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Consortia of *Bacillus subtilis* and *Purpuriocilium lilacinum* have Nematicidal activity against Root Knot Nematode (*Meloidogyne incognita*) in Tomato

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ABSTRACT: In agricultural eco systems, root-knot nematodes in tomato *(Solanum lycopersicum)* have caused significant crop loss and production. In this scenario, using helpful microorganisms to successfully manage *Meloidogyne* spp and helps to maintaining agricultural sustainability and decreasing the risk of using chemical pesticides. In this case, the study of fungal–bacterial interactions is a new area of study that employs modern microbial ecology tools. The major constrains of the study is Bacteria have both good and negative effects on fungal performance (and vice versa). Our results shows that *Purpuriocilium lilacinum and Bacillus subtilis* have a positive interaction in vitro, showing that the complementary processes may increase overall nematode treatment efficacy. In a dual plate experiment, *B. subtilis* and *P. lilacinum* had the highest juvenile mortality of 95.90 and 95.66 percent, respectively, and the egg hatching inhibition of 92.54 and 95.00 percent. We inoculated tomato roots with the nematophagus fungus *P. lilacinum* and *B. subtilis* together this will significantly reduced the egg masses (70.31%) and galling index (1.33) of *M. incognita*. After biocontrol inoculation, we observed nematicidal VOC (volatile organic compounds) produced by tomato plants, It is strongly increase the plant's resistance against plant parasitic nematodes.

Keyword: Compatibility, *B. subtilis, P. lilacinum, M. incognita,* Nematicidal activity, VOCs.

INTRODUCTION

Root knot nematodes are considered severe biotic limitations that cause significant damage and production loss to the majority of crops around the world (Forghani and Hajihassani 2020). Their potential use as biocontrol agents with protective activity against economically important plant pathogens has been highlighted in recent decades, making them a promising alternative to chemical pesticides (Expósito *et al*., 2017). Plants infected with *Meloidogyne* spp. exhibit typical root galling symptoms. Some infected plants show signs of deficiency. Rhizospheric microorganism play ancritical role in the management of soil-borne diseases such as nematodes. Most commercial *M. incognita* management biocontrol solutions currently on the market contain live microorganisms that target nematodes, such as *Bacillus subtilis* and *Purpuriocilium lilacinum*, and are a viable alternative to chemical pesticides (Lamovsek *et al*., 2013). Fengycins, surfactin, and iturin are produced by Bacillus lipopeptides, which exhibit antagonistic activity against a wide spectrum of phytopathogens and can reduce egg hatching and kill *M. incognita* J2 in vitro (Kavitha *et al.*, 2012).

The *Bacillus alvei* strain showed promise against nematode eggs and larvae, with hydrolytic enzymes hydrolyzing the worm eggs and larvae directly. The release of significant quantities of reducing sugars and lytic enzymes, such as proteases, chitinase, and chitosanase, is also linked to the effect of the strain or its metabolites on eggs and larvae (Abd-El-Khair *et al.*, 2016). *P. lilacinum* is an egg parasitic Antibiotics such as leucinostatin and lilacin are produced by fungus, as are enzymes such as protease and chitinase. Protease has nematicidal activity, causes eggshell degradation, and prevents hatching. The fungus enters the egg and grows profusely inside and on top of it, completely impeding juvenile development. The infected eggs swell and buckle as a result of the infection. As the egg continues to penetrate, the vitelline layer splits into three bands and a large number of vacuoles; the lipid layer disappears at this point. The rapidly growing hyphae destroy the developing juvenile inside the egg. *P. lilacinum* has been shown to produce a number of secondary metabolic products, which help to reduce *M. incognita* infection (Khan *et al*., 2006). The goal of our

study was to see if combining *B. subtilis* and *P. lilacinum* inoculation effectively reduced nematode infection, and we hypothesised that combining these beneficial microorganisms would benefit the crop more than individual applications. Biological control has resulted in a low effective strategy unless applied in combined inoculation (Moghaddam *et al*., 2015).

MATERIALS AND METHODS

Microbial strains and culture collection. *Bacillus subtilis* (Talc based) obtained from Department of Plant Pathology and *Purpuriocilicium lilacinum* (Talc based) product procured from Department of Nematology TNAU, Coimbatore. These biocontrol agents was used in the greenhouse experiments.

Maintenance of pure culture of root knot nematodes. RKN was cultured and maintained on tomato plants grown in sterilised garden soil in plastic pots (15 cm diameter × 12 cm depth) (sandy loam; pH 6.5). Tomato plants (PKM1) were artificially inoculated with M. incognita at a rate of two thousand nematodes per plant for this purpose. From the infected stock plants, second stage juveniles (J2) and egg masses were collected. Plants were carefully uprooted and thoroughly washed in running tap water before egg masses were collected. Hand-picked egg masses adhered to galls were collected in sterile water using forceps. They were surface sterilised for two minutes in 0.5 percent sodium hypochlorite, then washed three times with sterile water. Disinfected egg masses were allowed to hatch in sterile distilled water for two days before being collected in Petri plates using the Modified Bearmann Funnel Technique to collect J2 juveniles (Southey, 1986). (10 cm in diameter). After 48 hours of incubation at 28 + 20C, the extracted nematodes in the Petri plates were collected and used for further research. For our research purposes, the root knot nematodes pure culture is kept in the glass houses of the Department of Nematology, TNAU, and Coimbatore.

Compatibility interaction of *B. subtilis*, and *P. lilacinum*. The in vitro compatibility of *B. subtilis* and *P. lilacinum* was determined using the dual culture technique. The experiments were carried out in Petri dishes with a diameter of 100mm and a volume of 20 ml of LB Medium. A 48-hour-old *B. subtilis* culture was streaked on one side of the plate, and a 2-week-old *P. lilacinum* fungal disc was placed on the other. Plates were incubated in the dark at 250°C for 8-15 days to determine compatibility. When colonies were on the verge of merging, compatibility was determined. The rate of radial growth was then compared to the rate of colony growth on the respective controls (Fig. 1).

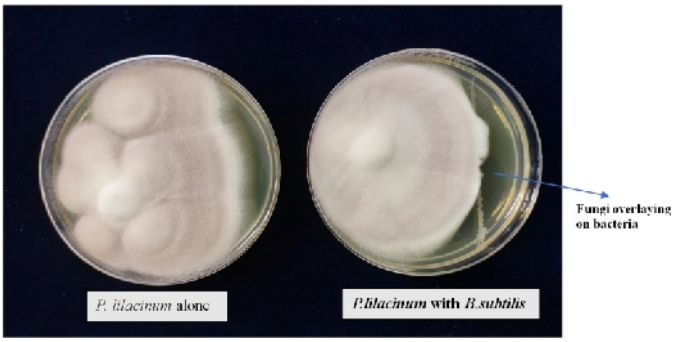
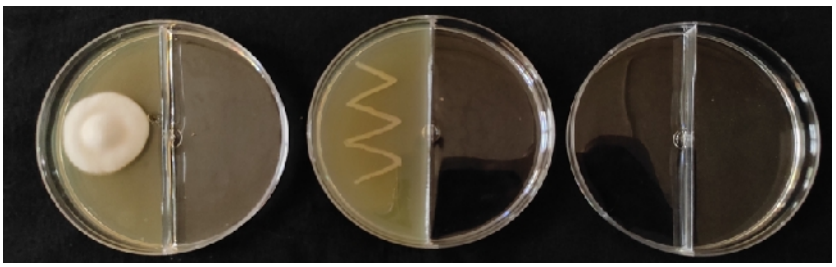


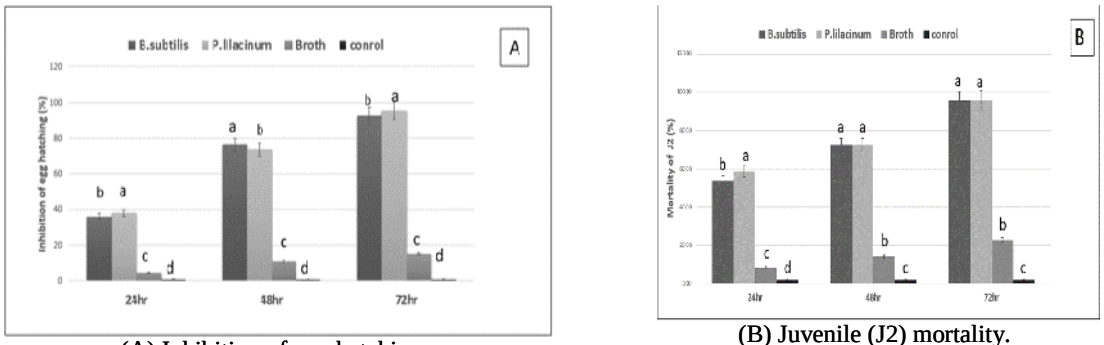
Fig. 1. Compatibility interaction of *Bacillus subtilis* with *Purpuriocilium lacinum.*

Dual plate assay for inhibition of egg hatching of *M. incognita.* The dual plate assay was carried out using the method previously described (Fokkema, 1978). For egg hatching inhibition, LB agar medium was poured one side of the plate after solidification, and *B. subtilis* culture streak over the surface of LB and *M. incognita* single egg masses were deposited on the other side of the plate. Distilled water and *M. incognita* egg plates served as controls. Plates were inspected under a stereo zoom microscope after 24, 48, and 72 hours of intervals to count the inhibition of egg hatching. *P. lilacinum* mycelial plugs measuring 8 mm in diameter were placed on one side of the plate, while *M. incognita* eggs were placed on the opposite side (Fig. 2, 3) (Table 1).



P. lilacinum *B. subtilis* Distilled water control

Fig. 2. *M. incognita* egg hatching and juvenile mortality assay using a dual partition plate.



(A) Inhibition of egg hatching. (B) Juvenile (J2) mortality.

Fig. 3. Dual plate assay for *M. incognita* egg hatching and juvenile mortality.

Dual plate assay for juvenile (J2) mortality of *M. incognita* *M. incognita* juvenile (J2) mortality assay were performed in two compartment plates. In this assay LB agar plates were streaked with *B. subtilis* in one sides and another side freshly hatched 500 juveniles (J2) were added. In the same way *P. lilacinum* mycelial plug placed in one side of PDA (Potato dextrose agar) plates and another side with *M. incognita* juveniles (J2). Distilled water with juveniles act as control. After 24, 48, and 72 hours of incubation, the juvenile mortality rate was calculated. In a completely randomised design, three replications were kept for each treatment. The experiment was carried out in a laboratory setting. A stereo zoom microscope can be used to examine juvenile mortality (Fig. 3) (Table 1).

Biocontrol of root-knot nematode on tomato in the glasshouse. Suppression of *M. incognita* determined under the greenhouse conditions. After 25 days, seedlings of tomato seeds (PKM 1 variety obtained from HC and RI, TNAU, Coimbatore) were transferred into 5 kg earthen pots with potting mixtures containing red soil, sand, and FYM in a 2:1:1 ratio. After 15 days of transplanting freshly hatched juveniles @ 1 J2 /g M. incognita was inoculated in soil around tomato plant roots by making three holes around the plant and covering with sterilised soil. Talc based formulation of effective bacterial (*Bacillus subtilis* TNAU (BS1)) and fungal (*Purpureocillium lilacinum* (TNAU PL01) antagonistic were applied at the rate of 10 g/pot were applied as per the treatment. The experiment consisted of 5 treatments with 3 replications in a completely randomized block design and the treatments include T1-*Bacillus subtilis*, T2-*Purpureocillium lilacinum*, T3-*Bacillus subtilis*, + *Purpureocillium lilacinum*, T4-Nematicidal check (carbofuran 1kg a.i. /ha), T5-Control. Pots were placed in green house conditions at the temperature of 35 ± 2°C (Fig. 4) (Table 2).

Observations on growth parameters and nematode multiplication factors were recorded at end of 60 days. Cobb's decanting and sieving procedure (Cobb, 1918) and the Modified Baermann funnel technique were used to process the obtained soil and root samples (Schindler *et al.*, 1961). Shoot and root length, as well as shoot and root weight, were measured 60 days after planting (Table 3) (Barker, 1985).

Table 1: *M. incognita* egg hatching and juvenile mortality assay on a dual partition plate

Treatments	% Egg hatching			% Juvenile mortality		
	24hr	48hr	72hr	24hr	48hr	72hr
B.subtilis	35.66(±0.20)^{b}	76.5(±0.54)^{a}	92.54(±1.22)^{a}	53.47(±0.27)^{a}	72.20(±0.29)^{a}	95.90(±1.31)^{a}
P.lilacinum	38.33(±0.14)^{a}	74.00(±1.73)^{a}	95.00(±0.31)^{b}	58.67(±0.77)^{b}	72.00(±0.55)^{a}	95.66(±0.18)^{a}
Broth	4.33(±0.06)^{c}	11.00(±0.01)^{c}	15.00(±0.08)^{c}	0.00(±0.00)^{c}	14.00(±0.04)^{b}	22.67(±0.13)^{b}
Control	0.00(±0.00)^{d}	0.00(±0.00)^{c}	0.00(±0.00)^{d}	0.00(±0.00)^{c}	0.00(±0.00)^{c}	0.00(±0.00)^{c}
SEd	0.179	1.279	0.892	0.580	0.439	0.941
CD=0.05	0.395	2.815	1.964	1.276	0.967	2.073

*Values are mean (± Standard error) (n=3) Values followed by the same letters are not significantly different from each other according to DMRT ($p \leq 0.05$).

Table 2: Compatibility efficacy of *B. subtilis* and *P. lilacinum* on *M. incognita* in tomato under glass house.

Treatments	Growth characteristics					
	Shoot			Root		
	Length (cm)	Fresh weight (g)	Dry weight (g)	Length (cm)	Fresh weight (g)	Dry weight (g)
T_1	48.00(±0.44)^{d}	31.22(±0.57)^{d}	18.42(±0.22)^{e}	13.55(±0.23)^{d}	12.30(±0.17)^{c}	5.74(±0.01)^{c}
T_2	46.31(±014)^{e}	24.39(±0.03)^{e}	15.82(±0.05)^{f}	13.00(±0.02)^{d}	12.00(±0.21)^{d}	5.55(±0.01)^{d}
T_3	53.10(±0.85)^{c}	36.75(±0.04)b	22.75(±0.12)^{c}	15.00(±0.21)^{b}	12.30(±0.08)^{c}	6.33(±0.02)^{b}
T_4	43.52(±0.10)^{g}	21.33(±0.18)^{g}	13.56(±0.32)^{g}	11.56(±0.05)^{f}	10.20(±0.03)^{f}	5.22(±0.05)^{f}
T_5	27.00(±0.06)^{h}	21.22(±0.25)^{g}	11.20(±0.07)^{h}	9.55(±0.15)^{g}	7.50(±0.01)^{g}	4.65(±0.08)^{g}
SEd	1.2760	0.7011	0.4844	0.1428	0.2537	0.1153
CD (0.05)	2.6809	1.4729	1.0177	0.3000	0.5331	0.2423

*Values are mean (± Standard error) (n=3) Values followed by the same letters are not significantly different from each other according to DMRT ($p \leq 0.05$).

Identification of volatiles derived from host plant roots (GC/MS). After microbial consortia inoculation, three-week-old tomato plants were used to capture volatiles. Plants were then gently uprooted and their roots were washed under running tap water to remove any adhered soil particles. To improve detection of compounds identified in trace amounts by gas chromatography/mass spectrometric (GC/MS) analysis, the roots of the respective plants were cut at the base of the stem and subjected to volatile analyses under liquid nitrogen (Table 4) (Rasmann *et al*., 2005).

Statistical analysis. Each experiment was examined independently. Duncan's Multiple Range-Test (DMRT) was used to compare treatment means for analysis (Gomez and Gomez 1984). IRRISTAT version 92-1 was used by the International Rice Research Institute Biometrics unit in the Philippines.

RESULTS AND DISCUSSION

It is pivotal to investigate the potential of biological control agents in agriculture because they are highly effective, inexpensive, and have an excellent shelf life, making them a viable alternative to chemical applications for long-term disease management without pesticide residues in food.

Compatibility interaction assay. In our study organisms have positive interaction in the compatibility test, with *P. lilacinum* mycelial growth overlapping on the bacterial (2.50 cm) in dual culture technique, indicating a positive connection. Furthermore, two-organism compatibility is an important method for biocontrol technology. The metabolites released by *B. subtilis* did not inhibit the growth of *P. lilacinum* when used together in the rhizosphere, according to the results of the dual culture plate assay on PDA agar plates (Fig. 1). The compatibility interaction was also demonstrated in ICAR: A Product – Containing Consortia Formulation of *Bacillus Subtilis* (NBAIM Accession No.: NAIMCC – 01211) And *Paecilomyces lilacinus* (NBAIM Accession No.: NAIMCC – 01211). (*Purpureocillium lilacinum*- IIHR PL-2, ITCC NO. 6887). Numerous bacteria and fungi, as well as microorganism combinations, collected from field tomato plants have been shown to be effective in controlling root knot nematodes and *Fusarium* wilt in tomato plants (Wanjohi *et al*., 2018). Similarly, Zaki & Mahmood (1993) proposed that in vitro compatibility between *Bacillus* and *Purpuriocilium* had a positive interaction among the cultures, which aids in the improvement of plant growth and yield. Many bacteria associated with plants (particularly root bacteria) release indole acetic acid (IAA), which works as a signal molecule in both fungus and plants and can also cause invasive development in *Saccharomyces cerevisiae* (Prusty *et al.,* 2004). It has been widely accepted that plants benefit from the bacterial involvement in the mycorrhiza. Interactions of plants, bacteria, and mycorrhizal fungi take place in that portion of soil, where microbial processes are primarily influenced by the root, i.e. the ‘‘rhizosphere’’ (Hiltner, 1904).

Dual plate assay. In our result clearly shows that both the microorganisms produced nematicidial volatiles can inhibit the egg hatching of *B. subtilis* (92.54 %) and *P. lilacinum* (95.00%) and juvenile (J2) mortality at 95.90 % and 95.66% respectively (Table 1) (Fig. 2). According to Huang *et al.* (2010), various studies have found that bacterial culture filtrates have nematicidal activity in vitro. In a preliminary experiment, different concentrations of culture filtrate from strain *Pseudomonas putida* 1A00316 were added to 96- or 24-well tissue culture plates to test 98.77 percent nematicidal activity against M. incognita eggs or J2 juveniles. The bacteria studied have a wide range of nematicidal activity. There were 37 isolates of *Bacillus simplex*, 56 of *Bacillus subtilis*, 52 of *Bacillus weihenstephanensis*, and one each of *Stenotrophomonas maltophilia*, *Microbacterium oxydans*, *Streptomyces lateritius*, and *Serratia marcescens*. There were 49 isolates with high NA (80%), including 22 isolates with 100% NA (six B. simplex, seven *B. subtilis*, eight *B. weihenstephanensis*, and one *B. subtilis*) (Gu *et al*., 2007). Many different bacterial strains have been found to have nematicidal effects on *Meloidogyne* species, including *Pseudomonas fluorescence* (Khan *et al*. 2005).

Biocontrol of root-knot nematode on tomato in the glasshouse. Beneficial microorganisms provide a favourable environment for bacterial motility that has been proposed as a criterion for active root colonisation (Bhattacharjee *et al.*, 2012). In this present study inoculation of culture filtrates of *B. subtilis* and *P. lilacinum* giving a maximum reduction in soil nematode population up to 77.12% and 76.03% of *B. subtilis* and *P. lilacinum* respectively, whereas inoculation *B. subtilis* with *P. lilacinum* recorded critical reduction of *M. incognita* population (78.48%) (Table 3). It’s noted that shoot length (53.10%) and dry weight (6.33 %) of biocontrol applied tomato plants produced significant growth compared to control. According to El-Hadad *et al*. (2011), *B. megaterium* was the most effective strain in reducing M. incognita populations and improving tomato growth. Siddiqui and Shahid (2003) reported that the combination of seed treatment and seedling dip treatment of culture filtrate resulted in a significant reduction (58.79 percent) in root galling, followed by seeding treatment (42.88 percent) and seed treatment (40.60 percent) via culture filtrate, one at a time The reproductive factor was significantly lower (1.06) when seed treatment and seedling dip application of culture filtrate were combined than in the infected control (2.10). Bacillus spp. has been linked to a reduction in nematode invasion, according to several studies. The potential of the fungal biocontrol agent *Paecilomyces lilacinus* strain 251 (PL251) to control the root-knot nematode *Meloidogyne incognita* on tomato was investigated. When compared to the inoculated control, a pre-planting soil treatment reduced root galling by 66%, the number of egg masses by 74%, and the final nematode population in the roots by 71% in growth chamber experiments (Kiewnick and Sikora 2006). *P. lilacinus's* production of leucinotoxin, chitinases,

proteases, and acetic acid has been linked to the infection process, which ultimately resulted in aborted embryonic development via a cascade of physiological disorders (Khan *et al.*, 2006). According to Aminuzzaman *et al.* (2018), in vitro compatibility between *Bacillus* and *Purpuriocilium* demonstrated a positive interaction among the cultures and improved plant growth and yield.

Table 3: Effect of biocontrol agents on root knot nematode multiplication under pot culture condition.

Treatments	Nematode population in 200cc	Number of female/5g root	Number of egg masses/5g root	Root knot index
B.subtilis	50.33(±0.54)^{c}	28.33(±0.06)^{c}	26.33(±0.04)^{c}	1.67(±0.03)^{c}
P.lilacinum	52.66(±0.83)^{b}	26.00(±0.33)^{b}	28.33(±0.50)^{d}	1.45(±0.02)^{b}
B.subtilis+ P.lilacinum	47.33(±0.02)^{a}	20.66(±0.24)^{a}	22.66(±0.19)^{a}	1.33(±0.01)^{a}
Carbofuron	54.00(±0.62)^{b}	26.67 (±0.41)^{b}	25.00(±0.02)^{b}	1.67(±0.02)^{c}
control	220(±0.19)^{d}	67.33(±1.21)^{d}	76.33(±1.47)^{e}	5.00(±0.04)^{d}
SEd	2.57	0.45	3.90	0.05
CD=0.05	5.28	0.94	1.86	0.01

*Values are mean (± Standard error) (n=3) Values followed by the same letters are not significantly different from each other according to DMRT ($p \leq 0.05$).

GC/MS analysis of root volatiles. Coupled GC/MS used for the analysis of tomato root volatiles. With these techniques, we identified 35 volatiles compounds 4-Pentenol 2-methyl-(15.79%) (E)-, Ethyl Acetate (6.94%), Benzene (6.90%)1,3-Pentadiene (4.36%) , Trimethylbicyclo (13.39%),was the most abundant volatiles. 4-Pentenol was released by plants in biotic /abiotic stress condition plants activated indirect plant defense signalling properties (Table 4). It may triggered pathogen and nematicidal activity (Zhou *et al.*, 2022). As a result, microbial VOCs have been found to have a considerable antagonistic effect against a variety of plant nematodes, promoting development and inducing induced systemic resistance (ISR) in afflicted plants (Zhang *et al.*, 2007). Infested tomatoes treated to pure volatiles (Pentenol and Pentadiene) had a much lower number of root galls, demonstrating that VOCs can diminish *M. incognita* infectivity and reproduction. Furthermore, these findings corroborate the theory that bacterial VOCs inhibit nematode migration toward roots, reducing their ability to produce severe root gall formation and egg production in tomato roots (Poveda *et al*., 2020). According to Barros *et al.* (2014) some alcohol found in the volatiles of brococoli have been demonstrated to be harmful to plant pathogens 4-Pentenol was found to be effective in immobilising and killing *M. incognita* J2 in water, but only at high concentrations (LC50=918ppm). However, several plant VOCs act as inhibitors of *Meloidogyne* spp. in their composition (Silva *et al.*, 2018). Compounds such as dimethyl disulfide, benzaldehyde, undecanone from tomato roots also reported as nematicidal volatilome (Miguel *et al*., 2015). The volatile contents of *Linum pubescens* aerial blooming portions were extracted using hydro-distillation and analysed using GC and GC/MS, yielding 4.99 percent 1-hexanol as the main constituent. They had high antimicrobial activity against Gram-positive bacteria (Bacillus cereus ATCC11778, *Enterococcus faecalis* ATCC 29212, and *Staphylococcus aureus* ATCC 2923) and moderate activity against Gram-negative bacteria (*Escherichia coli* ATCC 25922, (Al-Qudah *et al.*, 2013).

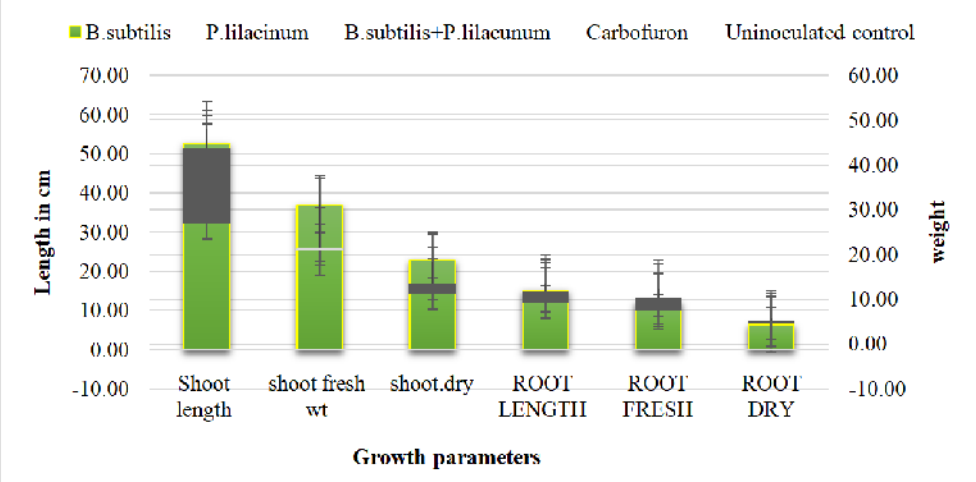
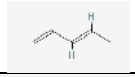
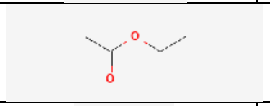
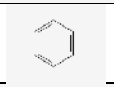
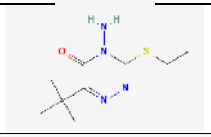
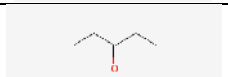
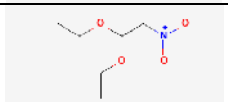
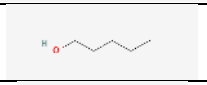
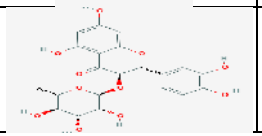
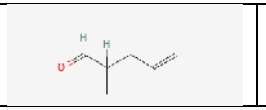
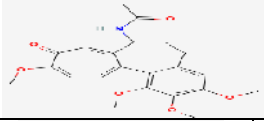
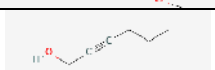
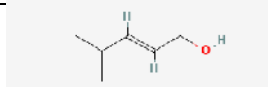
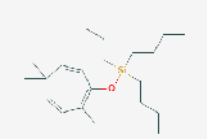
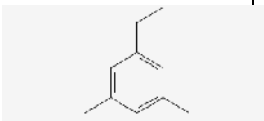
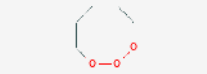
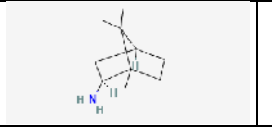
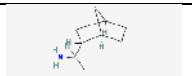
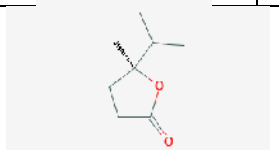
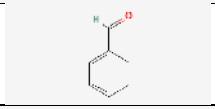
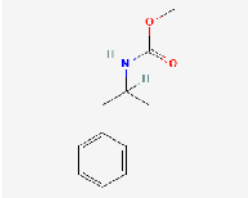
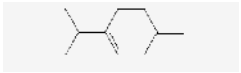
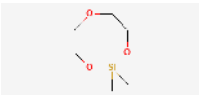
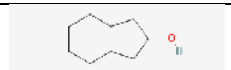
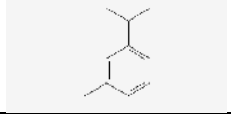


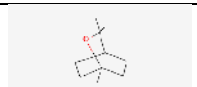
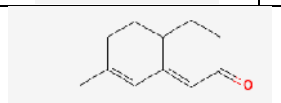
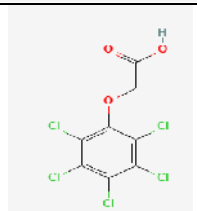
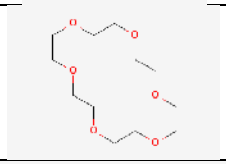
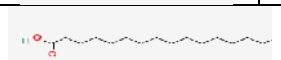
Fig. 4. Compatibility efficacy of *B. subtilis* and *P. lilacinum* on *M. incognita* in tomato under glass house.

Table 4: Metabolic profile of tomato root volatiles meadiated by *B.subtilis* and *P. lilacinum* consortia.

Sr. No.	Name of the compounds	RT	AREA %	Molecular formula	Molecular weight	Structure	FUNTION
1.	L-Alanine, 3-sulfo-	1.554	0.302	C3H7NO5S	169.16		**Antimicrobial, stress tolerance**
2.	1,3-Pentadiene	1.739	4.36	C5H8	68.12		Plant signalling
3.	Ethyl Acetate	1.974	6.949	C4H8O2	88.11		Antimicrobial
4.	Benzene	2.239	6.908	C6H6	78.11		Antimicrobial
5.	Ethiozin	2.354	2.906	C9H16N4OS	228.32		Pesticide
6.	3-Pentanone	2.414	2.628	C5H10O	86.13		Flavor and fragrance agent
7.	Ethane, 1,1-diethoxy	2.644	0.409	C6H13NO4	163.17		Antioxidant, antimicrobial
8.	1-Pentanol	2.769	0.535	C5H12O	88.15		Drought tolerance
9.	Cyclobut-1-enylmethanol	2.924	0.287	C5H8O	84.12		Antioxidant, anti inflammatory
10.	Cyclobutene, 2-propenylidene-	3.054	1.781	C7H8	92.14		Insecticide
11.	Astilbin	3.184	0.333	C21H22O11	450.4		Insecticide, anti-inflammatory

12.	4-Pentenol, 2-methyl-	3.369	15.799	C6H10O	98.14		Nematicidal, Antioxidant, anti-inflammatory
13.	Acetamide,	3.675	0.257	C22H25NO6	399.4		Anti-inflammatory, nematicide
14.	2-Hexyn-1-ol	3.97	0.545	C6H10O	98.14		Fragrance industry
15.	2-Hexenal, (E)-	4.07	0.578	C6H10O	98.14		Nematicide, antioxidant
16.	2-Penten-1-ol, 4-methyl-	4.18	0.24	C6H12O	100.16		Antioxidant
17.	Cycloheptatriene, 3,7,7-trimethyl-	4.28	0.435	C22H40OSi	348.6		
18.	m-Toluic acid, benzyl ester	4.705	0.113	C15H14O2	226.27		Plant growth regulator
19.	Benzene, 1-ethyl-3,5-dimethyl-	4.775	0.105	C10H14	134.22		Antioxidant, antibacterial
20.	Trioxocane	4.96	0.963	C5H10O3	118.13		Antimicrobial
21.	Trimethylbicyclo	5.35	13.391	C10H19N	153.26		

22.	Bicyclo	5.68	0.248	C9H17N	139.24		
23.	Furanone, dihydro-5-methyl	5.765	0.24	C8H14O2	142.2		Antioxidant
24.	Benzaldehyde	6.015	0.256	C7H6O	106.12		Antioxidant, anti-inflammatory
25.	Amphetamine, N-methoxycarbonyl	6.101	3.534	C11H15NO2	193.24		Fragrance component
26.	Cyclohexene, 4-methylene-1-(1-methylethyl)-	6.216	1.119	C10H16	136.23		Antioxidant
27.	1,3,6-Trioxa-2-silacyclooctane, 2,2,-dimethylsilyl-	6.481	0.241	C6H14O3Si	162.26		
28.	cis-8-Hydroxy-bicyclo(4,3,0)non-3-ene	6.986	0.133	C9H14O	138.21		Antibacterial activity
29.	Benzene, 1-methyl-3-(1-methylethyl)-	7.101	0.478	C10H14	134.22		Antioxidant, anti-inflammatory, antimicrobial

30.	Eucalyptol	7.256	1.1	C10H18O	154.25		Antifungal, antibacterial, antioxidant activity
31.	Naphthalenone,	7.446	0.096	C11H14O	162.23		Antimicrobial, anti-inflammatory, antitumour
32.	Acetic acid,	7.961	0.144	C8H3Cl5O3	324.4		Antioxidant
33.	Hexaoxacyclooctadecane	10.392	0.116	C12H24O6	264.31		Insecticidal, enzyme inhibition
34.	Stearic acid,	19.161	0.086	C18H36O2	284.5		Emulsifier
35.	Dimethyl	19.686	0.139	C2H6	30.07		

CONCLUSION

We offer evidence of plant performance benefits (both additive and synergistic) stemming from interacting effects between microbial inoculants. We propose that the observed beneficial effects may be due to the complimentary modes of action of their components rather than to recognised ingredients. Further field trials are necessary to fully confirm their effectiveness and potential in the field. As a result, more research is needed to look into a variety of root knot nematode management options using bio inoculant consortiums.

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Conflict of Interest. None.

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