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Research Article

Effects of Anaerobically Digested Slurry on *Meloidogyne incognita* and *Pratylenchus penetrans* in Tomato and Radish Production

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Since effective disposable way of anaerobically digested biogas slurry is expected, ADS was applied to soil to evaluate its effects on nematode damage. Damage index of tomato by root-knot nematode was significantly ($P < .05$) lower and the growth better in pots applied with ADS (100 and 200 mg NH_4^+-N kg^{-1}) than that in those with chemical fertilizer and control (no ADS). ADS was applied into radish cultivated fields infested with the root-lesion nematode: a single (100 kg NH_4^+-N ha^{-1}) in 2007 and 2008 and multiple applications (25, 50, 25 kg NH_4^+-N ha^{-1} soil) in 2009. Damage to radish was 30% and 50% lower in ADS-treated fields than that in the control in 2007 and 2009, respectively, although not in 2008. These results suggest that application of ADS to fields might be feasible for mitigating nematode damage, but the rate and timing should be considered further for the best application way.

1. Introduction

How to dispose byproduct of biogas plants into our environment is now becoming an important issue. Direct application to agricultural land is one of the most probable options. The byproduct, anaerobically digested slurry (ADS) from animal wastes, has been evaluated as a potentially important alternative source to synthetic inputs [1] in sustainable crop production. In addition, protection of plant-parasitic nematodes and phytopathogenic fungi [1–7] by ADS has been reported, and its ingredients such as ammonium and acetic acid are proposed as possible mechanisms [6]. In addition, it is discussed that plant-parasitic nematodes are controlled by certain kinds of organic amendment through the stimulation of naturally occurring antagonists [8, 9] for nematodes pests and/or change of the soil nematode community structure [10].

We have previously reported on the ability of different types of ADS to suppress the root-lesion nematode *Pratylenchus penetrans* and root-knot nematode *Meloidogyne incognita* in *in vitro* experiment without the host plants and the possible mechanisms involved [6]. The objective of this study was to evaluate the effectiveness of ADS on the plant-parasitic nematodes in larger scales of experiment, such as pot and field with the host plants. The Miura Peninsula in Kanagawa Prefecture is a major radish production area of Japan, and damage caused by *P. penetrans* is the major threat to the farmers since it reduces radish quality [11]. Therefore, potential nematicidal action of ADS was tested for the damage by nematode in conventional radish cultivated fields in the area. In addition, effect of ADS on damage to tomato by root-knot nematode disease *M. incognita* was also evaluated in a greenhouse.

2. Materials and Methods

2.1. ADSs Used. ADSs with different raw materials were used: cow slurry (ADCS), pig slurry (ADPS), and a mixture of

Table 1: Chemical properties of different types of anaerobically digested slurry.

Materials	Abbreviation	Total-N (mg/L)	NH_4^+-N (mg/L)	pH	EC (1 : 20) (mS/cm)	Solid matter content (g/L)
Cow manure slurry	ADCS	1,500	1,100	7.8	12	20
Pig slurry	ADPS	3,700	3,000	8.3	14	15
Municipla biowastes and excess slurry	ADMS	4,800	1,800	7.3	13	50

municipal biowastes, and excess slurry from activated sludge (ADMS). Some of chemical properties of ADSs are shown in Table 1.

2.2. Greenhouse Experiment

2.2.1. Damage to Tomato by Root-Knot Nematode. Pot experiments were conducted in a greenhouse at Tokyo University of Agriculture and Technology (TUAT), Japan. The effect of ADSs on root galling and final nematode population was studied in artificial inoculation experiments using a susceptible tomato variety (*Solanum lycopersicum* var. Fukuju). *M. incognita* was extracted from a sweet potato cultivated soil naturally infested with the nematode using the Baermann funnel method. Population density of the juvenile second stage of nematode was counted, and the pure *M. incognita* suspension was used as an inoculum after appropriate dilution.

2.2.2. Plant Material. Tomato seeds (*Solanum lycopersicum* var. Fukuju) were pregerminated in a Petri dish for 2 days at temperature conditions (25°C) in a biotron (LPH 200, NK System) (12 hr day and 12 hr night conditions). Then, three germinated tomato seedlings were planted in a vinyl pot (9 cm in diameter, 7.5 cm in height) containing 100 g of soil (no infested soil collected from Fuchu campus, TUAT, soil texture: clay loam (clay 39%, silt 33%, and sand 28%), pH (H_2O): 6.7 and grown for 30 days. Then, a one-month-old tomato plant was transplanted into a vinyl pot (11 cm in diameter, 10 cm in height) filled with 500 g of the soil.

2.2.3. ADS Application Rate and Time. One day prior to transplanting, ADCS and ADMS were applied into each pot at rates of 100 and 200 mg NH_4^+-N kg^{-1} soil. Chemical fertilizer (8-8-8, N-P-K) was applied at a rate of 100 mg NH_4^+-N kg^{-1} soil, and the control without fertilizer was also prepared. Each treatment was replicated seven times in a randomized complete block design.

2.2.4. Inoculation of J2. Tomato plants were inoculated by pouring 500 J2 in 10 mL of sterile distilled water around the root zone of a plant the next day after transplanting.

2.2.5. Nematodes Assessment and Disease Index. After one month cultivation in a greenhouse, individual plants were uprooted, and the roots were gently shaken to remove the adhering soil. Root knot index was recorded based on a 0–10 grade scale as described by Bridge and Page [12]. Soil in the pot was mixed well, together with the adhering soil with root and used for nematode extraction with the Baermann funnel method (seven days, room temperature). After the observation of root-knot index, nematodes were extracted from all the roots. After seven days incubation, nematodes were counted under microscope (×40).

2.3. Field Experiments

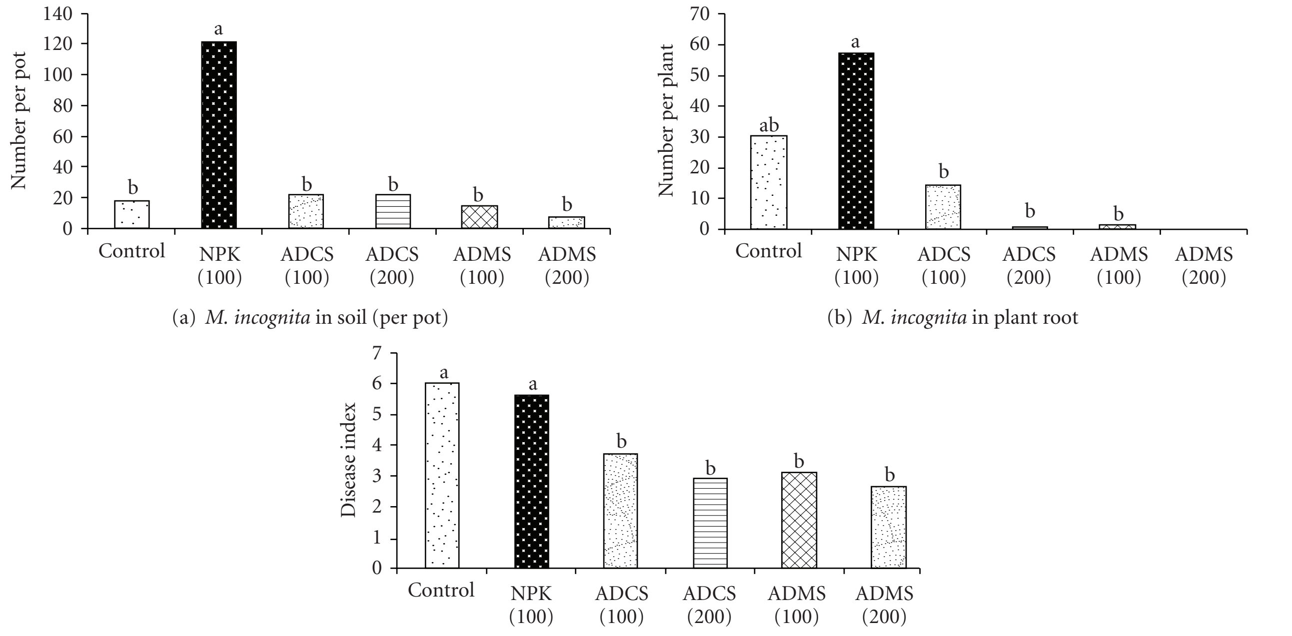
2.3.1. Experimental Site and Design. Experiment 1 was done in 2007 in a farmer field annually cultivated with radish in the Miura Peninsula, Kanagawa Prefecture, Japan. The soil of the experimental site is cumulic andosol (clay 6.0%, silt 62.2%, and sand 31.8%), pH (H_20): 6.1, total C: 43.9 g kg^{-1} soil, and total N: 3.5 g kg^{-1} soil. Total six plots each comprising 2 m × 2 m were divided into two treatments: untreated and ADS application.

ADCS was applied at a rate of 100 kg NH_4^+-N ha^{-1} soil on September 7, 2007 into each plot before sowing and, then, mixed manually with a shovel. In each plot, radish seeds were sown the next day of application with a conventional planting distance (25 cm × 50 cm; total 36 plants per plot). Radish was harvested at December 25, 2007.

Experiment 2 was done with the same experimental design and treatment in the same field but in a different place in 2008. ADCS was applied at September 9, 2008, radish was sown at September 12, 2008, and harvesting was done at December 25, 2008.

In experiment 3, split application was done in a different field of the same farmer field in 2009. Six plots, each comprising 1.5 m × 2 m, were set, and anaerobically digested pig slurry ADPS was applied three times with different application rates at October 6, 2009 as basal application (25 kg NH_4^+-N ha^{-1}), at one month growing period (November 12, 2009) as middle application (50 kg NH_4^+-N ha^{-1}) and at two months growing period as final application (25 kg NH_4^+-N ha^{-1}) (December 10, 2009) by pouring ADSs into the soil around the radish crop. In each plot, radish seeds were sown at 25 cm × 50 cm distance (total 24 plants per plot). Radishes were harvested at February 2, 2010. All experiments in Miura were planted with *Raphanus sativus* var. T465.

2.3.2. Soil and Plant Sampling. Soil sampling was done before cultivation time (prior to application of ADS) and at harvest in each year. Soil samples were collected from 0–30 cm depth using a root auger (4 cm in diameter, Daiki Riki Kogyo Co., Ltd., Tokyo, Japan). Five soil cores were taken



(a) *M. incognita* in soil (per pot)

(b) *M. incognita* in plant root

(c)

Figure 1: Population of *Meloidogyne incognita* in soil (a) and tomato roots (b) at one month after application of anaerobically digested slurry and root-knot gall index (c). Different letters indicate significant differences between the treatments at $P < .05$. NPK: chemical fertilizer (8 : 8 : 8 N : P : K), ADCS: cow slurry, ADMS: municipal biowastes and excess slurry, 100: 100 mg NH_4^+-N kg^{-1} soil and 200: 200 mg NH_4^+-N kg^{-1} soil.

from each plot, and the soil samples were mixed thoroughly to make a composite sample. Nematodes were extracted from 20 g in three replications using the Baermann funnel method (two days, room temperature). The numbers of total nematodes and *P. penetrans* were separately counted under a microscope (×40). Counting was using a composite solution consisting of three replicate tubes each plot in 2007 and 2008. In 2009, counting was separately done using each replicate tube.

At harvest, 10 to 20 plants were uprooted from the middle area of each plot and the following parameters measured: the number of spots caused by the root-lesion nematode by visual inspection and weight and length of radish.

2.4. Statistical Analysis. Differences among mean values of control and treatment were analyzed by analysis of variance (ANOVA) using a software Excel statistics 2002 (Social Survey Research Information, Tokyo, Japan). In field experiment, overall differences among means were tested using one-way ANOVA for nematode density. Damage (spot number) data were analyzed with two-way ANOVA including with treatment and plot interaction.

3. Results

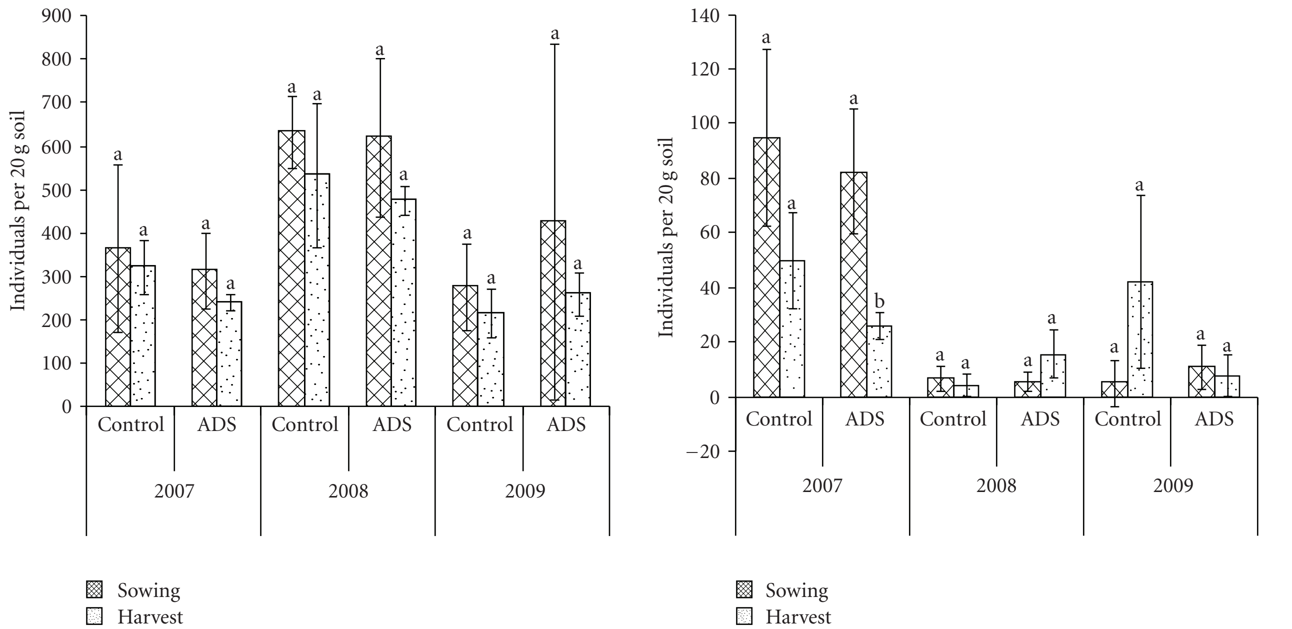
3.1. Nematode Population in Soil and Plant Root. Population of *M. incognita* was significantly lower ($P < .05$) in all ADSs-treated and control (no ADS) pots than in pots with chemical fertilizer both in soil and root at harvest (Figures 1(a) and 1(b)).

3.2. Effect of ADS on Damage to Tomato by the Root-Knot Nematode in Greenhouse Experiment. Gall formations were significantly lower ($P < .05$) in the plants treated with ADCS, MMS than in those in the control and treated with chemical fertilizer (Figure 1(c)). Significantly taller plant height was observed in the pots treated with ADMS (100 and 200 mg NH_4^+-N kg^{-1} soil) than in those with ADCS (100 and 200 NH_4^+-N kg^{-1} soil) and chemical fertilizer.

3.3. Field Experiment

3.3.1. Nematode Populations. In 2007 and 2008, population of free-living nematodes was not significantly different in control and ADS-treated plots at sowing time. At harvest, no increase in numbers was observed in both plots (Figure 2(a)). In 2007, *P. penetrans* population was lower both in the control (45%) and ADS plots (70%) at harvest than at sowing, and the difference was significant ($P < .05$) in the ADS plot. In contrast, there was no significant effect on *P. penetrans* in 2008.

In 2009, population of free-living nematodes was a little higher in the ADS plot than in the control at sowing although there was no significant difference. Like 2007 and 2008 results, a similar decreasing trend was observed at harvest. Initial population of *P. penetrans* in the ADS-treated plot was twice as that of control. At harvest, population of *P. penetrans* drastically increased (about 8 times) in the control plot, while 30% decrease was observed in the ADS plot although there was no significant difference (Figure 2(b)).



(a) Free-living nematode (b) *P. penetrans*

Figure 2: Populations of free-living nematodes (a) and *Pratylenchus penetrans* (b) at sowing and harvest in radish cultivated fields applied with anaerobically digested slurry. Error bars indicate standard deviation, and the different letters indicate significant difference ($P < .05$) between at sowing and at harvest.

Table 2: Effect of anaerobically digested slurry on root-lesion nematode infested spot number on radish at Miura, Kawashima field.

	2007		2008		2009	
	Control	ADS	Control	ADS	Control	ADS
Spot number	567 ± 65	425 ± 135	32 ± 34	52 ± 36	171 ± 135	87 ± 92
Plot	.000***		.000***		.000***	
Treatment	.000***		.212		.000***	
Treatment × plot	.000***		.000***		.000***	

Treatment with or without ADS.
***$P < .001$.

3.3.2. Damage by the Root-Lesion Nematode to Radish. There was a highly significant interaction effect ($P < .001$) between treatment and plot. Therefore, spot numbers on radish were not significantly different, but were lower by 30% and 50% in the ADS plot than in the control in 2007 and 2009, respectively. Higher spot numbers (60%) was found in the ADS plot than in the control in 2008 (Table 2).

4. Discussion

Although a number of studies have shown that anaerobically digested slurry may have potential for the control of plant-parasitic nematode, there is limited research for the management of root-lesion nematode in field condition. Our previous study [6] demonstrated acute toxicity of NH_3 and acetic acid in ADS for *P. penetrans* and *M. incognita* in *in vitro* experiment, in which their populations were reduced by 50% in one week incubation. Thus, ADS was considered as a valuable liquid fertilizer that might function as a nematicide for managing plant-parasitic nematode diseases as well as a fertilizer [7]. The present greenhouse and field study further supported the suppressive property of ADS to nematode damage.

In a greenhouse experiment, two types of slurry, ADCS and ADMS, were applied at rates of 100 and 200 mg NH_4^+-N kg^{-1}, within the conventional ranges of N fertilization. The results demonstrated that ADS significantly suppressed population of the root-knot nematode and damage to tomato caused by the nematode, concurrent with the previous study by Jothi et al. [5]. However, there were no significant differences in the effectiveness of ADS between 100 and 200 mg NH_4^+-N level, although the plant growth became more vigorous at 200 mg NH_4^+-N level.This result indicates that increase of the application rate of ADS may provide more nutrients, but not bring more nematicidal action.

Different response to the ADS amendment was observed in the management of *P. penetrans* in radish cultivated fields. In 2007, there was only a little reduction in the number of

P. penetrans at harvest in the ADS plots compared with that at sowing, it may be due to the weak toxicity of NH_3 and other volatile organic acids in the field condition, unlikely *in vitro* experiment [6]. But, nematode damage to radish was lower (30%) in the ADS plot than in the control. In this study, we used ADS as an organic material and expected increase in number of free-living nematodes in the soil at harvest. However, populations of free-living nematodes did not change by the application of ADS, contradicting with the previous findings that organic amendments stimulate free-living nematode [1, 13, 14]. Nahar et al. [14] found the increase in number of free-living nematodes following application of animal manure (100 kg N ha^{-1}). A similar trend was observed after application of liquid hog manure (ca. 260 kg N ha^{-1}) by Mahran et al. [1]. In contrast, Ferris and Matute [15] reported that significant changes in the nematode trophic groups and nematode abundance following organic amendments in field needed several years' application. McSorley and Gallaher [16] found that more consistent effects on nematode abundance are observed where organic amendments have been applied for several years. Our findings seemed to fall in the statements of these studies.

We conducted a field experiment again in 2008 in a different field with few numbers of *P. penetrans* and higher numbers of free-living nematodes. Likewise 2007 field result, a similar decreasing pattern of free-living nematodes was observed in both control and ADS plots at harvest. In contrast, the number of *P. penetrans* increased in the ADS plots at harvest and not in the control plot. The mechanism leading to increased numbers in the ADS plots is not clear. A possibility may be due to the presence of host and enhanced root biomass by the ADS application which could favor the multiplication of *P. penetrans* [15]. Since the initial inoculum density was fewer (ca. 6 and 80 individuals 20 g^{-1} soil in 2008 and 2007, resp.) in both plots in 2008, we expected lower damage and higher suppressive effect by ADS. The damage became lower in 2008 than that in 2007 in both plots but no suppressive effect was observed by ADS. The reason of inconsistent result with 2007 is not clear.

In this study, difference results of ADS were observed in greenhouse and field experiment. The reason for such difference in the ADS effects might be due to the different experimental condition. In greenhouse study, vinyl pots (11 cm in diameter and 10 cm height) were used and *M. incognita* was artificially inoculated into soil (1000 J2/kg soil). The effect of ADS on the limited soil volume condition was apparently seen by decreased population of inoculums at harvest and decreased gall formations. In the field condition, ADS was applied to the field soil and mixed well in a plough layer (0–15 cm). Although population changes during a growing season were not studied, no significantly reduced numbers of *P. penetrans* were observed at harvest compared with control. It suggested that NH_3 toxicity and other volatile acid from ADS can be weakened easily in field condition than in greenhouse. It was noticed that wind speed can contribute to NH_3 volatilization in field condition [17], suggesting that wind might stimulate volatilization of NH_3 in field condition. In addition, effect of ADS on *P. penetrans* in lower soil is not expected in the field experiment since it was mixed in a plough layer. It is known that *P. penetrans* living in lower soil (30–60 cm) may cause damage to radish [11]. In this study, the host root system was clearly seen in deeper soil up to 50 cm and is the limiting factor for the vertical distribution of *P. penetrans* [18]. It was considered that *P. penetrans* in lower soil layer can proliferate and, thus, cause damage to radish. Thus, the probable way that can reduce the damage to radish by ADS application must be further studied. Concentration of ammonium and volatile organic acids containing in ADS decreases with time after application into soil, and, thereby, the suppressive property of ADS will decrease. If NH_3 toxicity from ADSs is maintained in the soil throughout the time of cropping season, we can expect more reduction in population and reproduction of *P. penetrans* and thereby more reduction in the damage. Relating to this hypothesis, ADS was applied as split dosage in 2009 as an alternative application way to maintain the NH_3 toxicity in field condition throughout a growing season. Population of *P. penetrans* became 8 times higher in the control plot at harvest, but no increase was observed in the plot amended with ADS, suggesting that the suppressive property of ADS might be maintained by the split application. Numbers of spots was 50% lower in the ADS-treated plots than those in the control and this reduction of damage level was higher than that of single application in 2007. These results suggest that split application of ADS seems to suppress the activity of *P. penetrans* and its damage to radish more effectively, but further study should be conducted to decide the best application rate and timing.

In conclusion, there was no detrimental effect on the plant growth by the application of ADS both in the greenhouse and field trail. However, different results were observed in greenhouse and field experiment. Although reduction in the population of *P. penetrans* in field condition was not in the satisfactory range in all years, ADS decreased the damage to radish in field condition in 2 out of 3 years. Split application of ADS may be a feasible option for maintaining the suppressive mechanism which leads to minimum damage level but need to consider more probable ways for improving the nematicidal efficiency of ADSs to manage nematode disease.

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References

[1] A. Mahran, M. Tenuta, R. A. Lumactud, and F. Daayf, "Response of a soil nematode community to liquid hog manure and its acidification," *Applied Soil Ecology*, vol. 43, no. 1, pp. 75–82, 2009.

[2] M. Amari, K. Toyota, T. M. Islam, K. Masuda, T. Kuroda, and A. Watanabe, "Effect of the addition of anaerobically digested

slurry to soil and hydroponics on soil-borne plant diseases," *Soil Microorganisms*, vol. 62, pp. 106–113, 2008 (Japanese).

[3] M. Browning, D. B. Wallace, C. Dawson, S. R. Alm, and J. A. Amador, "Potential of butyric acid for control of soil-borne fungal pathogens and nematodes affecting strawberries," *Soil Biology and Biochemistry*, vol. 38, no. 2, pp. 401–404, 2006.

[4] M. H. Chantigny, P. Rochette, D. A. Angers, D. Massé, and D. Côté, "Ammonia volatilization and selected soil characteristics following application of anaerobically digested pig slurry," *Soil Science Society of America Journal*, vol. 68, no. 1, pp. 306–312, 2004.

[5] G. Jothi, S. Pugalendhi, K. Poornima, and G. Rajendran, "Management of root-knot nematode in tomato *Lycopersicon esculentum*, Mill., with biogas slurry," *Bioresource Technology*, vol. 89, no. 2, pp. 169–170, 2003.

[6] Y. Y. Min, E. Sato, T. Shirakashi, S. Wada, K. Toyota, and A. Watanabe, "Suppressive effect of anaerobically digested slurry on the root lesion nematode *Pratylenchus penetrans* and its potential mechanism," *Japanese Journal of Nematology*, vol. 37, pp. 93–100, 2007.

[7] B. Valocká, P. Dubinský, I. Papajová, and M. Sabová, "Effect of anaerobically digested pig slurry from lagoon on soil and plant nematode communities in experimental conditions," *Helminthologia*, vol. 37, no. 1, pp. 53–57, 2000.

[8] M. Akhtar and A. Malik, "Roles of organic soil amendments and soil organisms in the biological control of plant-parasitic nematodes: a review," *Bioresource Technology*, vol. 74, no. 1, pp. 35–47, 2000.

[9] Y. Oka, "Mechanisms of nematode suppression by organic soil amendments—a review," *Applied Soil Ecology*, vol. 44, no. 2, pp. 101–115, 2010.

[10] S. Sánchez-Moreno and H. Ferris, "Suppressive service of the soil food web: effects of environmental management," *Agriculture, Ecosystems and Environment*, vol. 119, no. 1-2, pp. 75–87, 2007.

[11] E. Sato, Y. Y. Min, K. Toyota, and A. Takada, "Relationships between the damage to radish caused by the root-lesion nematode *Pratylenchus penetrans*, its density prior to cultivation and the soil nematode community structure evaluated by polymerase chain reaction-denaturing gradient gel electrophoresis," *Soil Science and Plant Nutrition*, vol. 55, no. 4, pp. 478–484, 2009.

[12] J. Bridge and S. L. J. Page, "Estimation of root-knot nematode infected levels on roots using a rating chart," *Tropical Pest Management*, vol. 26, pp. 296–298, 1980.

[13] T. A. Forge, S. Bittman, and C. G. Kowalenko, "Impacts of sustained use of dairy manure slurry and fertilizers on populations of *Pratylenchus penetrans* under tall fescue," *Journal of Nematology*, vol. 37, no. 2, pp. 207–213, 2005.

[14] M. S. Nahar, P. S. Grewal, S. A. Miller et al., "Differential effects of raw and composted manure on nematode community, and its indicative value for soil microbial, physical and chemical properties," *Applied Soil Ecology*, vol. 34, no. 2-3, pp. 140–151, 2006.

[15] H. Ferris and M. M. Matute, "Structural and functional succession in the nematode fauna of a soil food web," *Applied Soil Ecology*, vol. 23, no. 2, pp. 93–110, 2003.

[16] R. McSorley and R. N. Gallaher, "Effect of compost and maize cultivars on plant-parasitic nematodes," *Journal of Nematology*, vol. 29, no. 4, pp. 731–736, 1997.

[17] S. G. Sommer, H. S. Østergård, P. Løfstrøm, H. V. Andersen, and L. S. Jensen, "Validation of model calculation of ammonia deposition in the neighbourhood of a poultry farm using measured NH concentrations and N deposition," *Atmospheric Environment*, vol. 43, no. 4, pp. 915–920, 2009.

[18] M. P. Pudasaini, C. H. Schomaker, T. H. Been, and M. Moens, "Vertical distribution of the plant-parasitic nematode, *Pratylenchus penetrans*, under four field crops," *Phytopathology*, vol. 96, no. 3, pp. 226–233, 2006.