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Effects of crop straws on root knot nematodes and soil fungi in continuous cropping of tomato

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ABSTRACT

Pot experiments were done with 3-types of straws (wheat straw, onion leaves straw and Jerusalem artichoke straw) as soil additives to determine their effects on plant nematodes. The straw application not only reduced the number of root knots in tomato, but also it also reduced the fungal species diversity. The fungal species diversity in the soil treated with onion leaves straw and Jerusalem artichoke straw differed significantly at 50 and 70 d. Furthermore, the classification analysis of the 23 fungal genera showed that four genera had an extremely positive correlation with the number of root knot nematodes and two genera had a significant positive correlation with the number of root knot nematodes.

Key words: Continuous cropping, crop straws, Jerusalem artichoke, *Meloidogyne incognita*, onion leaves, pot culture, root knot nematodes, soil, soil fungal community, *Solanum lycopersicon*, straw, tomato, wheat

INTRODUCTION

The continuous cropping of tomato (*Solanum lycopersicon* Mill) leads to serious development of root knot nematodes, which limits the growth of tomato industry. Thus, the ecological control of tomato root knot nematodes has attained importance. The extracts of African marigold (*Tagetes erecta*) (16), neem (*Azadirachta indica*) (11), *Crotalaria juncea* L. (4) and mint (1) kills the root knot nematode *Meloidogyne incognita*. Naz *et al*. (17) found that application of *Fumaria parviflora* to the soil as green manure reduced root knot nematodes and promoted the growth and yield of tomato. The application of *Streptomyces* sps could reduce root knot nematide disease in tomato by activating the systemic resistance and defensive mechanism against nematode infection (14). Gong *et al*. (6) reported that the addition of garlic straw to the root knot nematode infested soil and improved the soil food chain structure. Cao *et al*. (2) reported that the application of wheat straw to the soil, prevented the occurrence of root knot nematode by changing the diversity of the soil microbial community. Wen *et al.* (22) reported that maize straw compost modified the soil microbial environment and reduced the root knot nematode infection. The role of fungi in controlling the plant nematodes has also been examined. For example, application of *Paecilomyces lilacinus* reduces the root knot nematode infection in tomato (8) by infecting the larvae and adult nematodes and

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producing a chitinase (3,10). Similarly, *Verticillium chlamydosporium* parasitises the females, cysts, oocysts and eggs of the root knot nematode (20). Hence, this study aimed to examine if combined effects of crop straws and fungi could influence the root knot nematodes of tomato.

MATERIALS AND METHODS

The study was conducted in 2016, at the Horticulture Experiment Station, Northeast Agricultural University, Harbin, China (44°04′N, E125°42′, Altitude 171.7, Annual precipitation is 519.28 mm).

Straws

Wheat straw (XM), onion leaves straw (MC) and Jerusalem artichoke straw (JY) were used. These straws were collected from our Research Farm in August 2016. After harvest, the straws were air dried at room temperature for 6 d then powdered in the grinding mill to 2 mm size and stored for later use. The tomato variety 'Dongnong 722', was provided by the Institute of Tomato in our University. The experimental soil used was from the continuous tomato cropped area from our green house.

Root knot nematodes

(i). Eggs suspension: The nematode eggs were collected from the roots of tomato plants grown in infested soil with root knot nematodes in our greenhouse. Eggs of *M. incognita* were collected as per the method of Javed *et al*. (11). Galled roots with egg masses were washed free of soil and cut into 2-cm pieces. By using an anatomical needle, the oocyst of the root knot nematodes were carefully selected and placed in a dish. The oocyst surface was then sterilized with 2% sodium hypochlorite solution for 4 min, then rinsed and diluted with distilled water in Erlenmeyer flask, to give an egg suspension of approx 20 eggs/ml and stored at 4°C for later use**.**

(ii). **Numbers in soil:** The numbers of root knot nematodes in soil samples were determined by Buchner funnel method (12). At each sampling, 50 g soil samples was wrapped with cloth gauze and placed in funnel with a rubber tube and a clip. The water was added to submerge the soil in funnel to force out the nematodes to move into the rubber tubing. After 20 h, the stop clip was slowly opened and the water droplets were collected in 5 ml centrifuge tube. The numbers of nematodes were determined microscopically by using a 40X magnification.

(iii). **Numbers of root knots in tomato roots :** At each sampling, 100 ml water was poured to loosen the soil in pots. Three tomato plants were randomly selected and uprooted from each treatment. The adhearing soil was removed by gentle shaking of tomato roots, rinsed carefully with water until there was no soil left. The root surface was dried with absorbent paper and the number of root knots on the roots were counted.

Pot experiment: Pot experiment was done in our greenhouse. There were 4-experimental treatments: Control, Wheat straw, onion leaves straw and Jerusalem artichoke straw. The

treatments were replicated thrice in complete randomized design. The powdered straws were mixed individually with continuously cropped tomato soil at 2% (w/w). The soil without straw served as control. The soil: straw mixture (1 kg) was then put into plastic pots (14 cms × 16 cms) and kept for 10 d with watering. Tomato seedlings at 2-leaves stage were then transplanted at one seedlings per pot. Seven days after planting, four holes were made around each tomato seedling and 25 ml root knot nematode egg suspension was inoculated into each hole. Sampling was done at 30, 50 and 70 d after inoculation. Adequate amount of water was added to each pot before sampling, to ensure that the soil was free flowing and facilitated sieving. Three tomato seedlings were randomly selected to determine the number of knots on the roots. The soil from the pots was then passed through a 20-mesh sieve and stored in -80°C freezer for subsequent high-throughput sequencing.

Fungal communities in soil : Total DNA was extracted from 0.5 g aliquots of soil samples stored at -8 ^{0}C with a Fast DNA SPIN Kit for Soil (Bio 101, Carlsbad, CA, USA), with three replicates for each sample. The quality and concentration of DNA were verified by 1% agarose gel electrophoresis and a NanoDrop 2000. ultra micro UV spectrophotometer. The fungal primer pair was ITS1F (5'-CTT GGT CAT TTA GAG GAA GTA A-3') and ITS2 (5'-GCT GCG TTC TTC ATC GAT GC-3') (15). The PCR mixture (20 µl) contained 5× FastPfu buffer (4 µl), 2.5 mM dNTPs (2 µl), 5 µM of each primer (0.8 ml), 10 ng of DNA (variable), FastPfu polymerase (0.4 ml) and ddH_2O (TransGen Biotech, Beijing, China). The amplification programs were as follows: 95 °C for 3 min, then 27 cycles of 95 °C for 30 sec, 55 °C for 30 sec and 72 °C for 30 sec, with a final extension at 72 °C for 10 min and incubation at 10°C until halted by the user. However, for the fungal amplification, the number of cycles was increased to 30, but other steps remained the same.

The purified PCR products from different soil samples were pooled in equal quantities for pyrosequencing in one running time, which was done at Shanghai Majorbio Bio-pharm Biotechnology Co., Ltd. The fragments were sequenced using the Illumina PE300 Miseq platform.

Chemical analysis of soil extracts: The soil-straw water extracts were prepared at 70 d after inoculation and analysed for various phenolics using GCMS. The extracts were prepared by mixing the soil sample with water in 1:1 ratio (sample 20 g and water 20 ml) in a flask and placed on a shaker (120 rpm) for 24 h. The mixture was then centrifuged at 5000 rpm for 5 min and the supernatant was filtered through 0.45 µ membrane filter and diluted to 1/10 with butanol. All the filtered soil extract samples were subjected to GC-MS analysis fitted with a HP-5 MS capillary column (30m × 0.25 mm × 0.25 µm).

The GC-MS analyses were performed with a Gas Chromatograph Mass Spectrometer (20). Initially the oven temperature was at 40 ^{o}C for 2 min and then was gradually increased to 250 °C at 6 °C /min and this temperature was maintained for 30 min. All mass spectra were acquired in electron impact ionization (EI) mode (scan range m/z = 45-400, 5 scan/s, multiplier voltage 800 V, ionization energy 70 eV). Chromatogram peaks were identified by a mass spectra database search (NIST 98) and

each compound was identified by comparing their retention indices (RI), relative to C6-C18 n-alkanes.

Statistical analysis: Graphical and statistical analyses were done using Origin Pro 8.5.1 software, Microsoft Excel 97-2003 and SPSS 19.0 software; SAS 9.1 software was used to analyse for significant differences.

RESULTS AND DISCUSSION

Number of root knots on tomato plants

The number of root knots on the tomato plant roots from different treatments at various stages after the inoculation, were lower than control (Figure 1). Except the wheat straw treatment at 30 d after inoculation, all other treatments significantly decreased the number of root knots than control. This indicated that the straws reduced the nematodes infection of the tomato plants.

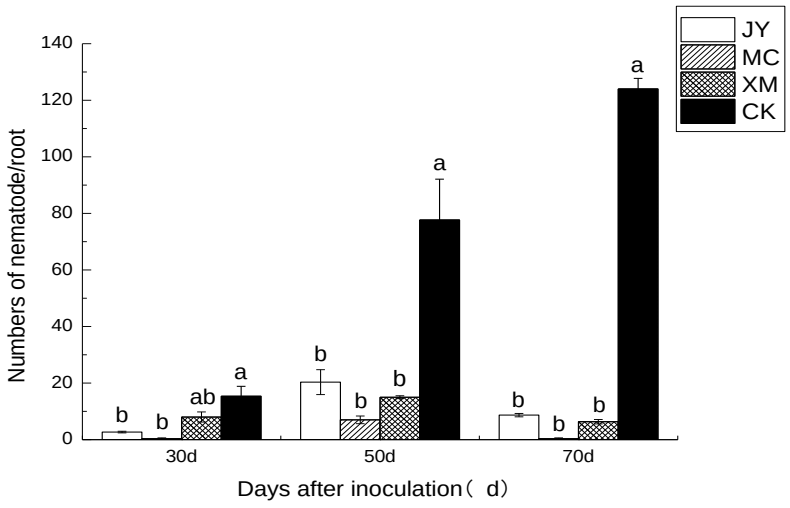

Figure 1. Effects of various straws on the number of root knots on roots at different sampling dates. CK:Control, JY: Jerusalem artichoke straw , MC: Onion leaves straw, XM: Wheat straw.
Data for each sampling date are the means of three replications. Vertical error bars and the letters (a-b) represent the standard errors of the differences of means ($p<0.05$).

Among the treatments, the onion leaves straw proved the best. Previous studies have shown that the application of garlic straw significantly reduces the intensity of root knot nematodes in tomato (5). The onion and garlic are related plants. The onion leaves straw suppressed the nematodes may be due to the fact that it not only provides a carbon source but also contains substances that are toxic to the nematodes.

Number of nematodes in soil

With the increase in inoculation time, the number of nematodes in soil treated with different plant straws, were lower than in control (Figure 2). This appears to be due to the decomposition of the straws and release of the organic chemicals which were harmful to the nematodes and also due to the microbiological changes brought about the addition of the straws. Thus addition of plant straws reduced the number of nematodes in the soil and the number of root knots on tomato plant roots.

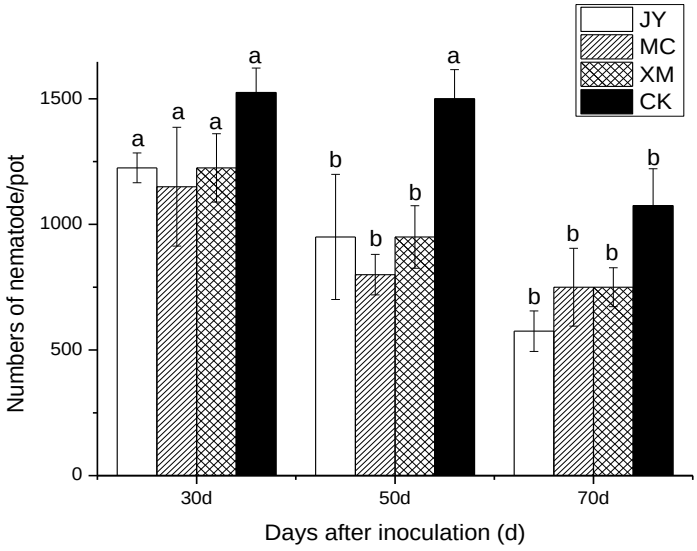

Figure 2. Effects of various straws on the number of nematodes in soil at different sampling dates. CK:Control, JY: Jerusalem artichoke straw , MC: Onion leaves straw, XM: Wheat straw. Data for each sampling date are the means of three replications. Vertical error bars and the letters (a-b) represent the standard errors of the differences of means ($p<0.05$).

The incorporation of plant straws into infected soil with root knot nematodes reduced the nematode numbers, which could be used as low-cost technology to control the root knot nematode. It is likely that the allelochemicals present in the plant residues and the abundant carbon content of the plant straws, improves the food chain structure in soil, which allows the soil ecosystem to change and that inhibits the root knot nematodes. This approach appears to be sustainable and effective in controlling the nematodes in soils.

Operational taxonomic unit (OTU) number and diversity indices of fungi

The high-throughput sequencing results with the library coverage of all samples, reached 99% (Table 1). During the sampling period, the Ace and Chao index, application of straws showed a tendency to reduce the fungal diversity significantly and also changed the structure of fungal community than in the control.

The analysis of fungal diversity in various treatments showed that the application of plant straws reduced the fungal diversity during all sampling periods. This is perhaps due to the fact that the plant allelochemicals released during the straw decomposition were detrimental to certain fungi. This is confirmed by the observation that although the application of straws reduced the fungal diversity, but it increased the number of Basidiomycetes that were beneficial to suppress the nematodes.

Table 1. Comparison of phylotype coverage and diversity indices of fungal community using 454 pyrosequencing analysis

Straw Treatments	Reads	OTUs	Ace	Chao	Coverage	Shannon	Simpson
	30 days after sowing						
Conrol	33334±844a	346±21a	441±22a	437±20a	0.997059	2.96±0.09a	0.1008±0.008a
Jerusalem artichoke	28796±2066a	236±9a	321±13a	326±3a	0.997276	2.32±0.14a	0.2061±0.029a
Onion leaves	37714±1156a	295±15a	341±13a	344±11a	0.998366	2.94±0.09a	0.1006±0.008a
Wheat	30564±4442a	270±36a	354±49a	349±59a	0.997388	2.55±0.19a	0.1812±0.042a
	50 days after sowing						
Conrol	34864±1802a	368±15a	468±11a	458±11a	0.997032	2.95±0.08a	0.1032±0.006a
Jerusalem artichoke	30669±4566a	248±5b↓	341±10ab	336±11b↓	0.997071	2.35±0.22a	0.1906±0.043a
Onion leaves	27966±3203a	243±31b↓	304±26b↓	312±28b↓	0.997691	2.73±0.32a	0.1299±0.036a
Wheat	2998±4133a	270±15b↓	385±52ab	350±37ab	0.997265	2.74±0.06a	0.1479±0.019a
	70 days after sowing						
Conrol	30867±450a	354±12a	451±23a	450±32a	0.996774	3.05±0.07a	0.0925±0.004a
Jerusalem artichoke	32687±3075a	211±41b↓	273±44b↓	272±39b↓	0.998123	2.03±0.45a	0.2814±0.084a
Onion leaves	32106±3385a	273±21ab	339±30ab	351±37ab	0.997598	2.9±0.05a	0.1026±0.004a
Wheat	31178±1968a	278±23ab	372±19ab	354±24ab	0.997512	2.93±0.23a	0.115±0.023a

Data for each sampling date are the means of three replicates. Values (mean ± standard error) in the same column followed by different letters are significantly different ($P<0.05$, Tukey's HSD test).

Fungal density

During growth period, the application of wheat straw had significant effects on the soil fungi at phylum level (Table 2). The main affected fungi were unclassified ones, which showed a significant difference than control. During the entire sampling period, the number of basidiomycetes in all plant straws treatments were higher than in control.

The number of unclassified basidiomycetes in Jerusalem artichoke straw treatment was high. Studies have shown that Basidiomycetes have a predatory and endoparasitic effects on nematodes and reducing the number of nematodes (22). The mechanisms by which the Basidiomycetes affect the nematodes include mycelium binding, toxin production as well as parasitisation and killing of the nematodes.

Table 2. Fungal density at the phylum level in different treatments

Phylum	Straw treatments at 30,50,70 days after application on Phylum level			
	Control	Jerusalem artichoke	Onion leaves	Wheat
	30 days after sowing			
Zygomycota	11167±749a	10377±4845a	12043±2294a	14031±4791a
Ascomycota	20368±591a	9888±1781a	17816±3309a	10159±3817a
Basidiomycota	1344±224a	7890±4239a	5706±524a	4686±2050a
Fungi_unclassified	439±83b	638±253b	579±62b	1678±277a↑
Chytridiomycota	16±8a	3±1a	1570±1387a	10±4a
	50 days after sowing			
Zygomycota	12823±1521a	12519±6887a	13024±3788a	11819±5199a
Ascomycota	20188±819a	10407±3074a	11053±3964a	10137±1618a
Basidiomycota	1387±156a	6997±2878a	3494±1121a	5988±2922a
Fungi_unclassified	459±49b	743±213b	390±131b	2033±449a↑
Chytridiomycota	7±3a	3±1a	5±3a	6±5a
	70 days after sowing			
Zygomycota	10579±1113a	19391±5562a	14441±3000a	8826±2615a
Ascomycota	18566±819a	10660±6810a	13902±2865a	11815±2984a
Basidiomycota	1318±80b	1958±1286ab	3075±169ab	8249±885a↑
Fungi_unclassified	396±40a	676±377a	672±242a	2282±905a
Chytridiomycota	7±2ab	3±2b	16±1a	6±1b

Data for each sampling date are the means of three replicates. Values (mean±standard error) in the same column followed by different letters are significantly different (P<0.05, Tukey's HSD test).

Fungal density at genus level

In Jerusalem artichoke straw treatment total of 23 fungal genera were detected at all three growth stages (Table 3). The unclassified Lasiosphaeriaceae population was significantly higher than in control at 30 d and 50 d, but non-significantly higher at 70 d. The number of unclassified Basidiomycetes was significantly higher than in control at 50 d but non-significantly higher at other stages. The number of *Thelebolus* was significantly lower than control at all three growth stages. The number of Unidentified and unclassified Pyronemataceae were significantly lower than control at 30 d and 50 d but non-significantly lower at 70 d.

In onion leaves straw treatment**,** the number of *Cryptococcus* increased. The number of *Arthrographis* and *Alternaria* were significantly higher than control at 30 d, but non-significantly higher at 50 d and 70 d. The number of *Cephaliophora* and unclassified Pyronemataceae were significantly lower than control at 30 d and 50 d but non-significantly lower at 70 d. The number of *Thelebolus* was non-significantly lower at 30 d but significantly lower at 50 d and 70 d compared to control.

In wheat straw treatment, the number of *Conocybe* increased with time, showing a non-significant increase at 30 d and 50 d but a significantly increased at 70 d. The number of unclassified fungi were significantly higher than in control at 30 d and 50 d but non-significantly higher number at 70 d. The number of unclassified Plectosphaerellaceae was non-significantly higher at 30 d, but showed a significant

increase at 50 d and 70 d. The fungi which showed significant decrease after treatment with wheat straw were *Cephaliophora* and *Thelebolus*. The number of unidentified and unclassified Pyronemataceae at 30 d and 50 d was non-significantly lower at 70 d compared to control. These results show that the addition of plant straws considerably changed the soil fungal community structure.

Table 3. Inhibitory/stimulatory effects of various straws application on the fungal density at the phylum level

Fungii phylum	Straws applied		
	Jerusalem artichoke	Onion leaves	Wheat
	30 days after sowing		
Zygomycota	-6.6	+8.3	+26.2
Ascomycota	-51.4	-12.5	-50.1
Basidiomycota	+487.0	+324.5	+245.87
Fungi_unclassified	+45.3	+31.7	+282.2
Chytridiomycota	-81.2	+971.3	-37.5
	60 days after sowing		
Zygomycota	-2.3	+1.6	-7.8
Ascomycota	-48.4	-45.2	-49.8
Basidiomycota	+504.0	+151.9	+771.9
Fungi_unclassified	+61.8	-15.0	+442.9
Chytridiomycota	-6.5	-28.6	-14.3
	90 days after sowing		
Zygomycota	+83.3	+36.5	-16.5
Ascomycota	-42.6	-25.1	-36.4
Basidiomycota	+48.6	-25.1	-36.4
Fungi_unclassified	+70.7	+69.7	+47.63
Chytridiomycota	-57.2	+128.6	-14.3

-: Inhibition over control, +: Stimulation over control

The number of *Mortierella* as the dominant genus in the treatments with straws (except XM) increased compared with that in the control. It has been reported that *Mortierella* can compete for resources with pathogenic soil bacteria by producing antibiotics that inhibit the growth of the pathogenic bacteria (13). Similarly, *Fusarium* spp isolated from the root-knot nematodes (9) also produced substances that affects the nematode vitality and egg development (17). The number of *Aspergillii* after treatment with onion leaves straw, was significantly higher than in control. The *Aspergillii* is endoparasite of nematodes (22) and produce toxins that kills the nematodes (19).

Correlation analysis of fungal abundance at genus level and number of root knots

At the genus level, there were 7 types of fungi which were positively correlated with the number of root knots. Among them, *Pyrenochaeta* and Unidentified were positively correlated and *Cephaliophora*, *Thelebolus*, *Pyronemataceae* unclassified and *Sordariomycetes* unclassified had an extremely significant positive correlation. There were 16 types of fungi which were negatively correlated with number of root knots on the plant roots and the correlation coefficient was -0.24, which was not significant (Table 4).

Table 3. Effects of straw treatments on the fungal density at the genus level

Genus	Straw treatments at 30,50,70 days after application on Phylum level			
	Control	Jerusalem artichoke	Onion leaves	Wheat
	30 days after sowing			
Mortierella	11158±749a	10374±4845a	12034±2290a	14028±4791a
Sordariomycetes_unclassified	7254±523a	1171±275a	6751±1910a	3521±1774a
Ascomycota_unclassified	2653±372a	6731±1296a	1840±818a	3935±1546a
Cryptococcus	1134±149b	336±13b	5436±518a↑	725±165b
Conocybe	85±69 a	4951±4253a	6±3a	3792±1972a
Cephaliophora	4936±204a	219±54b↓	822±184b↓	263±99b↓
Fungi_unclassified	439±83b	638±253b	579±62b	1678±277a↑
Fusarium	708±76b	104±18b	748±145ab	337±107b
Basidiomycota_unclassified	26±4a	2560±1339a	77±13a	112±48a
Unidentified	813±70a	237±56b↓	826±143a	246±88b↓
Pyronemataceae_unclassified	1395±69a	50±16c↓	501±76b↓	108±51c↓
Aspergillus	25±3b	12±2b	2922±1005a↑	47±14b
Preussia	47±1b	229±27ab	60±7ab	270±12ab
Humicola	364±45ab	43±20b	265±136ab	64±39b
Acremonium	206±38a	51±20	488±170a	92±32a
Lasiosphaeriaceae_unclassified	19±8b	396±57a↑	7±2b	131±37b
Schizothecium	15±2a	155±51a	16±6a	241±77a
Pyrenochaeta	168±100a	9±2a	403±192a	18±10a
Thelebolus	319±22a	22±5c↓	224±72ab	34±13c↓
Olpidium	16±8a	3±1a	1570±1387a	10±4a
Plectosphaerellaceae_unclassified	67±5ab	16±2b	144±43ab	219±51a
Arthrographis	32±5b	10±5b	267±57a↑	17±6b
Alternaria	6±1b	3±1b	184±39a↑	22±3b
	50 days after sowing			
Mortierella	12816±1521a	12514±6886a	13022±3789a	11816±5199a
Sordariomycetes_unclassified	6593±650a	1131±134b↓	4406±1842ab	2282±314ab
Ascomycota_unclassified	2642±300a	7300±2797a	766±319a	5004±1295a
Cryptococcus	1178±127a	344±63a	3318±1053a	483±13a
Conocybe	35±14a	2257±1366a	13±6a	4947±2825a
Cephaliophora	5824±118a	139±23c↓	367±83c↓	235±52c↓
Fungi_unclassified	459±49b	743±213b	390±131b	2033±449a↑
Fusarium	727±77b	92±20b	645±360b	320±10b
Basidiomycota_unclassified	28±4b	4362±1666a↑	46±23b	500±218ab
Unidentified	975±108a	205±11b↓	494±163ab	233±70b↓
Pyronemataceae_unclassified	678±48a	48±12c↓	377±68b↓	67±18c↓
Aspergillus	28±7b	12±1b	1178±418a↑	33±4b
Preussia	56±11a	391±69a	56±29a	411±165a
Humicola	412±47ab	34±6b	106±61b	69±39b
Acremonium	216±109a	67±32a	863±649a	139±61a
Lasiosphaeriaceae_unclassified	17±3b	405±110a↑	13±2b	142±48ab
Schizothecium	27±4b	119±15ab	12±3b	225±45a↑
Pyrenochaeta	101±47a	27±12a	197±87a	26±14a
Thelebolus	339±40a	19±3b↓	113±37b↓	35±6b↓
Olpidium	7±3a	3±1a	5±3a	6±5a

Plectosphaerellaceae unclassified	98±3ab	21±3b	109±64ab	262±36a
Arthrographis	50±6a	6±0.3a	275±161a	13±6a
Alternaria	6±1a	2±1a	239±168a	26±10a
	70 days after sowing			
Mortierella	10572±1114a	19385±5565a	14438±3001a	8822±2617a
Sordariomycetes_unclassified	7104±660a	1970±1215b↓	3462±664ab	2906±1034ab
Ascomycota_unclassified	2641±268a	6163±4049a	4270±2928a	5312±1007a
Cryptococcus	1138±45a	410±139a	2933±188a	580±111a
Conocybe	16±9b	190±166b	9±7b	6757±849a↑
Cephaliophora	3259±316a	103±17b↓	693±139b↓	192±15b↓
Fungi_unclassified	396±40a	676±377a	672±242a	2282±905a
Fusarium	798±109ab	137±81b	755±245ab	348±87b
Basidiomycota_unclassified	29±1a	1320±1016a	26±3a	813±58a
Unidentified	680±17a	326±194a	473±49a	198±52a
Pyronemataceae_unclassified	1086±399a	46±23a	618±212a	51±22a
Aspergillus	18±2a	13±6a	1152±593a	50±21a
Preussia	62±4a	787±593a	166±125a	529±68a
Humicola	385±68ab	40±23b	225±71ab	54±19ab
Acremonium	216±43a	31±22a	318±171a	280±164a
Lasiosphaeriaceae_unclassified	28±12a	365±203a	57±48a	304±39a
Schizothecium	30±9a	211±152a	306±264a	264±97a
Pyrenochaeta	472±215a	21±18a	80±44a	153±125a
Thelebolus	300±7a	11±3c↓	154±32b↓	27±12c↓
Olpidium	7±2b	2±1b	16±1a	6±1b
Plectosphaerellaceae_unclassified	100±15b	26±14b	50±7b	270±60a↑
Arthrographis	49±5a	17±12a	170±76a	18±10a
Alternaria	5±3a	2±1a	112±50a	39±9a

Data for each sampling date are the means of three replicates. Values (mean±standard error) in the same column followed by different letters are significantly different ($P<0.05$, Tukey's HSD test).

Relative abundance of different fungi at genus level

Over the entire sampling period, the proportion of fungi showed an extremely significant positive correlation with the number of root knots on plant roots in soil with straws, and were lower than in the control (Table 3,4). The proportions of fungi showed negative correlation with the number of root knots on plants in the soil with straws, which were higher than control.

During the entire sampling period, the proportion of fungi showing a highly positive correlation with the number of root knots on tomato plant roots in soils with straws decreased, while the proportion of fungi showing a negative correlation with number of root knots increased. The proportion of fungi showed significantly high positive correlation with the number of root knots with Jerusalem artichoke straw, which did not change nor did the proportion of fungi showed negative correlation with the number of root knots.

Table 4. Correlation analysis of relative abundance of different fungi at the genus level and the number of root knots on tomato plants

No.	Genus (Abbreviation)	Correlation coefficient
1	*Cephaliophora* (CEPH)	0.613**
2	*Thelebolus* (THEL)	0.576**
3	Pyronemataceae_unclassified (PYRO)	0.458**
4	Sordariomycetes_unclassified (SORD)	0.393**
5	*Pyrenochaeta* (PYRE)	0.368*
6	*Unidentified* (UNID)	0.323*
7	*Humicola* (HUMI)	0.191
8	Plectosphaerellaceae_unclassified (PLEC)	-0.009
9	*Acremonium* (ACRE)	-0.022
10	*Fusarium* (FUSA)	-0.027
11	*Olpidium* (OLPI)	-0.085
12	Ascomycota_unclassified (ASCO)	-0.106
13	*Arthrographis* (ARTH)	-0.115
14	*Mortierella* (MORT)	-0.123
15	Basidiomycota_unclassified (BASI)	-0.124
16	*Conocybe* (CONO)	-0.147
17	Fungi_unclassified (FUNG)	-0.178
18	*Alternaria* (ALTE)	-0.189
19	*Aspergillus* (ASPE)	-0.2
20	*Preussia* (PREU)	-0.2
21	Lasiosphaeriaceae_unclassified (LASI)	-0.22
22	*Schizothecium* (SCHI)	-0.228
23	*Cryptococcus* (CRYP)	-0.24

: The correlation coefficient was calculated based on the CORREL function in Microsoft Excel 97-2003.

The proportion of fungi showing an extremely significant positive correlation with the number of root knots with onion leaves straw decreased, while the proportion of fungi showing a negative correlation with the number of root knots increased. The proportion of fungi showing an extremely significant positive correlation with the number of root knots in wheat straw treatment, did not change significantly, nor did the proportion of fungi showing a negative correlation with the number of root knots.

Thus, the application of plant straws could reduce the relative abundances of the fungi, which showed an extremely significant positive and significant positive correlation with the number of knots on the plant roots, thus increasing the fungi that are conducive to the occurrence of root knot disease, reducing the number of root knot in the plants and relieving the disease. Soil microbes are a large community and the number of fungi in

soil is smaller than number of bacteria and actinomycetes. Some bacteria may be used to control root knot nematode. For example, Hu *et al*. (7) isolated an endophytic *Bacillus cereus* strain which colonized the tomato and effectively controlled the *M. incognita*. In this study, we have examined only the role of fungi in affecting the root knot nematode and the role of bacteria will be investigated subsequently.

Table 5. Chemical constituent of soil extracts at 70 d after inoculation

No.	Major compounds	Control	Straw		
			Jerusalem artichoke	Onion leaves	Wheat
1	Methyldimethoxysilane	3.378	-	-	3.294
2	Methyltrimethoxysilane	-	-	-	4.123
3	Hexamethylcyclotrisiloxane	4.82	4.728	4.745	4.734
4	Octamethylcyclotetrasiloxane	6.502	6.49	6.507	6.542
5	Decamethylcyclopentasiloxane	7.83	7.835	7.829	7.835
6	Undecane	-	7.446	7.457	7.463
7	Cyclohexasiloxane	9.1	9.088	9.099	9.105
8	Hexadecane	11.005	11.728	-	-
9	Octadecane	-	10.295	-	10.295
10	Eicosane	-		10.295	-
11	Silane, dimethoxydimethyl-	-	3.194	-	-
12	Silane, trimethoxymethyl-	-	4.123	-	-
13	2-Ethylhexanol	6.9		-	-
14	6-tert-butyl-4-methylphenol	18.644	18.615	18.644	18.65
15	1-Nonadecene	-	15.142	-	15.142
16	9-Tricosene, (Z)-	-	16.441	-	16.441
17	Methyl tetradecanoate	12.04	12.012	12.011	12.011
18	Diisobutyl phthalate	13.34	13.345	13.345	13.345
19	Methyl stearate	15.508	15.497	15.508	15.508
20	Bis(2-ethylhexyl) phthalate	20.652	20.664	20.664	20.664
21	Tetramethyl silicate	-	4.545	-	4.545
22	Dibutyl phthalate	-	14.181	-	-
23	Methyl hexadecanoate	13.74	-	13.74	-
24	Methyl 2-ethylhexyl phthalate	-	14.787	-	-
25	2-Dodecen-1-yl(-)succinic anhydride	-	14.381	-	14.381

Chemical analysis of soil extracts

A wide variety of compounds were detected in the soil extracts, of which 22 compounds were identified. The identified components included alkanes, alcohols, phenolic acids, alkenes, esters, etc. (Table 5). Compared with the control, some compounds were detected only in straw treatments. These are Methyltrimethoxysilane, Undecane, Octadecane, Eicosane, Silane, dimethoxydimethyl-, Silane, trimethoxymethyl-, 1-Nonadecene, 9-Tricosene, (Z)-, Tetramethyl silicate, Dibutyl phthalate, Methyl 2-ethylhexyl phthalate and 2-Dodecen-1-yl(-)succinic anhydride. These may have a role in the fungal structure and their specific relationship needs further Investigation.

The chemicals released during the decomposition of applied crop residues and the dominating fungi during the decomposition of the plant residues may control the nematodes as evident from the chemical composition of the soil extracts and the fungal community structure. This is a preliminary study and needs more detailed studies to see the effects of allelochemicals found in the soil extracts on the soil fungal communities.

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REFERENCES

1. Caboni, P., Saba, M., Tocco, G., Casu, L., Murgia, A., Maxia, A., Menkissoglu-Spiroudi, U. and Ntalli N. (2013). Nematicidal activity of mint aqueous extracts against the root-knot nematode *Meloidogyne incognita*. *Journal of Agricultural and Food Chemistry* **61**: 9784-9788.
2. Cao, Z.P., Zhou, L.X. and Han, X.M. (2010). Controlling tomato root-knot nematode disease by incorporating winter wheat straw to soil. *Acta Ecologica Sinica* **30**: 765-773. (Chinese)
3. Castro, L.G. (1998). Cutaneous *Paecilomyces lilacinus* infection. *Journal of American Academy of Dermatology* **39**: 516-517.
4. Chavan, S.N., Shakil, N.A., Sharma, H.K., Sirohi, A., Mhatre, P.H. and Singh, K. (2013). Bioefficacy of *Crotalaria juncea* L. extracts against rice root knot nematode, *Meloidogyne graminicola*. *Annals of Plant Protection Sciences* **21**: 408-411.
5. Gong, B., Blosziesb, S., Li, X., Wei, M., Yang, F.J., Shi, Q.H. and Wang, X.F. (2013). Efficacy of garlic straw application against root-knot nematodes on tomato. *Scientia Horticulturae* **161**: 49-57.
6. Gong, B., Zhang, L.L., Sui, S.L., Wang, X.F., Wei, M., Shi, Q.H., Yang, F.J. and Li, Y. (2016). Effects of garlic straw application on controlling tomato root-knot nematode disease and rhizospheric microecology. *Scientia Agricultura Sinica* **49**: 126-134. (Chinese)
7. Hu, H.J., Chen, Y.L., Wang, Y.F., Tang, Y.Y., Chen, S.L. and Yan, S.Z.. (2017). *Endophytic Bacillus cereus* effectively controls *Meloidogyne incognita* on tomato plants through rapid rhizosphere occupation and repellent action. *Plant Disease* **90**:711-722.
8. Hano, P., and Khan, M.R. (2016). Evaluation of fungal (*Paecilomyces lilacinus*) formulations against root knot nematode infecting tomato. *Bangladesh Journal of Botany* **45**:1003-1013.
9. Jansson, H.B., Tunli, D.A. and Nordbring-Hertz, B. (1997). *Biological Control of Nematodes in Fungal Biotechnology*. Chapman & Hall, Weinheim.
10. Jatala, P., Franco, J., Vilca, A. and Cornejo, W. (1979). Non-solanaceous hosts of *Globodera* in the Andes. *Journal of Nematology* **11**: 210-211.
11. Javed, N., Gowen, S.R., El-Hassan, S.A., Inam-ul-Haq, M., Shahina, F. and Pembroke, B. (2008). Efficacy of neem (*Azadirachta indica*) formulations on biology of root-knot nematodes (*Meloidogyne javanica*) on tomato. *Crop Protection* **27**: 36-43.
12. Li, H.X., Chen, X.Y. and Hu, F. (2002). Isolation and enrichment culture of a soil bacterial-feeding nematode. *Journal of Nanjing Agricultural University* **25**(2): 71-74. (Chinese)

13. Li, R., Shen, Z.Z., Sun, L., Zhang R.F., Fu, L., Deng, X.H. and Shen, Q.R. (2016). Novel soil fumigation method for suppressing cucumber *Fusarium* wilt disease associated with soil microflora alterations. *Applied Soil Ecology* 101: 28-36.
14. Ma, Y.Y., Li, Y.L., Lai. H.X., Guo. Q., Xue, Q.H. (2017). Effects of two strains of *Streptomyces* on root-zone microbes and nematodes for biocontrol of *root-knot nematode* disease in tomato. *Applied Soil Ecology* **112** : 34-41.
15. Natarajan, N., Cork, A., Boomathi, N., Pandi, R., Velavan, S. and Dhakshnamoorthy, G. (2006). Cold aqueous extracts of African marigold, *Tagetes erecta* for control tomato root knot nematode, *Meloidogyne incognita*. *Crop Protection* **25**: 1210-1213.
16. Naz, I., Palomares-Rius, J.E., Khan, S.M., Ali, S., Ahmad, M., Ali, A. and Khan, A. (2015). Control of Southern root knot nematode *Meloidogyne incognita* (Kofoid and White) Chitwood on tomato using green manure of *Fumaria parviflora* Lam (Fumariaceae). *Crop Protection* **67**: 121-129.
17. Nitao, J.K., Meyer, S.L. and Chitwood, D.J. (1999). *In-vitro* assays of *Meloidogyne incognita* and *Heterodera glycines* for detection of nematode-antagonistic fungal compounds. *Journal of Nematology* **31:** 172-183.
18. Siddiqui, I.A., Shaukat, S.S. and Khan, A. (2004). Differential impact of some *Aspergillus* species on *Meloidogyne javanica* biocontrol by *Pseudomonas fluorescens* strain CHA0. *Letters in Applied Microbiology* **39**: 74-83.
19. Stirling, G.R. and Kerry, B.R. (1983). Antagonists of the cereal cyst nematodes *Heterodera avenae* in Australian soil. *Australian Journal of Experimental Agriculture and Animal Husbandry* **23**: 318-324.
20. Wang, H.Y. and Guo, Y.L. (2004). Rapid analysis of the volatile compounds in the rahizomes of *Rhodiola sachalinensis* and *Rhodiola sacra* by staticheadspace-gas chromatopraphy-tandem mass spectrometry. *Analytical Letters* **37:** 2151-2161.
21. Wen, D., Gong, B., Guo, W., Wang, Y.F., Yu, W.J., Shi, Q.H. and Wang, X.F. (2011). Effects of tsugae bark, corn straw and compost on tomato root-knot nematode disease prevention and soil microbes. *China Vegetables* **20**: 39-44. (Chinese).
22. Zhang, K.Q., Liu, X.Z. and Li, T.F. (2001). *Biology of Nematophagous Fungi*. Science and Technology Press, China. (Chinese).