The combined effect of chemical nematicides and biofumigation on the control of *Meloidogyne incognita* in glasshouse tomato

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Summary. One trial was conducted to compare the effect of different chemical nematicides in combination with biofumigation on the control of *Meloidogyne incognita* on tomato plant in commercial glasshouses in both 2008 and 2009. Dazomet and avermectin applied separately or in combination with biofumigation were effective in reducing population density of *M. incognita* and root-galling index. Biofumigation with chicken manures and leaf mustard residuals reduced population density of the nematode and damage level to tomato, but the control effect of biofumigation was inferior to other treatments except the control. The tomato yield in dazomet or avermectine treatments with/without biofumigation was increased by up to about 30% and indicates a promising approach for tomato producers to manage root-knot nematodes. In general, the combination of avermectin with biofumigation could decrease the dose of chemical nematicides and appeared to be the most economical root-knot nematode control method.

Key words: Avermectin, biofumigation, dazomet, fosthiazate, *Meloidogyne* species.

Meloidogyne incognita is one of the most frequently observed root-knot nematodes (RKN) on glasshouse tomato plants in the northern part of China. It has detrimental effects in terms of both quantity and quality of tomatoes (Wang *et al*., 2007). The nematode is highly resistant to different climatic conditions and can survive in a wide range of soil moistures and temperatures (Sasser, 1979; Zhang *et al*., 1998). Control of plant-parasitic nematodes is difficult due to the unavailability of effective control methods and monitoring systems compared with those available for foliar diseases and insect pests (Oka *et al*., 2007). Wide host ranges and different biological races exist within RKN, so rotation, soil solarisation, biological control and soil amendment do not control species effectively (Kerry, 1990; McSorley *et al*., 1998; Ioannou, 2000). Biofumigation is an agronomic practice of using volatile chemicals, released from decomposing plant tissues and mixture of manures, to suppress soil-borne pests and pathogens (Li *et al*., 2007). Glucosinolate is a class of organic molecules that can be hydrolysed into isothiocyanates. It can be derived from the family Brassicaceae of some crop plants and acts as a chemical fumigant to control various soil-borne plant pathogens (Kirkegaard & Sarwar, 1998; Li *et al*., 2007; Lazzery *et al*., 2009). Biofumigation may be economic and effective because the materials used are readily available locally, but their performance is not always predictable and the results are not always comparable to those achieved by chemical control (Giannakou & Anasta, 2005; Piedra-Buena *et al*., 2006, 2007). To date, use of chemical nematicides is still the most popular and effective method of RKN management. However, many conventional soil chemical nematicides are hazardous and not environmentally friendly. Moreover, they have negative effects on beneficial soil organisms (Ijani *et al*., 2000; Ibekwe, 2004; Pandey, 2005). In addition, the application of chemical nematicides is expensive, and therefore not cost effective for glasshouse tomato cultivation. The application of fumigants also requires special techniques and equipment such as gas masks and polyethylene film. In order to increase the control efficiency and to decrease the cost of chemical nematicides, biofumigation is integrated into the

soil-borne disease control strategies. However, knowledge of how to combine such application effectively remains to be discovered in China. Therefore, evaluation of root-knot nematodes management by combining chemical nematicides with biofumigation is needed before it can be adopted on a widespread basis.

The purpose of this study was to evaluate the efficacy of different chemicals in combination with biofumigation on managing *Meloidogyne incognita* in glasshouse tomato in the northern part of China.

MATERIAL AND METHODS

Site details. The experiment was carried out in one commercial glasshouse naturally infested with *M. incognita* in Daxing district of Beijing, China. Tomato was cultivated twice each year in this glasshouse during the last 10 years, and the levels of *M. incognita* were recorded in early 1998 (Zhang *et al*., 1998). Disease incidence of 60-80% was detected and high galling indices (2.5-4.3) were recorded in May 2008. The sandy loam soil had the following properties: pH 7.6; electrical conductivity 3.6 dS m^{-1}; NH_4^+-N content 0.9 mg kg^{-1}; NO_3^-N content 57.1 mg kg^{-1}; total N 1.27 g kg^{-1}; extractable P 283.7 mg kg^{-1}, extractable K 152.0 mg kg^{-1}, organic C 16.8 mg kg^{-1}.

Field experiments. Ambient and soil temperatures (at the depth of 5, 10, 15 cm) were monitored during the experimental period with a data logger in 2009. Plants were fertilised with a compound fertiliser (N: 20%, P: 20%, K: 10%) and irrigated with ground water every 5 days according to local agronomic practice.

The experiment was carried out from July to December in 2008 and 2009 in the same commercial glasshouse. Each plot measured approximate 12 m^2 (2.4 m×5 m) and each treatment was arranged in a randomised complete block design with four replicates. Chemical treatments included avermectin (AVE) 1.8% EC (Zhongnongda Biotechnology Ltd; 6 ml m^{-2}); dazomet (DAZ) 98% w/w granules (Nantong Shizhuang Chemical Ltd; 45 g m^{-2}); fosthiazate (FOS) 10% w/w granules (Ishihara Sangyo Kaisha Ltd; 3 g m^{-2}); biofumigation (CHM) treatments were used with mixture of chicken manures, residual leaf mustard (1:3, 6 kg m^{-2}) in combination with solarisation. Residual leaf mustard was collected from a plot outside the glasshouse. The ratio of chicken manure and leaf mustard was decided by a previous experiment. Various combinations of these management methods were also used: DAZ (30 g m^{-2}) +CHM; FOS (2.3 g m^{-2}) +CHM, AVE (4 ml m^{-2}) +CHM; there was also a control without any treatment (CONT). The chicken manure properties were pH 7.4, EC 4.9 dS m^{-1}; organic C content 436 g kg^{-1}; total N 26.3 g kg^{-1}; total P 32.1 g kg^{-1} and total K 12.7 g kg^{-1}. The pre-crop treatments of DAZ, FOS, CHM, DAZ+CHM, FOS+CHM were applied 6 weeks before tomato plants were planted. Seedlings of tomato (*Solanum lycopersicum*) cv. Monte Carlo (Beijing Zhongyanyinong Seed Ltd) were cultured in a field without root-knot nematodes. Solid chemicals or mixture of chicken manures and residuals of leaf mustard were spread uniformly and cultivated with a rotavator into the upper soil (15-20 cm depth) and irrigated with about 35-40 l water m^{-2}. Avermectin was diluted with appropriate quantity of water (35-40 l m^{-2}) and irrigated in the soil twice in total. Initially, avermectin (3 ml m^{-2} in treatment AVE and 2 ml m^{-2} in treatment AVE+CHM) was irrigated 6 weeks before tomato plants were transplanted. Two days before tomatoes were transplanted, avermectin was irrigated a second time at the same dose. Before irrigation with avermectin, a mixture of chicken manures and residuals of mustard were spread uniformly and cultivated with a rotavator into the upper soil (15-20 cm depth) in plots of AVE+CHM. All the plots except control were covered with clear polyethylene film and the edges were buried with soil to seal the film edges. Polyethylene films were removed after 40 days and the soil was then cultivated to release any fumes. The soil in plots of CONT was cultivated 2 days before transplanting. After films had been removed for 2 days, 1-month-old tomato seedlings without root-knot nematodes were transplanted at 30 cm apart within the row and 50 cm between ridges on the same day in all 32 plots.

Prior to chemical applications and other control treatments, 10 soil cores (10 cm diamond and 25 cm deep) were collected from each plot using a cylindrical sampling tube for determining nematode population densities. Soil samples were collected in the transplanting period, mid-season period (60 days after transplanting) and near harvest period (120 days after transplanting, four times in total). Second-stage juveniles (J2) were extracted from 200 g soil by the Baermann funnel method and counted under a stereoscopic microscope. At mid-season and near harvest periods, roots were randomly collected from 10 plants per plot using a garden trowel. Root-knot galling was assessed according to a 0–10 scale (Barker, 1985). Roots were carefully washed and chopped into small pieces and left in water for 2 days until the tissue softened. Females and J2 were counted and the number expressed as nematodes per g root. Mature fruits were collected and weighed every 4 days from all the plants per plot until the end of harvest.

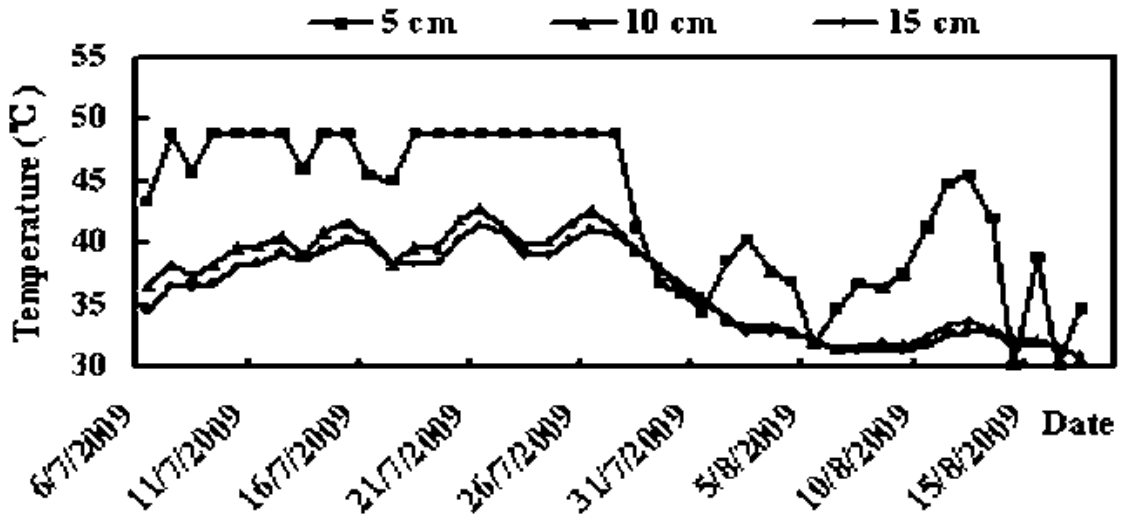

Fig. 1 Maximum soil temperatures (°C) in solarized plots during July 6 and August 18, at 5, 10, 15-cm depth.

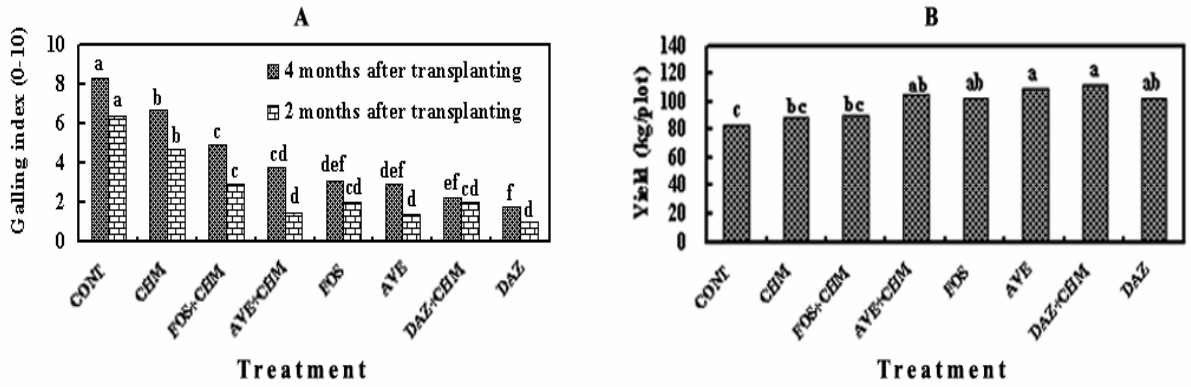

Fig. 2 Galling index (A) detected 2 months and 4 months after transplanting and fruit weight (B) of *Solanum lycopersicon* in glasshouse plots infested with *Meloidogyne incognita*.

Statistical analysis. The data were statistically analysed according to Duncan's t-test, and all analyses were performed by least significant difference (LSD) at the P=0.05 confidence level using the SAS software (SAS Institute 2001). Correlation coefficients between pairs of different variants of tomato yield, nematode population and galling index of root were also determined using SAS software.

RESULTS

The maximum soil temperatures at three soil depths in plots of CHM during July 6 and August 18 in 2009 are given in Figure 1. Soil temperature of the plots biofumigated with CHM was 3.8 to 9.2°C higher than that of control plots in the experimental period. Maximum soil temperature (48.9°C) was recorded at the 5-cm soil depth, and was 9.2°C higher than that of control plots. Temperature over 48.5°C lasted for 7.1±0.6 h during 13 days in the CHM plots. The maximum soil temperatures in biofumigation plots at the 10- and 15-cm soil depths were 42.9°C and 41.5◦C, respectively. However, in non-biofumigation plots, the corresponding soil temperatures were 39.1°C and 37.4°C, respectively. The average ambient temperature inside the glasshouse was 35.7±3.3°C during July 6 and August 18 in 2009. The average temperature in the biofumigated soils was 48.1±1.3°C at 5-cm soil depth during the first 22 days, and then gradually decreased to 37.1±3.5 °C during the next 22 days.

Soil population densities of *M. incognita* at different times as affected by the various treatments are shown in Table 1. Before application of any treatment, the

Table 1. Soil population densities of *Meloidogyne incognita* in nematicides treated and untreated tomato plots at pre-treatment, before transplanting, mid-season and near harvest.

Treatments	J2 200 cm^{-3} soil				
	T1[a]	T2	T3	T4	Treatment mean
Control	153.8(2.18) a[b]	719.3(2.84) a	1809.3(3.25) a	3818.8(3.58) a	3.21 a[c]
CHM	165.3(2.20) a	119.0(2.04) b	594.8(2.77) b	1198.0(3.07) b	2.72 b
FOS+CHM	136.5(2.14) a	31.3(1.5) c	399.3(2.60) a	845.8(2.92) bc	2.55 bc
AVE+CHM	140.5(2.15) a	16.0(1.22) de	78.5(1.87) d	271.0(2.42) d	2.12 d
DAZ+CHM	116.5(2.06) a	12.5(1.11) def	53.5(1.72) de	212.5(2.29) d	2.06 d
FOS	122.8(2.09) a	21.3(1.33) cd	351.0(2.54) c	714.0(2.84) c	2.48 bc
AVE	132.0(2.12) a	10.8(1.05) ef	71.8(1.85) d	253.5(2.39) d	2.09 d
DAZ	108.5(2.03) a	7.8(0.93) f	49.3(1.68) e	182.8(2.23) d	1.94 d
Time mean	2.12 b	1.50 c	2.29 ab	2.72 a	–
LSD values for treatment	0.19	–	–	–	–
Time	0.51	–	–	–	–

[a] T1, T2, T3 and T4 refer to pre-treatment, before transplanting, mid-season (60 days after transplanting) and near harvest (120 days after transplanting); [b] $Log10^{x}$ transformed data used in ANOVA in parenthesis; [c] In each column and row, data followed by the same letter are not significantly different according to Duncan's t-test.

Table 2. Effect of nematicides and untreated control on nematodes g^{-1} of root of tomato (*Solanum lycopersicon*) in a glasshouse at 30 days, 60 days and 120 days after transplanting.

Treatment	Nematodes g^{-1} root			
	30 days after transplanting	60 days after transplanting	120 days after transplanting	Treatment mean
CONT	25.3(1.40) a[a]	94.8(1.97) a	123.3(2.09) a	1.82 a[b]
CHM	12.8(1.09) b	26.5(1.43) b	27.5(1.44) b	1.32 b
FOS+CHM	11.8(1.05) bc	15.5(1.19) c	25.8(1.41) bc	1.21 bc
AVE+CHM	9.8(0.96) bcd	13.3(1.13) cd	20.3(1.31) bc	1.13 cd
DAZ+CHM	6.3(0.75) de	8.8(0.96) de	10.3(1.04) d	0.92 cde
FOS	7.3(0.83) bcd	12.5(1.10) cd	18.5(1.28) bc	1.08 d
AVE	7.3(0.81) cd	12.8(1.12) cd	16.8(1.24) c	1.06 d
DAZ	3.5(0.54) e	6.8(0.86) e	7.8(0.93) d	0.76 e
Time mean	0.93 b	1.22 ab	1.34 a	
LSD values for Treatment	0.12	–	–	–
Time	0.33	–	–	–

[a] $Log10^{x}$ transformed data used in ANOVA in parenthesis; [b]In each column and row, data followed by the same letter is not significantly different according to Duncan's t-test.

Table 3. Correlation coefficients between pairs of root-knot nematode populations and different variants measured on tomato (*Solanum lycopersicon*) under field conditions.

Parameter	1	2	3	4	5
1. Galling index of root at 2 months after transplanting	1.000				
2. Galling index of root at 4 months after transplanting	0.961*	1.000			
3. Number of nematodes g^{-1} root	0.867*	0.828*	1.000		
4. Number of nematodes 200 cm^{-3} soil	0.921*	0.879*	0.984*	1.000	
5. Yield (kg per plot)	-0.865*	-0.896*	-0.714*	-0.792*	1.000

* Significant at $P < 0.05$.

field was uniformly infested with root-knot nematodes. The mean population density of *M. incognita* was 135 200 cm^{-3} of soil in all treatments. There was no significant difference in the mean initial population densities in individual treatments (P<0.05). Before transplanting, the number of J2 in the control plots was higher than that of pre-treatment (T2, Table 1). The number of J2 in all the other plots before transplanting was significant lower than that of pre-treatment (P<0.05). The nematode population increased rapidly at 60 days after transplanting in the control plots, following by CHM, FOS and FOS+CHM (T3, Table 1). RKN populations increased slowly and a significantly lower mean population density was found in treatments with DOZ, DOZ+CHM, AVE and AVE+CHM compared with the control, CHM and FOS+CHM treatments (P<0.05). At the end of the cropping season (120 days after transplanting), significantly higher population densities were found relative to that of mid-season in all the treatment (P<0.05) (T4, Table 1). The highest root-knot nematode population densities were recorded in untreated plots following by plots with CHM, FOS and FOS+CHM. Significantly lower numbers of nematodes (P<0.05) were recovered in plots treated with DAZ and AVE with/without CHM compared with the control, although nematode populations in these plots had increased beyond the initial population to about 80-130 200 cm^{-3} of soil.

In two complete seasons of 2008 and 2009, significantly higher numbers of nematodes were observed in roots at 120 days after transplanting compared with those of 30 days and 60 days after transplanting (P<0.05). The highest number of nematodes (P<0.05) in roots was recorded in the control treatment at all sampling dates, followed by the CHM treatment (Table 2). The lowest number of nematodes (P<0.05) in roots was recorded in DAZ treatment in both seasons. This result was reflected in the galling index of roots at 2 and 4 months after transplanting (Fig. 2 a). At 2 months after transplanting, the galling indices of tomato plants in the control plots were higher than those in other plots (P<0.05). Galling index of plants in plots with DAZ or DAZ+CHM was significantly lower than those of control plots, CHM and FOS+CHM treatments, but was similar to those of AVE and AVE+CHM treatments (P<0.05). Four months after transplanting, the galling index of different treatments showed a similar trend to those of 2 months after transplanting (Fig. 2 a). The most effective control method against *M. incognita* in reducing root galling was DAZ and AVE, followed by DAZ+CHW and AVE+CHW. Yields in all plots with chemicals did not differ from those with chemicals in combination with CHM, but yields in the plots with AVE, DAZ, AVE+ CHM and DAZ + CHM were higher than that of control plots (Fig. 2 b). Among all the treatments, plots with DAZ+CHM resulted in the highest yield, but significant differences were observed in the control plots, CHM- and FOS+CHM-treated plots (P<0.05).

Multiple regression analysis indicated that there was a significant negative correlation (P<0.05) between tomato yield and the number of root-knot nematodes 200 cm^{-3} soil, number of nematodes per g root, and the galling index of roots at 2 and 4 months after transplanting (Table 3). In addition, there were significant positive correlations (P<0.05) between galling index and the number of nematodes in soil and in roots of tomato.

DISCUSSION

Glasshouse-grown tomato is an important crop in Beijing with a total yield of 510,000 t from 6,000 ha. The major factors restricting yield are root-knot nematodes and *Bemisia tabaci* (Wu *et al*., 2004; Wang *et al*., 2007). Previous studies revealed that dazomet, fosthiazate, metham-sodium, chloropicrin and 1, 3-dichloropropene were effective as alternatives to MeBr to control the pests and diseases of tomato, tobacco and pepper (Csinos *et*

al., 2000; Yücel *et al*., 2007a). The combinations of chemicals with biofumigation are potentially synergistic and effective in reducing soil-borne pathogens (Freeman and Katan, 1988; Gamliel and Stapleton, 1993). Soil solarisation combined with chicken manure and mustard residuals is highly pesticidal and significantly lower amounts of root-knot nematode damage were found relative to those plants grown in non-biofumigated soil. The present study indicated that dazomet and/or avermectine in combination with CHW could effectively control *M. incognita*. Low dose of DAZ (30 g m^{-2}) combined with CHW reduced nematode numbers by 80% to a level similar to that of AVE+CHM. In these plots, nematode population densities recovered slowly until tomato harvest. A related study in Turkey revealed that nematodes in tomato plots reduced from 948 to 32 J2 200 cm^{-3} soil during 5 weeks after treating with DAZ at 40 g m^{-2} combined with solarisation (Yücel *et al*., 2007b).

After chemical nematicides were applied to plots, covering the soil with plastic films trapped higher concentrations of poisonous gas, and thus enables it to diffuse in the soil more effectively. The soil temperature at 5-cm soil depth in the present study is 2°C lower than those in organic farming systems using organic amendments in the eastern Mediterranean region, but similar to those conducted with non-chemical nematicides (Giannakou *et al*., 2007; Yücel *et al*., 2007b). Soil temperature over 48.5°C at the depth of 5 cm in the present study can last 7.1 ± 0.6 h every day for 13 days. The increased soil temperatures might increase the poisonous vapour pressure and increase the number of nematodes that are killed (Oka *et al*., 2007). In the previous work, biofumigations appeared effective in controlling fungal diseases and root-knot nematodes (Stapleton *et al*., 1991; Gamliel & Stapleton, 1993; Guo, 2004; López-Pérez *et al*., 2005; Giannakou *et al*., 2007; Oka *et al*., 2007; Lazzeri *et al*., 2009). Guo (2004) found that biofumigation with chicken manures could not only decrease the hatch of *M. incognita* but also promote the growth of tomato. Solarisation combined with defatted Brassicaceae meal were reported to limit nematode infestation and resulted in plant roots with a lower root galling index (Lazzeri *et al*., 2009). Data in the present study indicate that effective soil disinfestations could be achieved by a combination of physical with chemical applications to control *M. incognita* in the northern part of China. When chemical nematicides were added to the soil combined with biofumigation, nematodes were controlled more effectively and the population recovery was slower. In addition, the tomato roots were significantly less galled under the combined treatment than with biofumigation alone.

This study showed that biofumigation combined with low doses of chemical nematicides was effective in reducing tomato root damage, and gave similar results to treatment with high doses of chemicals. DAZ or AVE treated with/without biofumigation resulted in the best control results at 4 month after transplanting. The highest yield increase (36%) was observed when DAZ was used combined with CHM. This was followed by AVE (33%), AVE+CHM (27%) and DAZ (24%). In 2009, the costs of the chemicals per hectare were $900 for AVE, $1600 for DAZ, $600 for FOS; the increased yields by using these chemicals, expressed as value of tomatoes per hectare, were US$3200, US$2400, US$2300, respectively. Therefore, biofumigation combined with AVE or DAZ can not only decrease the cost of chemical nematicides by US$300 and US$500 per hectare, respectively, but also increase the yield value of tomato by US$2300 and US$800 per hectare. Therefore, such control methods as AVE+CHM and DAZ+CHM are a promising approach for tomato producers to manage root-knot nematodes.

The application of chemical nematicides in combination with biofumigation is one of the most practical RKN control methods in glasshouses of different countries (Chavarría-Carvajal *et al*., 2001). In some regions of the northern part of China, a combined treatment of solarisation and chicken manure was applied in a glasshouse with a history of minor disease contamination. Most of the farmers prefer to use solarisation in combination with low doses of chemical fumigants in glasshouses where sequential cropping is practiced and there is a high nematode incidence. According to the availability, cost, control efficiency and residual effect on the crop, the combination of AVE with biofumigation of CHW is the most economical root-knot nematode control method in organic glasshouse farming systems in the northern part of China. Further studies should be planned to determine whether or not there is a substantial decrease in the effect of AVE+CHM in the following years of repeated applications.

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Wen-Kun Huang, De-Liang Peng, Hong-Yun Jiang, Hai-Bo Long, Huan Peng, Gaofeng Wang. Совместное применение химических нематицидов и биофумигации для контроля *Meloidogyne incognita* на томатах при тепличном выращивании.

Резюме. В 2008-2009 годах были проведены испытания для сравнения эффективности воздействия различных химических нематицидов вместе с биофумигацией для контроля *Meloidogyne incognita* на томатах в промышленных тепличных хозяйствах. Дазомет и авермектин по отдельности и вместе с биофумигацией эффективно сокращали численность *M. incognita* и индекс галлообразования. Биофумигация с использованием куриного помета и остатков листьев горчицы снижали численность популяции нематод и их вредоносность для томатов, но общая эффективность биофумигации была самой низкой, по сравнении со всеми другими экспериментами, за исключением контроля. Выход томатов при обработке дазометом и авермектином с биофумигацией на 30% превышала таковую без биофумигации, что указывает на перспективность ее использования. Предполагается, что совмещение авермектина и биофумигации позволит сократить расход химического средства, и потому представляется наиболее эффективным в экономическом отношении методом контроля галлообразующих нематод.