) and used as bacterial inoculums (Thompson, 1996). These strains were kept at -80°C in 44 per cent glycerol and 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 10⁶ 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 infested with root knot nematode. The spacing adopted for gerbera was 40 cm X 30 cm, accommodating 8 plants/m². 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%/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.

**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 m² 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 was counted. 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* strain 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 Ardhnanareeswaran (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 PtYb 22 + *B. subtilis* Bbv 57 at 1000 ml/ha.

Significant reduction in number of adult females (12.83 5 g⁻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 5 g⁻¹ root), which attributed for 63.19 per cent decrease over control. The plants treated with carbofuran resulted in 27.17 5 g⁻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 5 g⁻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 5 g⁻¹ root and 21.83 5 g⁻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 100 nos/m² with 129.83 per cent increase over the control. This was followed by a yield of 93 nos/m² in the liquid formulation of *B. subtilis* strain BG37 treated plants. Flower yield recorded in control plots were 43.5 nos/m². 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>
<thead>
<tr>
<th>Treatment</th>
<th>Stalk length (cm)</th>
<th>Per cent increase over control</th>
<th>Flower diameter (cm)</th>
<th>Per cent increase over control</th>
<th>Flower Colour and Visual grade</th>
<th>Vase life (No. of days)</th>
<th>Per cent increase over control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil drenching of liquid formulation of <em>B. subtilis</em> strain BG42 @ 1% m⁻²</td>
<td>58.48</td>
<td>55.74</td>
<td>9.77</td>
<td>53.86</td>
<td>2</td>
<td>11.17</td>
<td>76.46</td>
</tr>
<tr>
<td>Soil application of talc formulation of <em>B. subtilis</em> strain BG42 @ 1% m⁻²</td>
<td>56.64</td>
<td>50.84</td>
<td>9.48</td>
<td>35.84</td>
<td>2</td>
<td>9.67</td>
<td>52.76</td>
</tr>
<tr>
<td>Soil drenching of liquid formulation of <em>B. subtilis</em> strain BG37 @ 1% m⁻²</td>
<td>56.48</td>
<td>50.41</td>
<td>9.30</td>
<td>46.46</td>
<td>1.5</td>
<td>10.33</td>
<td>63.19</td>
</tr>
<tr>
<td>Soil application of talc formulation of <em>B. subtilis</em> strain BG37 @ 1% m⁻²</td>
<td>54.50</td>
<td>45.14</td>
<td>8.41</td>
<td>32.44</td>
<td>2</td>
<td>8.67</td>
<td>36.97</td>
</tr>
<tr>
<td>Soil drenching of liquid formulation of <em>B. amyloliquefaciens</em> strain B4 @ 1% m⁻²</td>
<td>49.31</td>
<td>31.32</td>
<td>7.72</td>
<td>21.57</td>
<td>2</td>
<td>8.00</td>
<td>26.38</td>
</tr>
<tr>
<td>Soil application of talc formulation of <em>B. amyloliquefaciens</em> strain B4 @ 1% m⁻²</td>
<td>49.32</td>
<td>31.34</td>
<td>7.23</td>
<td>13.86</td>
<td>2</td>
<td>7.50</td>
<td>18.48</td>
</tr>
<tr>
<td>Soil application of Carbofuran @ 3.3g/m²</td>
<td>43.43</td>
<td>15.66</td>
<td>7.24</td>
<td>14.02</td>
<td>2</td>
<td>7.83</td>
<td>23.70</td>
</tr>
<tr>
<td>Untreated control</td>
<td>37.55</td>
<td>6.35</td>
<td>3</td>
<td>6.33</td>
<td>2</td>
<td>1.45</td>
<td>1.45</td>
</tr>
</tbody>
</table>

* Colour and visual grade: 1 – Very good; 2 – Good; 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)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of females/g root</th>
<th>Per cent of Nematode population decrease</th>
<th>No. of egg masses/10 g soil</th>
<th>Per cent of root knot Yield/m² increase</th>
<th>Per cent of Root gall index decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil drenching of liquid formulation of B. subtilis strain BG42 @ 1%/m²</td>
<td>12.83</td>
<td>66.68</td>
<td>3.33</td>
<td>30.65</td>
<td>28.75</td>
</tr>
<tr>
<td>Soil application of talc formulation of B. subtilis strain BG42 @ 1%/m²</td>
<td>15.33</td>
<td>60.18</td>
<td>5.00</td>
<td>34.83</td>
<td>3.63</td>
</tr>
<tr>
<td>Soil drenching of liquid formulation of B. subtilis strain BG37 @ 1%/m²</td>
<td>14.17</td>
<td>63.19</td>
<td>9.17</td>
<td>68.71</td>
<td>1.83</td>
</tr>
<tr>
<td>Soil application of talc formulation of B. subtilis strain BG37 @ 1%/m²</td>
<td>17.50</td>
<td>54.55</td>
<td>12.50</td>
<td>70.42</td>
<td>2.17</td>
</tr>
<tr>
<td>Soil drenching of liquid formulation of B. amyloliquefaciens strain B4 @ 1%/m²</td>
<td>24.67</td>
<td>35.92</td>
<td>19.83</td>
<td>36.03</td>
<td>3.00</td>
</tr>
<tr>
<td>Soil application of talc formulation of B. amyloliquefaciens strain B4 @ 1%/m²</td>
<td>27.00</td>
<td>29.87</td>
<td>21.50</td>
<td>34.83</td>
<td>2.67</td>
</tr>
<tr>
<td>Soil application of Carbofuran @ 3.3g/m²</td>
<td>27.17</td>
<td>29.43</td>
<td>21.83</td>
<td>34.33</td>
<td>3.33</td>
</tr>
<tr>
<td>Untreated control</td>
<td>38.5</td>
<td>31.00</td>
<td>616.67</td>
<td>4.50</td>
<td>43.50</td>
</tr>
</tbody>
</table>

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 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.

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REFERENCES


