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Biological services by *Streptomyces lavendulae* MTCC 706 to control invasion, development and reproduction of root-knot nematode (*Meloidogyne incognita*) infecting tomato cv Pusa Ruby

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ABSTRACT

The drench application of *S. lavendulae* MTCC706 @ 2% v/w (SLT) resulted in less than 50% juvenile invasion of *M. incognita* J2s in tomato (cv. Pusa Ruby), delayed development of J4s, 50.7% reduction in root galling and 42.7% reduction in egg mass production, compared to control. The reproduction factor was 1.2 in SLT, compared to 2.4 in control in 45 days. SLT was compatible with the nematicide carbofuran, as increase in bioefficacy of SLT was observed with 60% reduction in root galling, 61.5% reduction in egg mass production, and a reproduction factor of 1.1 in the combined application with carbofuran @0.5 kg a.i/ha. Replanting of tomato in the pre-treated soil revealed a further reduction in root galling (81.3%), egg mass production (80.5%) and reproduction factor (0.6), indicating establishment of the actinomycete in the soil.

Key words: Biological control, *Meloidogyne incognita*, Residual effect, *Streptomyces lavendulae*, Tomato

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 fuchsin-lactophenol

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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 in10 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 (Fig1a)

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

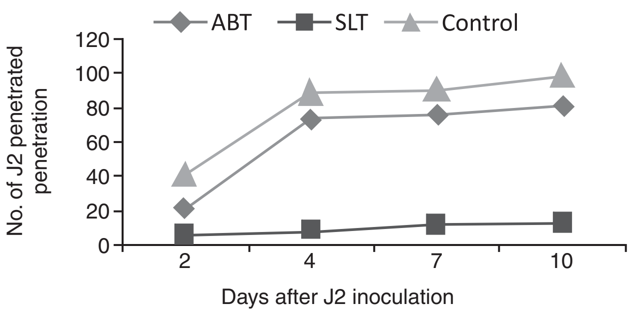

Fig 1 Effect of *S. lavendulae* MTCC 706 (SLT) and abamectin (ABT) on invasion in tomato (cv Pusa Ruby) roots.

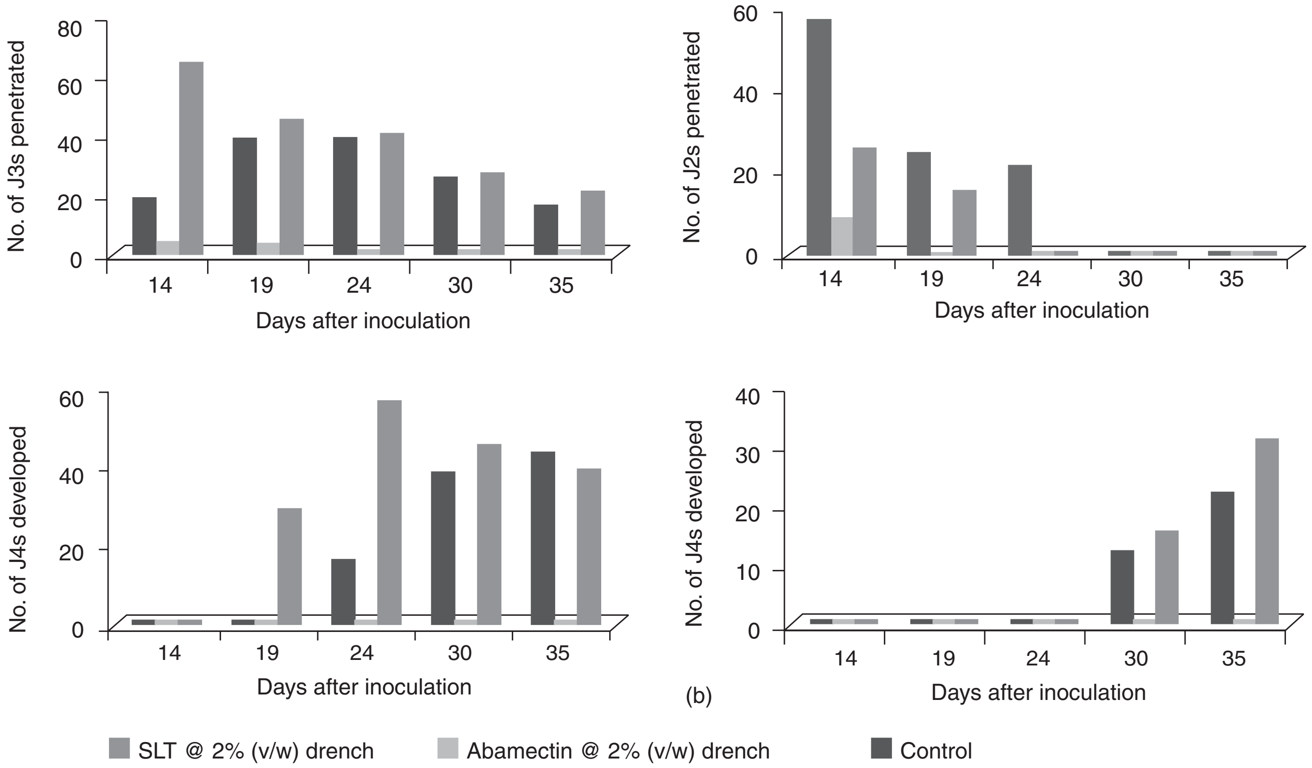

Fig 1 Effect of *S. lavendulae* MTCC 706 and abamectin on (b) development of root-knot nematode *M. incognita,* in tomato cv Pusa Ruby.

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 1 Bioefficacy 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

Treatment	No. of galls/ plant	% Decrease over control	Egg masses/ plant	% Decrease over control	Eggs/ egg control	% Changes over control	J2/200cc soil	RF (Pf/Pi)	Shoot length (cm)	Root length (cm)	Fresh shoot weight (g)	Fresh root weight (g)	Dry shoot weight (g)	Dry root weight (g)
SLT	283.2	50.7	34.6	42.7	478.0	-0.12	471.2	1.2	43.5	37.4	22.1	19.2	4.5	1.1
CBF	272.6	52.5	29.8	50.6	467.6	2.05	463.4	1.1	44.8	36.5	22.9	19.2	4.5	1.1
SLT+CBF	230.4	59.9	23.2	61.5	456.8	4.31	445.0	1.1	48.4	40.1	25.2	20.0	4. 8	1.2
ABT	128.8	77.6	12.6	79.1	395.0	17.2	187.8	0.5	53.2	41.5	26.2	22.3	6.5	1.2
Control	575.0	NA	60.4	NA	477.4	NA	952.4	2.4	40.6	32.0	16.9	15.2	3.5	0.6
SEm	15.2		3.3		11.8		13.5		1.8	2.2	3.2	1.6	0.4	0.1
CD (P=0.05)	45.1		10.0		35.1		40.2		5.6	N.S	5.7	N.S	1.2	0.4

* 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 populaton

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

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

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 (1 916.4), 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 (21.1g), SLT+CBF (22.1g), CBF (19.9g) but was significant with control (13.9g) [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 (0.9g) and SLT+CBF (1.0 g) and was statistically higher than that in CBF (0.7 g) or control (0.3 g) [CD (00.05P) =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-amino butyric 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

Treatment	No. of galls/ plant	% Decrease over control	Egg masses/ plant	% Decrease over control	Eggs/ Egg masses	% Changes over control	J2/200cc soil	RF (Pf/Pi)	Shoot length (cm)	Root length (cm)	Fresh shoot weight (g)	Fresh root weight (g)	Dry shoot weight (g)	Dry root weight (g)
SLT	218.6	81.3	23.6	80.5	470.2	-3.6	315.8	0.6	41.8	34.9	21.1	16.3	4.1	0.9
CBF	496.8	57.5	55.7	54.1	468.4	-3.2	865.8	1.8	38.0	29.5	19.9	14.0	3.7	0.7
SLT+CBF	208.6	82.1	22.4	81.5	460.8	-1.5	427.2	0.9	46.2	37.3	22.1	17.0	4.4	1.0
ABT	68.8	94.1	8.8	92.7	462.8	-1.9	137.8	0.7	49.0	39.5	23.1	19.8	5.8	1.0
Control	1171.4	0.0	121.6	0.0	453.8	0.0	1916.4	2.0	35.4	26.4	13.9	9.4	3.0	0.3
SE (m)	32.7		3.4		9.1		18.2		6.6	2.0	1.8	1.5	0.2	0.3
CD (P=0.05)	97.3		10.3		N.S		54.3		2.2	5.9	5.4	4.4	0.6	0.1

*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), CBF: 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.

ion 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 reported to be 43-117 days as the degradation depends upon the soil type, soil pH, moisture content and microbes present in the soil (www.cdpr.ca.gov/docs/emon/pubs/fatememo/carbofuran.pdf, 32). In the present study, *S. lavendulae* MTCC 706 exhibited some residual effect with respect to number of galls or eggmass production per plant and reproduction factor of the nematode, on second planting of tomato in the pretreated soil (Table 2). The per cent reduction in galling on application of *S. lavendulae* MTCC 706 increased from 51.0% in the first planting to 81.3% in the second planting and in the combined application from 59.9 to 82.1%. Long persistence effect (4 years) has been reported for S*treptomyces* strain 93 (Bowers *et al*. 1996). This effect on increased percent reduction in galling was also observed in abamectin from 77.6 to 94.1%, although Bull *et al*. 1984 have reported chemical detection of abamectin for only 47 days. The persistence of avermectin in the field is generally reported to be low (Burg *et al.* 1979, Bull *et al.* 1985)

In the second planting, the reproduction factor decreased from 1.2 to 0.6 in SLT and from 1.1 to 0.9 in CBF, whereas it increased from 1.1 to 1.8 in CBF treated soil. These changes were however not reflected in the plant growth parameters on second planting.

The application of *S. lavendulae* MTCC 706 thus can be exploited for management of root knot nematode, *M. incognita* as it showed antagonistic effects on nematode invasion, development and multiplication. Besides, it is compatible with carbofuran and its bioefficacy showed residual effect on root-knot nematode. The quantification of the isolate in the soil at different time periods would confirm its establishment and rate of propogation in the soil. The 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.

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