[Original Article]

A rapid method of DNA detection of the beet cyst nematode, *Heterodera schachtii*, in infested field soil

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Infestation of vegetable fields by the beet cyst nematode, *Heterodera schachtii*, was first reported in Japan in 2017. After pesticide treatments, fields should be monitored for the possible re-establishment of the nematode population. This paper describes a rapid method of DNA detection by direct extraction of DNA from soil using saturated phosphate buffer, followed by real-time PCR using *H. schachtii*-specific primers. Accurate handling of many soil samples collected from large fields is possible; however, the method has a limited success rate of one second-stage juvenile (J2) or ten cysts per 10 g of soil, which was determined by adding J2s or cysts *in vitro*. Using this method on 141 soil samples collected from an infested field, a clear correlation between DNA level and the number of hatched J2s was found. This method enables rapid detection and semi-quantitative measurement of cyst nematodes in the soil.

Key words: soil DNA, infested field, phosphate buffer, real-time PCR, dormant cyst

INTRODUCTION

Cyst nematodes are endoparasites with relatively narrow host ranges that infect plant roots. The most economically important species are found in the genus *Heterodera*, including soybean cyst nematodes (*H. glycines*), cereal cyst nematodes (*H. avenae*), and beet cyst nematodes (*H. schachtii*), as well as the genus *Globodera*, including potato cyst nematodes (*G. pallida* and *G. rostochiensis*). These organisms induce specialized feeding structures, called syncytia, through which they can extract nutrients from their hosts. Females with fertilized eggs inside their swollen bodies eventually form cysts. Cysts fall off the roots into the soil, and after their surface cuticles harden, they protect the eggs inside for several years. In response to exudates from new host roots, second-stage juveniles (J2s) hatch from eggs inside cysts and reinitiate the infection life cycle (Lilley *et al*., 2005).

The beet cyst nematode, *H. schachtii*, was first reported in 2017 in fields of brassica located in Hara village, Nagano Prefecture, Japan (Sekimoto *et al*., 2019). Immediately after the infestation was identified, the fields were treated with pesticides, including fumigants, to exterminate the nematodes. However, cysts can survive these chemical treatments, and nematode populations in these fields can recover, especially with renewed cultivation where the vegetables become hosts for the nematodes. It is important to carefully monitor these fields for the re-establishment of nematode populations. Rapid nematode detection methods are therefore needed.

Numerous methods of identifying cyst nematodes, based on DNA extraction followed by PCR, have been described. These methods involve the separation of nematodes, either as cysts or juveniles, from the soil, followed by direct DNA extraction from nematode bodies. Specific primers have been developed for species identification. PCR-RFLP of ITS-rDNA can distinguish between the four *Heterodera* species, *H. schachtii*, *H. betae*, *H. trifolii,* and *H. medicaginis* (Amiri *et al*., 2002). DNA has also been extracted from soil samples. Species-specific primers were used in combination with real-time PCR to quantify *H. glycines* in 30 g samples of dried soil pulverized with a ball mill (Shirai and Toyota, 2019).

Methods of extracting DNA from soil samples and identifying eukaryotic organisms in these samples include environmental DNA sequencing, also called metabarcoding. Soil samples are immersed in saturated phosphate buffer (0.12 M Na_2HPO_4/NaH_2PO_4, pH 8.0) to release DNA bound to soil

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particles bridged by cations. Taberlet *et al.* (2012) used this method to extract extracellular DNA from soil samples for the identification of complex, heterogeneous plant communities in the French Alps. Saturated phosphate buffer has also been used to extract nematode DNA from soil samples (Peham *et al*., 2017; Cheng *et al*., 2018). This simple DNA extraction method was found to be as sensitive as conventional method based on DNA extraction with hexadecyltrimethylammonium bromide (CTAB), followed by DNA precipitation with polyethylene glycol 8,000 (PEG) in detecting DNA by real-time PCR (Cheng *et al*., 2018).

In this study, the phosphate buffer method was used to extract DNA from *H. shachtii*-infested field soil, and the resulting DNA was quantified by real-time PCR using originally developed species-specific primers. Because of the importance of detecting live cysts that could re-establish infestation after recultivation, the association of the detected DNA level with the number of hatched J2s in each soil sample was evaluated.

MATERIALS AND METHODS

Soil samples

Heterodera shachtii-infested fields at Hara village, Nagano Prefecture, Japan (35°57′ 51.8″ N 138°13′ 2.6″ E), which measured 42 × 20 m, were divided into 3 × 4 m test blocks separated from each other by 0.5–1.0 m wide rows. In 2018 the blocks were treated with the pesticides dazomet (96.5% active component, Basamid 30 kg/10 a; Agro Kanesho Co., Ltd.), imicyafos (1.5% active component, Nemakick 20 kg/10 a; Agro Kanesho Co., Ltd.), fosthiazate (1.5% active component, Nematorin Ace 20 kg/10 a; ISK Biosciences), or the fumigants methyl isothiocyanate and 1,3-dichloropropene (20.0% for methyl isothiocyanate and 40.0% active component for 1,3-dichloropropene, Ditrapex 30 L/10 a; Bayer CropScience), 1,3-dichloropropene (97.0% active component, D-D 20 L/10 a or 30 L/10 a; Agro Kanesho Co., Ltd.), or chloropicrin (70.0% active component, 1 tablet/30 cm^2; Nankai Chemical Co., Ltd.). Each block was then planted with a different vegetable crop, including broccoli (*Brassica oleracea* var. *italica*), cauliflower (*B. oleracea* var. *botrytis*), cabbage (*B. oleracea* var. *capitata*), Chinese cabbage (*B. pekinensis*), and spinach (*Spinacia oleracea*). As controls, certain test blocks were not treated with chemicals, and crops were either cultivated or not. Based on the treatment and the cultivated crop, the soil samples were classified as listed in Table 1.

Soil samples were collected from August to October 2018, after treatment with pesticides and subsequent vegetable

Table 1. Classification of soil samples

Treatment	Crop	No. of Samples	Sub total
dazomet	broccoli	7	
	cauliflower	2	9
chloropicrin	broccoli	3	
	cabbage	4	
	cauliflower	3	
	Chinese cabbage	1	
	spinach	1	
	no data*	6	18
1,3-dichloropropene (20 L/10 a)	Chinese cabbage	6	
	spinach	7	13
1,3-dichloropropene (30 L/10 a)	cabbage	4	
	Chinese cabbage	8	12
methyl isothiocyanate and 1,3-dichloropropene	cabbage	5	5
imicyafos	cabbage	4	
	spinach	6	10
fosthiazate	broccoli	6	
	cabbage	1	
	cauliflower	8	
	Chinese cabbage	1	16
no treatment	broccoli	2	
	cabbage	17	
	cauliflower	5	
	Chinese cabbage	9	
	spinach	12	
	no data*	2	47
no treatment	no crop	11	11
total			141

*Record has been lost.

crop cultivation. Samples of approximately 100 g each were taken from a maximum of nine spots within each test block. The soil samples were air-dried and kept at room temperature until used. As a positive control, *H. schachtii* cysts isolated from infested fields in Hara village were maintained in a 23.6 L container filled with commercial compost, Kenbyo (Yae Nogei Co. Ltd.), containing growing Brassicaceae plants (Chinese cabbage) in a growth chamber (16 h light/8 h dark, 25°C) at Ryukoku University.

DNA extraction

Ten grams of each soil sample were pulverized in a 50 mL polypropylene tube (ST-5010PPR) with a metal cone (MC-5038R) at 3,000 rpm for 10 sec using a Multi-beads Shocker MB3000 (Yasui Kikai Co.). Pulverized soils were suspended in 15 mL phosphate buffer (120 mM, pH 7.58) and incubated with rotation at 80°C for 15 min to separate soil-bound DNA. The suspended samples were separated using NucleoSpin Soil (Takara Bio Inc.), and a 2 mL aliquot of each supernatant was used for DNA purification according to the manufacturer's protocol. Extracted DNA was dissolved in a 100 µL TE buffer. As controls, J2s or cysts were collected from the control container, mixed with 10 g of freshly prepared Kenbyo soil (Yae Nogei Co. Ltd.), and DNA extracted as described above.

Real-time PCR

Real-time PCR, performed on DNA directly extracted from three replicate soil samples, was used to test for the presence of *H. schachtii*. Extracted DNA was diluted 1:10 with distilled water and used as a template in the real-time PCR with the primers Hs_C1f (5'-GACTAATGAGGATTTATGGTACAC-3') and Hs_C2r (5'-GTGCTACGACATAATAGGTATC-3') amplifying a sequence of the *H. schachtii* mitochondrial cytochrome c oxidase subunit I (COI) gene. The specificity of the amplified 159 bp fragment was confirmed by sequencing, which showed that its sequence was a 100% match with that of the *H. schachtii* COI gene with the GenBank accession number MK093165. PCR reactions were performed using a 20 µL solution containing 1 x SsoAdvanced Universal SYBR Green Supermix (Bio-Rad), 500 nM of each primer, and 2 µL of template DNA. The amplification protocol entailed an initial denaturation at 95.0°C for 2 min, followed by 40 cycles of denaturation at 95.0°C for 5 sec and annealing and extension at 60.0°C for 30 sec, and was monitored by a CFX Connect Real-Time PCR Detection System (Bio-Rad). SYBR Green fluorescence was assessed at the end of each cycle. Melt curve analysis was used to determine the presence of a single expected fragment with a melting temperature of 77.0 °C. Distilled water was used as a negative control in each real-time PCR reaction plate to confirm a no-signal start-up without the presence of a DNA template. Quantification cycle (C_q) values were calculated from the real-time PCR data following the MIQE Guidelines (Bustin *et al*., 2009).

Induction of J2s hatching from cysts

A 10 g aliquot of each soil sample was added to tap water and filtered through 500 µm and 100 µm mesh sieves to obtain a small volume of debris containing cysts. The hatching experiment was performed twice using different root diffusate solutions. To obtain root diffusate solutions, Chinese cabbage, broccoli, or cauliflower seeds were sown in 25-cell plug trays (280 × 280 mm) filled with commercial compost Kenbyo (Yae Nogei Co. Ltd.). The Chinese cabbage seedlings were grown in a glasshouse for 3 weeks, and the broccoli and cauliflower seedlings were grown for 43 days, after which each tray was submerged in tap water to a depth of about 2 cm for 24 h. The root diffusate solutions were filtered using 150-mL Sterile Analytical Filter Units (pore size 0.2 µm, Thermo Fisher Scientific) and stored at 4°C until use. Debris containing cysts was spread on 150 mm Ø filter paper, which was submerged in water for 3 days in the first experiment and for 4 weeks at room temperature (20–25°C) in the second experiment to simulate the end of dormancy. Hatched J2s were collected continuously for 2 weeks using the Baermann funnel method with Chinese cabbage root diffusate solution in the first experiment and broccoli or cauliflower root diffusate solution in the second experiment.

Statistical analysis

The correlations between C_q values and numbers of J2s were calculated using Pearson's product-moment correlation test in R (R Core Team, 2020).

RESULTS

Quantification of *H. schachtii* DNA by real-time PCR

DNA was successfully detected in 10 g of control soil containing 1, 5, or 10 J2s with average C_q values of 35.5, 36.6, or 35.6, respectively, or ten cysts with an average C_q value of 36.6 (Suppl. Table 1), but not in 10 g of soil containing one cyst (data not shown) when testing for the lowest limits of DNA detection. The range of C_q values from field soil samples was 25.0–38.6 (Suppl. Table 1), and the average C_q values associated with pesticide treatments were between 28.5 and 31.2 (Table 2).

Induction of hatching from cysts

Hatching from cysts was induced by using the root diffusate obtained from Chinese cabbage in the first experiment and broccoli or cauliflower in the second

Table 2. Average C_q values of the realtime PCR

Treatment	Average C_q
fosthiazate	28.5
imicyafos	29.2
dazomet	29.4
no treatment (with crop cultivation)	29.4
methyl isothiocyanate and 1,3-dichloropropene	29.5
chloropicrin	30.2
1,3-dichloropropene (30 L/10 a)	30.5
no treatment (no crop cultivation)	31.1
1,3-dichloropropene (20 L/10 a)	31.2

experiment. A total of 1,995 and 2,173 J2s, respectively, were hatched (Table 3). The number of hatched J2s varied between different soil samples even when treated with the same agent, with a wide range observed in both experiments (Fig. 1). Almost the same number of J2s hatched from samples from the treatment plots 'fosthiazate', 'imicyafos', 'no treatment (with crop cultivation)', and '1,3-dichloropropene (20 L/10 a)' in both experiments, but fewer J2s hatched from 'methyl isothiocyanate and 1,3-dichloropropene' and

Table 3. Number of hatched juvenilies (J2s) from 10 g soil samples

	No. samples	First experiment[a]	Second experiment[b]	Average J2s in 10 g soil
dazomet	9	91	345	24.2
methyl isothiocyanate and 1,3-dichloropropene	5	121	61	18.2
fosthiazate	16	280	260	16.9
imicyafos	10	173	164	16.9
no treatment (with crop cultivation)	47	726	720	15.4
1,3-dichloropropene (30 L/10 a)	12	288	64	14.7
1,3-dichloropropene (20 L/10 a)	13	159	173	12.8
chloropicrin	18	111	259	10.3
no treatment (no crop cultivation)	11	46	127	7.9
Total	141	1995	2173	

[a]First experiment: Root diffusate of Chinese cabbage was used.
[b]Second experiment: Root diffusate of broccoli or cauliflower was used.

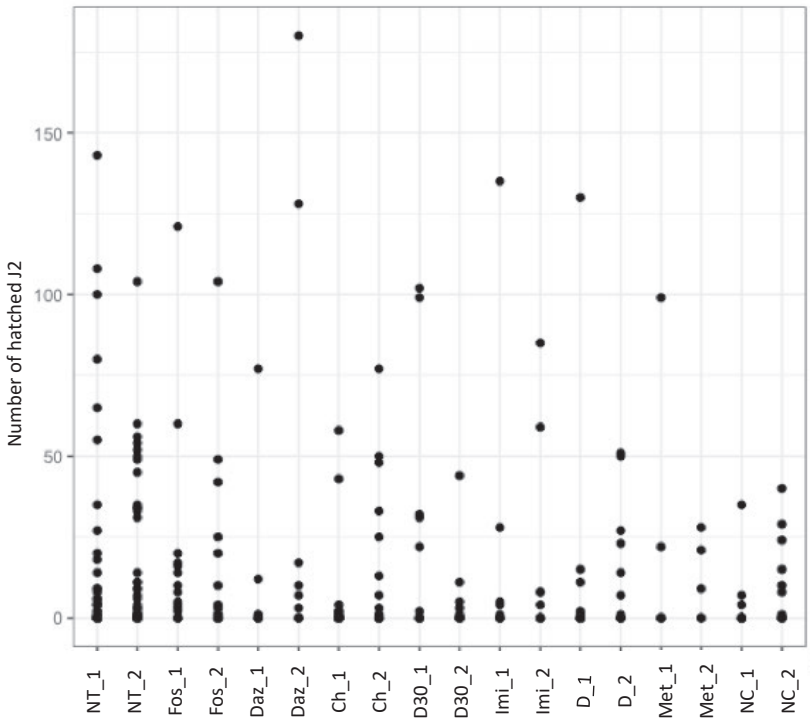

Fig. 1. Dot plot graph indicating the number of hatched J2s from each 10 g soil sample. Abbreviations are: NT, no treatment (with crop cultivation); Fos, fosthiazate; Daz, dazomet; Ch, chloropicrin; D30, 1,3-dichloropropene (30 L/10 a); Imi, imicyafos; D, 1,3-dichloropropene (20 L/10 a); Met, methyl isothiocyanate and 1,3-dichloropropene; and NC, no treatment (no crop cultivation). The numbers 1 and 2 after each type of treatment represent the first (1) and second (2) experiments, respectively.

'1,3-dichloropropene (30 L/10 a)' samples, and more J2s hatched from 'dazomet', 'chloropicrin', and 'no treatment (no crop cultivation)' samples, in the second experiment. The average number of J2s hatched from 10 g soil in the two hatching experiments is shown in Table 3.

Relation between the C_q value and the number of hatched J2s

A significant correlation between the amount of soil-detected DNA and the number of *H. schachtii* J2s hatched from these soil samples is illustrated by the correlation coefficient between the C_q value and the number of hatched J2s, which is -0.67 ($P < 0.05$) (Fig. 2).

DISCUSSION

The re-establishment of *H. schachtii* populations is a threat to vegetable growers aiming to restart cultivation. Compared with the separation of cysts from the soil, the direct extraction of DNA from soil samples is a less laborious method to handle many samples. The present study evaluated the feasibility of detecting DNA in 141 soil samples collected after pesticide treatment of *H. schachtii*-infested fields in Hara village, Nagano Prefecture.

The level of DNA detection was quantified using the C_q value of real-time PCR analysis. One J2 per 10 g of soil from the control experiment was detected, resulting in an average C_q value of 35.5. Average C_q values for 5 and 10 J2s were 36.6 and 35.6, respectively, indicating that these low-density samples do not provide accurate quantitative data. The ability to detect DNA was also assessed by adding cysts to the soil. Ten cysts per 10 g of soil yielded an average C_q value of 36.6. However, DNA could not be detected when one cyst was mixed with 10 g of soil. These results suggested that when soil samples were pulverized, J2s were more likely to be damaged than cysts, which have hard cuticle surfaces. The C_q values of field soil samples ranged from 25.0–38.6 (Suppl. Table 1), indicating that this method not only detects the presence of *H. schachtii* DNA but also enables its quantitation. Because of the importance of evaluating the effects of chemical treatments, DNA levels were compared with the number of J2s hatched from treated soil samples.

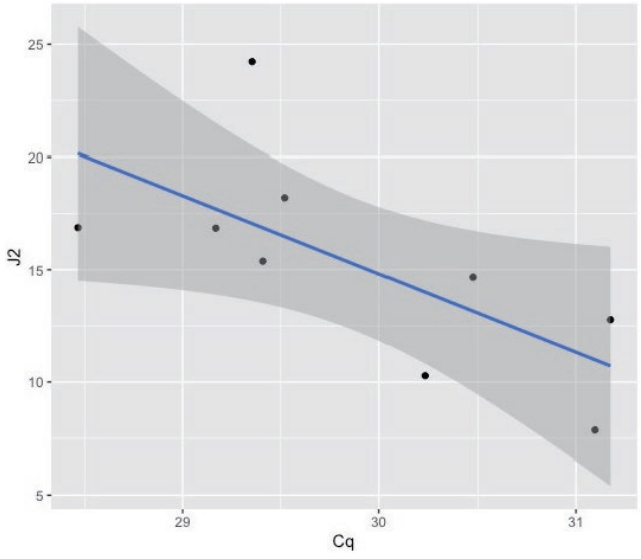

Fig. 2. Dot plot graph indicating the correlation between the real-time PCR results (C_q values) and number of hatched J2s from 10 g soil samples with respect to the treatment types. The blue line indicates the Pearson's correlation coefficient of -0.67 ($P < 0.05$), with 95% confidence intervals shown as dark gray area.

To assess the relationship between the amount of detected DNA and the potential reestablishment level, hatching was induced in the 141 soil samples. The numbers of J2s hatching from field soil samples varied widely (Fig. 1). Comparisons of the numbers of J2s hatched from the various soil samples using the Tukey-Kramer test showed no significant difference between any two pairs of test samples (data not shown). These results suggest that it is difficult to determine the reestablishment level based on hatching experiments alone. The differences in hatching may have been due to the low density of live cysts in the soil samples or to difficulties in synchronizing *H. schachtii* hatching, as their survival strategy includes the retention of part of the population in diapause for years to overcome environmental changes. For example, immersion of cysts in root diffusate or 5 mM zinc chloride for 12 weeks resulted in the emergence of juveniles from 83% and 87% of these cysts, respectively (Steele *et al*., 1982). Recently, when testing *H. schachtii* hatching efficiency in 4 cm-deep soil treated with sugar beet exudate, Ngala *et al.* (2021) found that 80% of eggs per cyst hatched. Although these studies evaluated newly formed cysts, the hatching rate did not reach 100%. The soil samples used in our study were collected in 2018 and kept dry for 2 years before hatching was induced. The storage conditions did not favor the hatching of J2s, indicating that they might have become dormant. Future studies are needed to examine the dormancy state of cysts in field soil samples and the conditions for ending dormancy.

The DNA extraction method using saturated phosphate buffer was initially designed to isolate extracellular DNA from soil (Taberlet *et al*., 2012). In the present study, this method was used to detect DNA in control soil samples spiked with cysts *in vitro*. The level of detected DNA was associated with the number of hatched J2s in field soil samples, and the C_q value range was 28.5–31.2 (Table 2 and Fig. 2). The pulverization step was effective in pulverizing cysts and their eggs and was followed by efficient separation

of DNA in phosphate buffer. The results suggest that this method can rapidly detect *H. schachtii* DNA in infested field soil and provide semi-quantitative information, enabling a rough estimation of the infestation level while handling many soil samples.

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Supplemental Table 1. Complete list of the realtime PCR results

ID	C_q_rep1	C_q_rep2	C_q_rep3	C_q_average	Treatment	Crop
3-1-Q7	27.5	27.3	27.1	27.3	methyl isothiocyanate and 1,3-dichloropropene	cabbage
3-1-Q5	27.7	27.8	27.4	27.6	fosthiazate	broccoli
3-1-P8	27.8	28.1	28.0	28.0	no treatment (with crop cultivation)	broccoli
3-2-L5	28.2	28.3	28.4	28.3	fosthiazate	broccoli
3-1-P5	27.7	27.6	27.5	27.6	imicyafos	spinach
9-C7	29.7	28.9	30.0	29.5	chloropicrin	broccoli
9-M7	28.8	29.1	28.7	28.8	no treatment (with crop cultivation)	broccoli
3-1-P6	26.4	26.5	26.6	26.5	no treatment (with crop cultivation)	cabbage
9-J7	33.1	33.3	34.1	33.5	no treatment (with crop cultivation)	cabbage
3-2-N7	28.4	28.3	28.5	28.4	1,3-dichloropropene (30 L /10 a)	cabbage
9-P7	32.4	33.0	32.1	32.5	no treatment (with crop cultivation)	cabbage
3-2-N6	28.9	29.5	29.3	29.2	1,3-dichloropropene (20 L /10 a)	Chinese cabbage
9-I7	30.1	30.0	30.3	30.1	chloropicrin	broccoli
12-1-H6	30.9	31.1	30.5	30.8	1,3-dichloropropene (20 L /10 a)	Chinese cabbage
12-1-B6	31.1	31.2	31.3	31.2	1,3-dichloropropene (20 L /10 a)	Chinese cabbage
9-C5	26.3	26.0	26.2	26.2	no treatment (with crop cultivation)	cabbage
9-I5	25.7	25.3	25.8	25.6	no treatment (with crop cultivation)	cabbage
10-H7	27.8	28.1	28.5	28.1	1,3-dichloropropene (30 L /10 a)	cabbage
3-2-M6	27.4	27.5	27.6	27.5	dazomet	broccoli
10-Q3	28.2	27.8	27.6	27.9	fosthiazate	broccoli
10-E3	35.2	37.3	33.9	35.5	fosthiazate	broccoli
10-B7	35.3	32.3	33.4	33.7	1,3-dichloropropene (30 L /10 a)	cabbage
9-O5	25.9	25.5	25.7	25.7	no treatment (with crop cultivation)	cabbage
3-2-L7	30.2	30.3	30.4	30.3	methyl isothiocyanate and 1,3-dichloropropene	cabbage
9-O7	31.5	29.3	29.5	30.1	chloropicrin	broccoli
10-D6	30.3	29.9	30.1	30.1	imicyafos	spinach
10-P7	30.0	30.4	30.0	30.1	no treatment (with crop cultivation)	cabbage
10-D7	29.7	29.3	29.6	29.5	no treatment (with crop cultivation)	cabbage
9-M5	29.7	29.7	29.6	29.7	dazomet	broccoli
3-1-O8	28.6	28.6	28.4	28.5	chloropicrin	cabbage
10-C5	32.8	32.5	32.2	32.5	no treatment (with crop cultivation)	cabbage
10-I5	34.8	35.6	35.4	35.3	no treatment (with crop cultivation)	cabbage
9-C7	25.5	25.3	25.3	25.4	chloropicrin	cabbage
9-I7	30.0	30.3	30.3	30.2	chloropicrin	cabbage
9-O7	29.1	28.9	28.7	28.9	chloropicrin	cabbage
11-I5	30.7	30.5	29.8	30.3	no treatment (with crop cultivation)	cabbage
9-O7	34.2	33.3	34.3	33.9	chloropicrin	cauliflower
11-D1	30.3	30.0	30.1	30.1	no treatment (with crop cultivation)	cabbage
11-C5	31.5	31.3	31.2	31.3	no treatment (with crop cultivation)	cabbage
11-P1	30.0	29.8	29.8	29.9	no treatment (with crop cultivation)	cabbage
9-C7	36.8	ND	35.6	36.2	chloropicrin	cauliflower
11-J1	29.7	29.7	29.8	29.7	no treatment (with crop cultivation)	cabbage
9-I7	27.4	27.2	27.4	27.3	chloropicrin	cauliflower
9-I8	27.8	27.9	27.9	27.9	chloropicrin	Chinese cabbage
3-1-O7	28.8	29.0	29.1	28.9	chloropicrin	no_data
1-K1	28.2	28.5	28.7	28.5	no treatment (with crop cultivation)	cabbage
10-N7	31.8	31.5	31.4	31.6	1,3-dichloropropene (30 L /10 a)	cabbage
1-E1	30.3	30.3	30.2	30.3	no treatment (with crop cultivation)	cabbage
3-2-M7	29.0	28.9	28.1	28.7	no treatment (with crop cultivation)	cauliflower
1-J3	30.7	30.5	30.7	30.6	imicyafos	spinach
12-1-N6	29.8	30.1	30.0	30.0	1,3-dichloropropene (20 L /10 a)	Chinese cabbage
10-K3	29.5	29.2	29.4	29.4	fosthiazate	broccoli
9-M8	29.5	29.4	29.1	29.3	no treatment (with crop cultivation)	cauliflower
12-1-L5	29.8	30.4	29.4	29.9	fosthiazate	broccoli
1-D3	29.5	29.2	29.3	29.3	imicyafos	spinach
12-2-H6	27.7	28.1	28.1	27.9	1,3-dichloropropene (20 L /10 a)	Chinese cabbage
9-M6	28.1	27.9	28.0	28.0	no treatment (with crop cultivation)	cauliflower
1-G2	ND	28.2	28.0	28.1	no treatment (with crop cultivation)	cauliflower
1-A2	26.8	26.8	26.9	26.8	no treatment (with crop cultivation)	cauliflower
1-P3	27.6	27.9	28.1	27.9	imicyafos	spinach
1-Q1	28.1	27.6	27.9	27.9	fosthiazate	cabbage
3-2-M8	29.0	28.5	28.7	28.7	no treatment (with crop cultivation)	Chinese cabbage
12-1-O7	31.7	31.6	31.6	31.6	chloropicrin	no_data
3-1-Q8	28.4	28.4	28.3	28.4	1,3-dichloropropene (30 L /10 a)	Chinese cabbage
3-1-Q6	26.3	26.9	26.7	26.6	fosthiazate	cauliflower
3-2-L8	28.4	28.9	28.9	28.7	1,3-dichloropropene (30 L /10 a)	Chinese cabbage
12-2-B6	29.4	29.3	29.4	29.4	1,3-dichloropropene (20 L /10 a)	Chinese cabbage
3-2-N5	30.5	30.1	30.2	30.2	no treatment (with crop cultivation)	Chinese cabbage
3-2-L6	28.0	27.5	27.4	27.6	fosthiazate	cauliflower
9-O8	24.9	25.1	25.2	25.0	no treatment (with crop cultivation)	Chinese cabbage
9-C8	27.5	27.4	28.1	27.7	no treatment (with crop cultivation)	Chinese cabbage
12-1-N5	29.9	29.5	29.7	29.7	no treatment (with crop cultivation)	Chinese cabbage
11-F2	32.3	32.1	32.8	32.4	no treatment (no crop cultivation)	no_crop
12-1-B5	30.4	30.1	30.6	30.3	no treatment (with crop cultivation)	Chinese cabbage
12-1-H5	32.0	31.8	32.6	32.1	no treatment (with crop cultivation)	Chinese cabbage
12-2-H5	28.6	28.6	28.8	28.7	no treatment (with crop cultivation)	Chinese cabbage

ID	C_q_rep1	C_q_rep2	C_q_rep3	C_q_average	Treatment	Crop
1-P2	26.2	26.3	26.6	26.4	imicyafos	spinach
12-1-L7	29.3	29.3	29.0	29.2	methyl isothiocyanate and 1,3-dichloropropene	cabbage
10-K4	28.0	28.0	28.0	28.0	fosthiazate	cauliflower
12-1-L8	32.1	32.8	32.7	32.6	1,3-dichloropropene (30 L /10 a)	Chinese cabbage
3-1-B8	32.3	32.7	32.4	32.5	1,3-dichloropropene (20 L /10 a)	spinach
12-2-B5	29.6	28.7	28.6	29.0	no treatment (with crop cultivation)	Chinese cabbage
3-2-N8	29.4	29.5	29.6	29.5	1,3-dichloropropene (20 L /10 a)	spinach
12-1-G5	31.7	32.0	31.4	31.7	dazomet	broccoli
12-1-Q7	28.9	28.9	28.9	28.9	methyl isothiocyanate and 1,3-dichloropropene	cabbage
1-M2	27.3	27.2	27.6	27.4	no treatment (with crop cultivation)	no_crop
12-1-E7	31.6	32.1	31.8	31.9	methyl isothiocyanate and 1,3-dichloropropene	cabbage
12-1-E8	33.9	35.3	34.8	34.7	1,3-dichloropropene (30 L /10 a)	Chinese cabbage
12-1-I7	30.2	30.1	30.5	30.2	chloropicrin	no_data
3-1-O6	30.6	30.3	30.7	30.5	no treatment (with crop cultivation)	no_data
12-1-M5	30.4	30.9	31.3	30.9	dazomet	broccoli
12-1-A5	32.0	32.1	31.9	32.0	dazomet	broccoli
12-1-C7	31.2	30.8	31.2	31.1	chloropicrin	no_data
12-2-C7	27.7	26.6	26.9	27.0	chloropicrin	no_data
12-1-Q8	29.1	29.0	28.7	28.9	1,3-dichloropropene (30 L /10 a)	Chinese cabbage
10-N8	31.8	31.8	31.5	31.7	1,3-dichloropropene (20 L /10 a)	spinach
12-2-I7	28.6	28.3	28.5	28.5	chloropicrin	no_data
12-2-G5	29.1	29.0	28.9	29.0	dazomet	broccoli
12-2-A5	27.1	26.9	27.0	27.0	dazomet	broccoli
3-1-O6	28.3	28.4	28.4	28.3	no treatment (with crop cultivation)	spinach
12-2-K6	28.8	28.8	28.6	28.7	no treatment (no crop cultivation)	no_crop
12-2-E8	27.1	27.1	27.0	27.1	1,3-dichloropropene (30 L /10 a)	Chinese cabbage
10-B8	32.9	33.1	32.7	32.9	1,3-dichloropropene (20 L /10 a)	spinach
3-1-P7	26.3	25.7	25.8	25.9	no treatment (with crop cultivation)	spinach
10-O6	ND	37.6	39.6	38.6	chloropicrin	spinach
12-2-K5	28.2	27.8	27.6	27.9	no treatment (no crop cultivation)	no_crop
10-H8	32.5	31.9	31.7	32.0	1,3-dichloropropene (20 L /10 a)	spinach
12-3-L2	29.8	29.6	29.3	29.6	no treatment (no crop cultivation)	no_crop
12-3-L1	ND	34.7	33.4	34.1	no treatment (no crop cultivation)	no_crop
12-3-L3	28.1	28.3	28.3	28.3	no treatment (no crop cultivation)	no_crop
12-4-L1	33.6	34.0	33.7	33.8	no treatment (no crop cultivation)	no_crop
12-4-K6	31.4	32.2	31.6	31.8	no treatment (no crop cultivation)	no_crop
12-4-L2	31.1	31.9	32.2	31.7	no treatment (no crop cultivation)	no_crop
12-4-L3	31.6	31.6	32.4	31.9	no treatment (no crop cultivation)	no_crop
12-4-K5	32.0	32.0	32.0	32.0	no treatment (no crop cultivation)	no_crop
1-N8	34.2	34.7	34.1	34.3	1,3-dichloropropene (20 L /10 a)	spinach
10-Q4	26.8	26.8	27.0	26.9	fosthiazate	cauliflower
1-H8	33.7	34.3	33.7	33.9	1,3-dichloropropene (20 L /10 a)	spinach
1-K4	27.2	27.2	27.4	27.2	fosthiazate	cauliflower
1-Q4	28.6	28.9	28.8	28.8	fosthiazate	cauliflower
9-J5	30.4	30.4	30.5	30.4	imicyafos	cabbage
10-P5	28.1	27.9	28.0	28.0	imicyafos	cabbage
1-K2	31.1	32.0	32.0	31.7	1,3-dichloropropene (30 L /10 a)	Chinese cabbage
10-C6	31.4	31.9	31.6	31.6	no treatment (with crop cultivation)	spinach
10-D8	30.7	31.4	30.7	31.0	no treatment (with crop cultivation)	spinach
1-E4	28.3	28.8	29.0	28.7	fosthiazate	cauliflower
1-Q4	ND	25.1	25.2	25.1	fosthiazate	cauliflower
1-A4	ND	27.0	27.1	27.1	dazomet	cauliflower
10-P8	29.2	28.5	29.1	28.9	no treatment (with crop cultivation)	spinach
1-M4	29.3	29.3	29.3	29.3	dazomet	cauliflower
10-D5	29.1	29.3	29.1	29.1	imicyafos	cabbage
1-E2	31.6	32.0	31.8	31.8	1,3-dichloropropene (30 L /10 a)	Chinese cabbage
11-D5	32.4	32.3	32.2	32.3	imicyafos	cabbage
10-P6	29.6	29.5	30.0	29.7	no treatment (with crop cultivation)	spinach
1-Q2	29.9	30.2	30.3	30.1	fosthiazate	Chinese cabbage
10-I6	31.5	31.8	31.3	31.5	no treatment (with crop cultivation)	spinach
1-P8	31.1	31.6	31.1	31.3	no treatment (with crop cultivation)	spinach
1-C6	29.5	29.5	29.4	29.5	no treatment (with crop cultivation)	spinach
1-J2	30.9	31.0	31.2	31.0	no treatment (with crop cultivation)	spinach
1-D2	29.0	28.8	28.7	28.8	no treatment (with crop cultivation)	spinach
1-I6	30.0	30.1	29.5	29.9	no treatment (with crop cultivation)	spinach
Control_J2_1	35.3	37.1	34.2	35.5	1 J2 added to 10 g Kenbyo Soil	
Control_J2_2	34.6	36.3	35.4	35.4	1 J2 added to 10 g Kenbyo Soil	
Control_J2_3	36.2	37.0	35.2	36.1	5 J2 added to 10 g Kenbyo Soil	
Control_J2_4	36.6	37.9	36.5	37.0	5 J2 added to 10 g Kenbyo Soil	
Control_J2_5	35.8	36.6	35.4	35.9	10 J2 added to 10 g Kenbyo Soil	
Control_J2_6	36.0	ND	34.6	35.3	10 J2 added to 10 g Kenbyo Soil	
Control_soil_1	32.4	ND	34.4	33.4	10 cysts added to 10 g Kenbyo Soil	
Control_soil_2	38.2	37.4	37.6	37.7	10 cysts added to 10 g Kenbyo Soil	
Control_soil_3	38.3	ND	38.0	38.2	10 cysts added to 10 g Kenbyo Soil	
Control_soil_4	37.0	ND	37.3	37.2	10 cysts added to 10 g Kenbyo Soil	

ND: no data