Microbial organisms are extremely important in regulating nematode populations like root-knot. The suppressive effect of a microbial bioagent is dependent on the strain, its density and virulence in the soil (Kamra and Rao 2013, Sellaperumal et al. 2014). Streptomyces spp. are gram positive filamentous bacteria that produce a wide array of biologically active compounds some of which are nematicidal (Chubachi et al. 1999, Takatsu et al. 2003). Many of them effectively colonize plant roots, influence plant growth and protect plant roots from pathogens. S. avermitilis produces nematicidal macrocyclic lactones such as avermectins sold under the tradename AVICTA by Syngenta (www.avicta.com). However, avicta is recommended for seed treatment and is currently registered for cotton in US. It moves from the treated seed alongside the growing root protecting the young plant from nematode parasitisation. Avermectin B1, also known as abamectin, is registered as an insecticide, acaricide and nematicide in more than 50 countries. Its liquid and granular formulations for control of plant parasitic nematodes, have been studied in wide range of crops such as tobacco, tomato (Garabedian and Van Gundy 1983), garlic (Roberts and Mathews 1995), banana (Jansson and Rabatin 1997, 1998), melon (Moreira and Barbosa 2002) and cotton (Faske and Starr 2006). It is known to inhibit egg hatching and paralyse juveniles (J2s) of M. arenaria but its persistence in the soil is low (Cayrol et al. 1993, Faske and Starr 2006).

Limited investigations have been carried out on nematicidal potential of indigenous isolates of Streptomyces spp. in India (Jayakumar et al. 2005, Subhashini et al. 2009, Patidar et al. 2012). The present work on evaluation of antagonistic effects of this indigenous isolate of S. lavendulae MTCC 706, (isolated from compost sample) against M. incognita has not been reported so far, although Takatsu et al. (2003) have reported a nematocide from S. lavendulae isolate from Japan, SANK 64297 that caused inhibition of root galling on cucumber due to M. hapla at a MIC of 0.05 ppm. In the present study, the nematicidal effect of S. lavendulae MTCC 706 was significant on the nematode in laboratory bioassays, causing significant juvenile mortality and egg hatch inhibition (Perumal et al. 2014). Therefore, its bioefficacy as a drench treatment was evaluated in pot trials against root-knot nematode, M. incognita, infecting tomato cv Pusa Ruby.

MATERIALS AND METHODS

Root-knot nematode, Meloidogyne incognita collected from brinjal field of IARI, New Delhi was raised on brinjal var. Pusa purple long. Roots with conspicuous galls were washed gently with tap water. Females with egg masses were removed and kept individually in Syracuse cups half filled with water, stained in hot acid fuchsins-lactophenol.
stain and their identity was verified by preparing semi-permanent mounts of the perineal pattern. After confirmation of the species as *M. incognita*, a single fresh egg mass was used to initiate the nematode culture. The second stage juveniles (J2s) collected from Syracuse cups were inoculated in the rhizosphere of 20 day old brinjal seedlings raised in sterilized soil, and transplanted in five earthen pots each containing 5 kg sterilized sand-soil mixture. The plants were maintained for three months after inoculation, uprooted and roots were gently washed free of soil. Well developed egg masses were collected and incubated at 25-30°C, to collect the freshly hatched J2s which were used for *in vitro* bioassays.

*S. lavendulae* MTCC 706 was grown axenically in sterilized KM broth at 28±2°C and 4000 lux light with a 16/8 light and dark period in an incubator shaker (15 rpm), in thirty 500 ml flasks containing 100 ml medium. After 13 days, the crude extract of the actinomycete was collected after filtration through Whatman no.1.

Nursery of tomato cv Pusa Ruby was raised in sterilized soil. Four week old seedlings were planted in 10 cm diameter earthen pots containing sterilized sand: sandy loam soil in the ratio of 2:1. The nematode was inoculated @ 2 J2/g of soil around root zone of the seedlings after 2 days of transplanting. Simultaneously, the soil was drenched with crude filtrate of *S. lavendulae* (2% v/w). The penetration of J2s in the root systems was determined at 2, 4, 7 and 10 DAI (Days after inoculation) by staining the roots with acid fuchsin (Byrd *et al*. 1983) and observing under stereo binocular microscope at 40x. The seedlings inoculated @ 2J2/g soil but without drench application served as control. Each treatment was replicated four times.

The different developmental stages, i.e. J2s, J3s, J4s and adults of the nematode in the root system were observed and counted per plant at 14, 19, 24, 30 and 35 DAI, under a stereo binocular microscope at 40x. The experiment was arranged in a complete randomized block design with each treatment replicated five times.

Tomato seedlings (4 weeks old) were planted in pots (15 cm diameter) filled with 2.5 kg of steam sterilized soil. One week after planting, seedlings were thinned to one healthy plant per pot and inoculated with freshly hatched *M. incognita* @ 2 J2/g soil. The following treatments were given: (1) SLT: *S. lavendulae* (Crude extract drench @ 2% v/w), (2) CBF: Carbofuran 3G @ 1.0 kg a.i./ha, (3) SLT + CBF: *S. lavendulae* (Crude extract drench @ 2%) + Carbofuran @ 0.5 kg a.i./ha, (4) ABT: Abamectin drench @ 2% v/w, (5) Control (*M. incognita* @ 2 J2/cc soil).

The above treatments were replicated five times and the pots were maintained in the glass house at a temperature varying from 18-35°C. After 45 days of planting, the plants were removed carefully without disturbing the root system and washed gently in running water to remove the soil adhering on root surface. Observations were made on number of galls/plant, number of egg masses/root, number of eggs/egg mass, shoot length, fresh shoot weight, dry shoot weight, root length, fresh root weight, dry root weight, and J2 population in soil. As the efficacy and mode of action of abamectin is already reported, it was taken as a control treatment in the present studies.

The observations were repeated in the same pots by replanting fresh tomato seedlings cv Pusa Ruby to observe the residual effect of the treatments.

### RESULTS AND DISCUSSION

**Effects of soil drenching with *S. lavendulae* crude extract and abamectin**

The average number of J2s/plant in SLT plants were significantly low (19.3) compared to inoculated control (41.3) in first 48 hr of inoculation. The trend of reduced J2 penetration was observed on all four days of observation, indicating the antagonistic effect of the actinomycete. The reduced penetration was possibly because of impaired mobility in the nematodes in the soil. An average 62.6% immobility and 36.6% mortality it was observed in J2s in laboratory trials (Perumal *et al*. 2014)

The average number of J2s/plant increased with progress in time from day 2 to 10 in all three treatments, although the number of J2s penetrated in SLT was consistently lower than the control treatment (Fig 1a)

**Effect of *S. lavendulae* on development of root knot nematode**

The relative numbers of developmental stages of root-knot nematode (J2s, J3s, J4s and adults) showed differences in SLT with respect to untreated inoculated control and ABT (Fig 1b). On 14 DAI in SLTs, the maximum number of nematodes was in J2 stage, while in inoculated control, most of the penetrated nematodes had developed into J3. On 19 DAI, no J4 was observed in SLTs, while an average of 28.3 J4s/plant could be observed in control. On 24 DAI too, an average of 21.5 J2s/plant were observed in SLTs, compared to none in inoculated control. On 30 DAI, averages of 15.0 adult females were dissected from inoculated control, no female or J4 were observed in SLTs. The number of adults were significantly less in SLTs on 35 DAI, compared to control. Thus an antagonistic effect of *Streptomyces* drench on development of the nematode inside the tomato roots was observed. The microbe or its product affected nematode

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![Fig 1 Effect of *S. lavendulae* MTCC 706 (SLT) and abamectin (ABT) on invasion in tomato (cv Pusa Ruby) roots.](image-url)
physiology as reported for AVM B1 in *M. arenaria* infecting tomato plants (Stretton *et al.* 1987).

**Bioefficacy of *S. lavendulae* MTCC 706, carbofuran and abamectin**

The bio-efficacy of SLT alone and SLT+CBF on number of galls/plant, egg masses/plant, eggs/egg masses and soil population were evaluated at 45 DAI and compared with CBF and ABT alone (Table 1). A decrease of 50.7% in number of galls/plant was observed in SLT alone, compared to 52.5% with CBF alone and 59.9% in combined treatment compared to inoculated control. Thus, a synergistic effect of SLT with CBF was observed.

The number of egg masses/plant decreased by 42.7%, compared to 50.6% in CBF and 61.5% in combined treatment, again reflecting a synergistic effect of the combined treatment. The numbers of eggs/egg masses in SLTs were at par with control while a decrease of 2.05% was observed in CBF and 4.31% in the combined treatment. The soil population of J2s was significantly low in all the

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of galls/ plant (%)</th>
<th>Egg masses/ plant (%)</th>
<th>Eggs/ egg masses (%)</th>
<th>J2/200c soil (PFPi)</th>
<th>Shoot length (cm)</th>
<th>Root length (cm)</th>
<th>Fresh shoot weight (g)</th>
<th>Fresh root weight (g)</th>
<th>Dry shoot weight (g)</th>
<th>Dry root weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLT</td>
<td>283.2</td>
<td>50.7</td>
<td>34.6</td>
<td>42.7</td>
<td>478.0</td>
<td>0.12</td>
<td>471.2</td>
<td>1.2</td>
<td>43.5</td>
<td>37.4</td>
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<tr>
<td>CBF</td>
<td>272.6</td>
<td>52.5</td>
<td>29.8</td>
<td>50.6</td>
<td>467.6</td>
<td>2.05</td>
<td>463.4</td>
<td>1.1</td>
<td>44.8</td>
<td>36.5</td>
</tr>
<tr>
<td>SLT+CBF</td>
<td>230.4</td>
<td>59.9</td>
<td>23.2</td>
<td>61.5</td>
<td>465.8</td>
<td>4.31</td>
<td>445.0</td>
<td>1.1</td>
<td>48.4</td>
<td>40.1</td>
</tr>
<tr>
<td>ABT</td>
<td>128.8</td>
<td>77.6</td>
<td>12.6</td>
<td>79.1</td>
<td>395.0</td>
<td>17.2</td>
<td>187.8</td>
<td>0.5</td>
<td>53.2</td>
<td>41.5</td>
</tr>
<tr>
<td>Control</td>
<td>575.0</td>
<td>60.4</td>
<td>60.4</td>
<td>NA</td>
<td>NA</td>
<td>17.2</td>
<td>187.8</td>
<td>0.5</td>
<td>53.2</td>
<td>41.5</td>
</tr>
<tr>
<td>SEM</td>
<td>15.2</td>
<td>3.3</td>
<td>3.3</td>
<td>11.8</td>
<td>477.4</td>
<td>NA</td>
<td>952.4</td>
<td>2.4</td>
<td>40.6</td>
<td>32.0</td>
</tr>
<tr>
<td>CD</td>
<td>45.1</td>
<td>10.0</td>
<td>10.0</td>
<td>35.1</td>
<td>40.2</td>
<td>13.5</td>
<td>1.8</td>
<td>2.2</td>
<td>3.2</td>
<td>1.6</td>
</tr>
</tbody>
</table>

* The figures are square root transformed values (@ Sqrt (x+0.5); Average of 5 replications. SLT: *S. lavendulae* (Crude extracts drench @ 2% v/w), CBF: Carbofuran @ 1kg a.i/ha, SLT+CBF: *S. lavendulae* (Crude extract drench @ 2% v/w) + Carbofuran @ 0.5 kg a.i/ha, ABT: Abamectin drench @ 2% v/w, RF: Reproduction factor; Pf, Final J2 population; Pi, Initial J2 population*

Fig 1 Effect of *S. lavendulae* MTCC 706 and abamectin on (b) development of root-knot nematode *M. incognita*, in tomato cv Pusa Ruby.
treatments, compared to control. The reproduction factors were 1.2, 1.1 and 1.1 in SLT, CBF and SLT+CBF, respectively, compared to 2.4 in control.

The average shoot length was the highest (53.2 cm) in ABT, followed by SLT+CBF (48.4 cm), CBF (44.8 cm) and SLT (43.5 cm), all of which were significantly different from each other, as well as compared to inoculated control (40.6 cm) [CD (0.05P) = 0.6]. The average root length of the plants was the highest (41.5 cm) in ABT followed by SLT+CBF (40.1 cm), SLT (37.4 cm) and CBF (36.5 cm). The difference were however non-significant statistically. The average fresh shoot weight was the highest (26.2 g) in ABT, at par with SLT+CBF (25.2 g) SLT (22.1 g) or CBF (22.9 g) and but significantly higher than control (16.9 g), [CD (0.05P) = 5.7]. The average fresh root weight was the highest (22.3 g) in ABT, followed by SLT+CBF (20.0 g), CBF (19.2 g) and SLT (19.2 g). The difference were however non-significant statistically.

The average dry shoot weight was the same in SLT and CBF (4.5 g), at par with that an SLT+CBF (4.8 g) significantly higher than inoculated control (3.5 g) [CD (0.05P) = 1.2]. The average dry root weights were at par with each other in SLT (1.1 g), CBF (1.1 g), SLT+CBF (1.2 g) and ABT (1.3 g) but were significantly higher than the values observed in control (0.6 g).

Residual effect of S. lavendulae MTCC 706, carbofuran and sequence abamectin

A perusal of Table 4 shows good residual effect with respect to per cent reduction in root galling in SLT (81.3%) and SLT + CBF (82.1%) compared to control. The CBF treated plants showed only 57.5% reduction in galls.

The per cent decrease in egg masses/plant with respect to control, were significantly high in SLT (80.5%) and SLT + CBF (81.5%), compared to 54.1% in CBF treatment.

The average of eggs/egg masses did not exhibit a significant difference among the treatments. The average density of J2/200cc soil was significantly low (315.8) in SLT, compared to SLT+CBF (427.2) or CBF alone (865.8) compared to control (1916.4), indicating establishment of S. lavendulae in the soil. This population density was reflected in a low reproduction factor of 0.6 in SLT and 0.9 in SLT+CBF.

The average shoot length was the maximum in SLT+CBF (46.2 cm), followed by SLT (41.8 cm) and CBF (38.0 cm), compared to a low of 35.4 cm in control. The values were statistically significant with each other and control [CD (0.05P) = 2.2] (Table 2).

The average root length also followed the same pattern as the average shoot length, the maximum in SLT+CBF (37.3 cm), followed by SLT (34.9 cm) and CBF (29.5 cm), compared to a low of 26.4 cm in control. The values were statistically significant with control [CD (0.05P) = 5.9].

The average fresh shoot weight (g) was at par in SLT (21.1 g), SLT+CBF (22.1 g), CBF (19.9 g) but was significant with control (13.9 g) [CD (0.05P) = 5.4].

The average root weight (g) was higher in SLT (16.3 g) or SLT+CBF (17.0 g), compared to CBF (14.0 g) or control (9.4 g) [CD (0.05P) = 4.4]. This trend was also observed in average dry shoot weight with a higher value in SLT (4.1 g) and SLT+CBF (4.4 g), than CBF (3.7 g) or control (3.0 g). The dry root weight (g) was at par in SLT (0.9 g) and SLT+CBF (1.0 g) and was statistically higher than that in CBF (0.7 g) or control (0.3 g) [CD (0.05P) = 0.1].

Soil microorganisms residing in and around the plant root show significant positive influence on plant growth. Streptomyces is one such group residing in the soil that is reported to produce 10^5-10^7 colony forming units/g of soil (Smither-Kopperl 2002). Biocontrol of plant diseases by Streptomyces spp., was well documented worldwide (Campbell et al. 1983). However a fewer attempts was made to manage plant parasitic nematodes using these microbes, except for abamectin which is a commercial fermentation product obtained from Streptomyces avermitilis. It causes neurotoxicity in nematodes by blocking the gamma-aminobutyric acid-stimulated chloride channels causing an

Table 2  Residual effect of S. lavendulae MTCC 706, carbofuran and abamectin alone and in combination on root knot nematode M. incognita infestation and plant growth characters in tomato cv Pusa Ruby

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of galls/plant</th>
<th>% Decrease over control</th>
<th>Egg masses/plant</th>
<th>% Decrease over control</th>
<th>Eggs/ Egg masses</th>
<th>% Changes over control</th>
<th>J2/200cc soil</th>
<th>Root length</th>
<th>Shoot length</th>
<th>Fresh shoot weight</th>
<th>Fresh root weight</th>
<th>Dry shoot weight</th>
<th>Dry root weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLT</td>
<td>218.6</td>
<td>81.3</td>
<td>23.6</td>
<td>80.5</td>
<td>470.2</td>
<td>-3.6</td>
<td>315.8</td>
<td>41.8</td>
<td>34.9</td>
<td>21.1</td>
<td>16.3</td>
<td>4.1</td>
<td>0.9</td>
</tr>
<tr>
<td>CBF</td>
<td>496.8</td>
<td>57.5</td>
<td>55.7</td>
<td>54.1</td>
<td>468.4</td>
<td>-2.2</td>
<td>865.8</td>
<td>8.8</td>
<td>29.5</td>
<td>19.9</td>
<td>14.0</td>
<td>3.7</td>
<td>0.7</td>
</tr>
<tr>
<td>SLT+CBF</td>
<td>208.6</td>
<td>82.1</td>
<td>22.4</td>
<td>81.5</td>
<td>460.8</td>
<td>-1.5</td>
<td>427.2</td>
<td>0.9</td>
<td>37.3</td>
<td>22.1</td>
<td>17.0</td>
<td>4.4</td>
<td>1.0</td>
</tr>
<tr>
<td>ABT</td>
<td>68.8</td>
<td>94.1</td>
<td>8.8</td>
<td>92.7</td>
<td>462.8</td>
<td>-1.9</td>
<td>137.8</td>
<td>0.7</td>
<td>39.5</td>
<td>23.1</td>
<td>19.8</td>
<td>5.8</td>
<td>1.0</td>
</tr>
<tr>
<td>Control</td>
<td>1171.4</td>
<td>0.0</td>
<td>121.6</td>
<td>0.0</td>
<td>453.8</td>
<td>0.0</td>
<td>1916.4</td>
<td>2.0</td>
<td>26.4</td>
<td>13.9</td>
<td>9.4</td>
<td>3.0</td>
<td>0.3</td>
</tr>
<tr>
<td>SE (m)</td>
<td>32.7</td>
<td>3.4</td>
<td>9.1</td>
<td>9.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>CD</td>
<td>97.3</td>
<td>10.3</td>
<td>N.S</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<td>0.3</td>
</tr>
</tbody>
</table>

(P=0.05) *The figures in the parentheses are square root transformed values (@Sqrt (x+0.5); Average of 5 replications. SLT: S. lavendulae (Crude extracts drench @2% v/w) + Carbofuran @1.5kg a.i/ha, SLT+CBF: S. lavendulae (Crude extract drench @2% v/w) + Carbofuran @1.5kg a.i/ha, ABT: Abamectin drench @2% v/w.*
ior imbalance in the nervous system, resulting in paralysis and finally death of nematode (Jansson and Dybas 1998). It thus disturbs the behavioural sequence of the nematode preceding invasion resulting in reduced penetration of the juveniles in the roots, and consequently reduced densities in the soil. The bioefficacy of abamectin is already reported (Monfort et al. 2006) and so was taken as a control treatment in the present studies.

In the present experimental trial, nematode reproduction parameters like number of galls/plant, development of egg masses in root system, soil population etc. were significantly reduced on application of S. lavendulae MTCC 706. The average number of egg masses was significantly low in SLTs (34.6/plant) or carbofuran (29.8), and reduced to 23.2 in the combined application resulting in 61.5% reduction in egg mass production, supporting the compatibility of the actinomycete with the nematicide.

The J2 density in the soil also reduced further in the combined application of SLT and half the dose CBF, resulting in a RF of 1.1, at par with that of CBF @ 1 kg a.i/ha. Actinomycetes or their product are reported to improve the plant growth characters (Chubachi et al. 1999, Jayakumar et al. 2005). In the present investigation too, significant enhancement of average shoot length, fresh and dry shoot weights, with no significant difference in root length or fresh root weight compared to control was observed on application of SLT alone or in combination with CBF. Applications of microbes are sometimes reported to have a longer lasting effect than the chemicals that are effective for a short term. The half life of carbofuran is effective for a short term. The half life of carbofuran is 23.2 days in the soil (www.cdpr.ca.gov/docs/emon/pubs/ 38). In the present study, it is reported to be 43-117 days as the degradation depends on abiotic factors also influence the bioefficacy of microbial bioagents and need to be investigated (Nagesh et al. 2013). Attempts can be made to characterise the nematicidal metabolite(s) in the isolate with the possibility to explore its synthetic production.

REFERENCES


