농약과학회지 (Korean J. Pestic. Sci.)
Vol. 20, No. 4, pp. 293-299 (2016)
Open Access https://doi.org/10.7585/kjps.2016.20.4.293
ORIGINAL ARTICLES / SYNTHESIS / FORMULATION / CONTROL

Online ISSN 2287-2051
Print ISSN 1226-6183

Efficacy of Different Nematicidal Compounds on Hatching and Mortality of *Heterodera schachtii* Infective Juveniles

Jeongeun Kim*, Abraham Okki Mwamula*, Faisal Kabir, Jin Hee Shin[1], Young Hwa Choi[2], Jae-Kook Lee[3] and DongWoon Lee**

Department of Ecological Science, Kyungpook National University, Sangju, Gyeongsangbuk-do 37224, Korea
[1]Hanearl Science LTD, Seobuk-gu, Cheonan, Chungcheongnam-do 31055, Korea
[2]School of Ecology and Environmental System, Kyungpook National University, Sangju, Gyeongsangbuk-do 37224, Korea
[3]Crop Protection Division, National Academy of Agricultural Science, RDA, Wanju, Jellabuk-do 55365, Korea

(Received on November 1, 2016. Revised on November 29, 2016. Accepted on November 29, 2016)

Abstract Effect of nematicidal compounds on hatchability of sugar beet cyst nematode, *Heterodera schachtii* and its infective juveniles was investigated. The sugar beet cyst nematode was isolated from Chinese cabbage field in Samcheok in Korea. Acute toxicity of nematicidal compounds against infective juveniles was also tested to find the LC_{50} by exposing juveniles to given dilutions of each compound. Hatchability and mortality of infective juveniles of *H. schachtii* were influenced by nematicidal compounds (Fluopyram 40% SC, imicyafos 30% SC, fosthiazate 30% SC, abamectine 1.68% SC, terthiophene, and *Eclipta prostrata* extract). Fluopyram and imicyafos yielded the lowest rates of hatching. Total hatched infective juveniles were significantly different among nematicidal compounds. Positive correlation in percentage reduction of hatching was observed in fluopyram. Furthermore, the highest mortality was also observed in the treatments of fluopyram and imicyafos (LC_{50} of 0.0543 and 0.0178 ppm respectively). The study, therefore, demonstrated available alternative nematicidal compounds which could be used in the control of *H. schachtii*.

Key words Acute toxicity, Chinese cabbage, *Eclipta prostrata*, nematicide, sugarbeet cyst nematode

<< ORCID
DongWoon Lee
http://orcid.org/0000-0001-9751-5390

Introduction

Chinese cabbage, *Brassica rapa chinensis* L. is one of the most highly valued economic crops and the leading vegetable in both production and consumption in Korea (Chang et al., 2008; Kim et al., 2014). It is ranked third after rice and fresh vegetables in annual production due to the high demand of the crop in the production of Kimchi; a Korean national dish and the most important processed food product in the country (FAO, 2013, Park et al., 2014). However, there is pressure exerted on Chinese cabbage production by pests and diseases especially in the highland areas (Kim et al., 2012, 2014). Besides insect pests and diseases caused by bacteria, fungi and viruses, an important plant pathogenic nematode, sugar beet cyst nematode (SBCN), *Heterodera schachtii* has recently been considered a serious pest causing severe damage and yield reduction in the Gangwon province, Korea (Lee et al., 2013; Kabir et al., 2015).

SBCN is a well-documented major pest of sugarbeet, causing severe yield losses (Griffin, 1987; Hafez and Seyedbagheri, 1997). The pest has a relatively wide host range and the symptoms include wilting, yellowing and eventual necrosis of the leaves. In the roots, the taproot may fail to develop and numerous root hairs are sometimes formed, on which white lemon-shaped females can be

*Equal contribution authors
**Corresponding author
E-mail: whitegrub@knu.ac.kr

observed. Once fully grown, the females turn to brown cysts and dissociate from the root. The brown cysts can survive in irrigated fields for several years without a host (Hafez and Seyedbagheri, 1997). Management of SBCN involves integration of different strategies like prevention, cultural practices, resistant cultivars, trap crops and chemical control (Griffin, 1987; Turner and Rowe, 2006). Chemical control using aldicarb and 1, 3-dichloropropene is considered more potent depending on soil properties though their use is currently under restriction due to their side effects on non-target organisms and groundwater contamination (Holden, 1986; Ritter, 1990; Gray et al., 1992; Urwin et al., 2000). Aldicarb in particular was discontinued in 2015 (Cone, 2010). Non fumigants like imicyafos and fosthiazate are generally known to pose limited effect on non-target microbes though not widely used on SBCN (Wada and Toyota, 2008; Wada et al., 2011). Other alternatives in nematode control include abamectin, terthiophene and plant extracts from various plant species with nematicidal effect (Akhtar and Irshad, 1993; Zhang et al., 2010; Kang et al., 2012). However, limited information is known on the efficacy of these nematicidal compounds on SBCN.

Recent reports indicate that SBCN is rapidly spreading within the highland Chinese cabbage growing areas of Gangwon-do province (from 11.2 ha in 2011 to 70 ha in 2015) and cabbage production is greatly affected (Kweon et al., 2015). Efforts to understand the ecology of the pest in highland cabbage growing areas are underway through various laboratory and field studies (Kabir et al., 2015). However, current management practices are limited to prevention of spread and multiplication through proper disposal of infested waste soil and sanitation to avoid transfer of cysts to non-infested fields through farm machinery (Kweon et al., 2015). Thus, without a readily available reliable control strategy, the economic efficiency of Chinese cabbage under the increasing SBCN infestation in the main growing areas is under threat. Chemical control is considered as one of the most effective and reliable integrative method of SBCN control. However, no nematicides have been tested and registered for the control of SBCN in Chinese cabbage fields in Korea (KCPA, 2016). In addition, there is a need to detect more environmentally friendly non-fumigant nematicidal compounds in the control of SBCN due to the increasing global regulatory pressure on well- known effective nematicidal compounds (aldicarb and 1, 3-dichloropropene), This study therefore aimed at testing the efficacy of selected environmentally friendly nematicidal compounds for the control of SBCN on Chinese cabbage.

Materials and methods

Nematicides

The nematicidal compounds used in the study were fluopyram 40% SC, imicyafos 30% SC, fosthiazate 30% SC, abamectin 1.68% SC, terthiophene and *Eclipta prostrata* extract. These compounds have well-known nematicidal activity and some of them have been tested and applied as nematode control means. Fluopyram is a broad spectrum fungicide and effective on plant parasitic nematodes (Faske and Hurd, 2015). Imicyafos, fosthiazate and abamectine are registered as nematicides against other plant parasitic nematodes in Korea (KCPA, 2016). Terthiophene and *Eclipta prostrata* extract have nematicidal activity against pine wood nematode, *Bursaphelenchus xylophilus* (Shin et al., 2016). Chemical nematicides used in the study were obtained from pesticide market and terthiophene was obtained from Sigma-Aldrich. *Eclipta prostrata* extract was made using the same material and procedure described by Shin et al. (2016).

Cyst collection

Healthy Cysts were collected from soil samples of Chinese cabbage fields in Samcheok area (collected in August 2014). One hundred grams of soil from each sample was washed using 20 and 60 mesh sieves. Cysts were collected from 60 mesh sieve and filtered with Whatman no. 100 filter paper to remove excess water. The filter paper was then placed in a Petri dish and observed under Nikon SM2 1000 electron microscope, to isolate healthy cysts.

Preparation of plant root extract

In the previous hatching studies done by Kabir et al. (2015), SBCN infective juveniles were hatched in great numbers in Chinese cabbage root exudates than in water. Thus, root exudates were adopted for the experiment. Healthy Chinese cabbage plants were collected from green house and whole plant including root was washed using tap water to remove all dirts. Twenty washed plants along with their roots were soaked in 1-liter of water for 24 hours. The residual water was then elutriated by filtering

with clean solid-liquid separation filter cloth to remove any dirty particles, after which the extract was preserved for further use.

Testing of effect of nematicidal compounds on hatching

In this experiment, the actual concentrations of the compounds used were as follows; fluopyram 100 ppm, imicyafos 75 ppm, fosthiazate 75 ppm, abamectin 3.36 ppm, terthiophene 100 ppm and *Eclipta prostrata* extract 1000 ppm.

Nematicidal compound activity against hatching of infective juveniles was tested in two ways. In the first experiment, healthy cysts were soaked in the nematicidal compound throughout the experimental period. An aperture was trimmed on the underside of 15 ml tube cover cork separated from the tube. The aperture was wrapped with fine filter mesh to prevent free passage of cysts but allow free movement of the hatched infective juveniles. Ten healthy cysts were then placed in a single cover cork before being inserted in each single well of a 12 multi-well culture plate. Each well was filled with 1.5 ml of 6 different nematicidal compounds in 4 replicates. Four replicates of a control were set up in the same way but with only root extract solution. The multi-well plates were then kept in growth chamber (Han Baek HB 303 DH-0, Korea) at 25ºC and newly hatched infective juveniles were counted under a dissecting microscope every 24-hour. Fresh nematicidal compounds and root extract solutions were replaced in each well on each counting interval. Counting of the newly hatched infective juveniles was continued for 14 days.

In the second experiment, healthy cysts were soaked in the nematicidal compounds on the 5th day after initiation of hatching. The same experimental design was set up as described in above experiment. However, all the cysts were initially treated with a 1.5 ml root extract solution for the first four days to enhance uniform hatching. On the 5th day, each well was filled with 0.75 ml of each nematicidal compound plus 0.75 ml of root extract solution. Control replicates were filled with 1.5 ml of root extract solution. Counting of infective juveniles was made as described above.

Acute toxicity of nematicidal compounds on infective juveniles

Infective juveniles of *H. schachtii* were obtained from healthy cysts using modified Baermann technique (EPPO, 2013) at room temperature, with root extract as the medium of extraction. After 24 hours, the hatched infective juveniles were collected from the funnel tube into a 250 ml beaker. With the help of an electric stirrer machine and dissecting microscope, a 0.5 ml root extract solution containing 100 infective juveniles was taken from the beaker using a pipette for further use in the experiment.

For further test of the efficacy of the nematicidal compounds on mortality rates of infective juveniles, a 0.5 ml root extract solution containing 100 infective juveniles was filled in each well of a 12 multi-well culture plate. An equal volume of nematicidal compound in varying dilutions was added in each well, filled initially with the root extract solution containing infective juveniles. Four replicates were set up for each nematicidal compound concentration. The multi-well culture plates were wrapped with aluminum foil and kept at 25ºC in the growth chamber (Han Baek HB 303 DH-0, Korea) and the number of both live and dead infective juveniles was counted under Nikon SM2 1000 electron microscope after 24 hours.

Statistical analysis

Data were subjected to analysis of variance using SAS statistical package version 9.4. Treatment means were compared using Tukey's honestly significant difference (HSD) at $P \leq 0.05$. Percentage correlations were also calculated. LC_{50} and LC_{90} values were determined using probit analysis.

Results

Effect of different nematicidal compounds on hatching of infective juveniles

Hatching rate of *H. schachtii* was significantly different depending on nematicidal compounds (Fig. 1). No hatching was observed in the first 3 days after treatment and the highest number of infective juveniles was recorded from cysts treated with terthiophene (Fig. 1).

Hatching peaks were varied among different nematicidal compounds with the highest peak in the treatment of terthiophene on the 6th day after treatment (Fig. 1). Highest hatching peaks for imicyafos and abamectin were observed on 7th and 9th day after treatment, respectively. Both *Eclipta prostrata* extract and fosthiazate treated cysts showed maximum hatching on the 11th day after treatment (Fig. 1). There was hardly any hatching from the healthy cysts treated with fluopyram. Daily hatching rates from cysts were significantly different among nematicidal compounds ($P \leq 0.05$), except on the last day when there

Table 1. Lethal concentrations of nematicidal compounds treated against infective juveniles of *Heterodera schachtii*

Nematicidal compound	LC_{50} (95% FL) ppm	LC_{90} (95% FL) ppm	x^2	*P*
Eclipta prostrata	183.97*	397.47	9.14	0.0025
Fluopyram 40% SC	0.0543 (4.21E-3~0.5456)	2.63 (0.3152~3435.7)	21.65	0.0001
Imicyafos 30% SC	0.0178 (1.75E-3~0.2129)	1.29 (0.1285~1730.6)	18.99	0.0001
Terthiophene	0.7574 (0.00641-100.94)	119.01 (4.95-3.01E12)	11.48	0.0007

*Could not calculate FL.

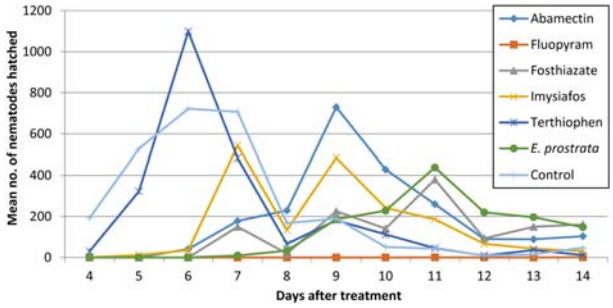

Fig. 1. Average number of infective juveniles of *Heterodera schachtii* recovered from cysts treated with nematicidal compounds. Cysts were soaked in each nematicidal compound with Chinese cabbage root exudates.

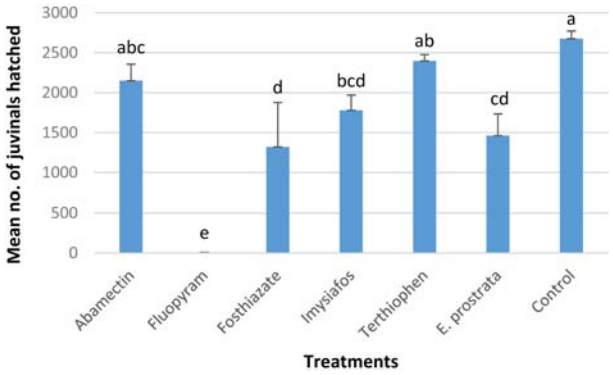

Fig. 2. Total mean numbers of infective juveniles of *Heterodera schachtii* recovered from cysts treated with nematicidal compounds after 14 days. Error bars show standard error and letters indicate significant differences among means (Tukey's HSD test, $P<0.05$).

were no significant differences among treatments (P= 0.056). Total infective juvenile hatched populations were also significantly different among nematicidal compounds (*df*=6, 14, *F*=34.58, *P*<0.0001) (Fig. 2).

In second experiment, initial treatment of the cysts with root extract solution increased hatching rates of infective juveniles. However, treatment of cysts with nematicidal compounds on the 5th day resulted in varying hatching rates among nematicidal compounds (Fig. 3).

There was a positive correlation in percentage reduction of hatching rates among nematicidal compounds (Fig. 3).

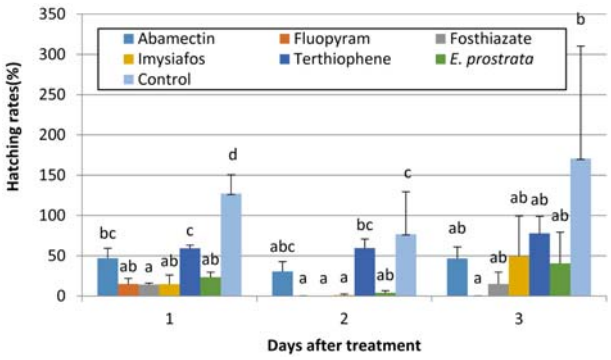

Fig. 3. Percentage of hatching increase from cysts initially treated with root extracts for 4 days before synergistic treatment with nematicidal compounds and root extract solution. Bars (±SD) with the same letter in each day after treatment are not significantly different (Tukey's HSD test, $P<0.05$).

Hatching hardly occurred in the treatment of fluopyram as seen in Fig. 3 (almost 100% no hatching) and there was significant differencs in the number of infective juveniles hatched in the first two days after treatment with the nematicidal compounds (1 day after treatment; *df*=6, 14, *F*=37.79, *P*<0.0001 and 2 days after treatment; *df*=6, 14, *F*=7.06, *P*<0.0013). However, differences were not significant on the third day after treatment (*df*=6, 14, *F*=2.71, *P*< 0.0586).

Acute toxicity of nematicidal compounds on infective juveniles

Treatment of cysts with various concentrations of the nematicidal compounds showed variations in the acute toxicity of compounds depending on concentrations. Imicyafos and fluopyram showed 100% control efficacy with LC_{50} as low as 0.0178 and 0.0543 ppm, respectively (Table 1).

Discussion

Hatching of SBCN was affected by treatment of nematicidal compounds at varying levels. Initial treatment of cysts with root extract accelerated the hatching rates and this was in agreement with the findings published by Kabir et al. (2015). There was a positive correlation in percentage

reduction in hatching rates in the treatment of nematicidal compounds. Hatching was slowed down depending on the nematicidal compound.

Fluopyram showed the greatest inhibitory effect and acute toxicity on the infective juveniles. Hatching was hardly noticed and there was a 100% mortality of infective juveniles. Faske and Hurd (2015) showed the effect of fluopyram on motility of *Meloidogyne incognita* and *Rotylenchulus reniformis*. Fluopyram showed a strong effect on *M. incognita*. Their results also indicated that immobility could be reversible; a mode of action similar to aldicarb (Faske and Starr, 2006). However, this study demonstrated that fluopyram could be a nematistatic compound with irreversible mode of action on SBCN. This was supported by the lethal nematicidal effect on infective juveniles even at the lowest concentrations tested (LC_{50}= 0.0543 ppm), a characteristic which can be vital along with its hatching inhibitory effect in the control of SBCN on Chinese cabbage.

Fosthiazate is an acetylcholinesterase inhibitor that negatively affects nematode hatching and movement (Woods et al., 1999). Thus, this could explain the slow progress of hatching compared to the control in our experiment. Additionally, treatment of cysts with fosthiazate does not permanently inhibit hatching. It only slows down the hatching rate and this is in agreement with the findings by Woods et al. (1999) on potato cyst nematodes. While as inhibition of acetylcholinesterase by oxime carbamates like aldicarb and oxamyl is reversible, the process is thought to be irreversible with fosthiazate (Richard, 1960; Yu et al., 1972) and this was supported by the limited number of hatched infective juveniles in comparison to the control.

Imicyafos on the other hand, equally showed a significant effect on infective juvenile hatching rates compared with the control. More notably, it had a high toxic effect on the juveniles with LC_{50} of 0.0178 ppm. Imicyafos has been shown to have a similar significant effect on plant parasitic nematodes like *Pratylenchus penetrans* (Wada et al., 2011) and *Meloidogyne* spp. (Kim et al., 2015). Its mode of action is similar to that of fosthiazate. The low LC_{50} shown by the two compounds strongly indicate the great potential of these organophosphates for the control of *H. schachtii* on Chinese cabbage.

Abamectin and terthiophene had limited effect on hatching of SBCN. Despite the fact that abamectin showed limited influence on hatching rates of SBCN, it is noted to be one of few non-fumigant nematicides with a true nematicidal effect as nematode paralysis is irreversible on treatment (Faske and Starr, 2006). According to our results, terthiophene had a nematicidal property on infective juveniles (LC_{50}=0.7574 ppm). These compounds did not adversely affect infective juvenile hatching but they could be an alternative in cyst nematode control program integrated with other management strategies.

Eclipta prostrata considerably reduced the hatching rates of infective juveniles and additionally had a nematicidal effect on infective juveniles (LC_{50}=183.97 ppm). This is in agreement with Zarina et al. (2003), who also showed similar results on inhibition of hatching and respective mortality of *Meloidogyne javanica* infective juveniles. Kim et al. (2015) showed that terthiophene could be isolated from the aerial parts of *E. prostrata*. However, given the fact that terthiophene showed limited effect on infective juvenile hatching, it is therefore possible that different compound (s) within the *E. prostrata* extract is involved in inhibiting hatching of SBCN. Probably, a closer analysis of the different phytochemicals constituting *E. prostrata* extracts could reveal the compound involved in hatching inhibition. Botanical nematicides are generally considered to be a secure alternative to synthetic nematicides (Dang et al., 2012), and with the increasing call to phase out or limit use of synthetic nematicides, the emergence of promising botanical nematicides could be a solution.

Wada and Toyota (2008) demonstrated that the application of imicyafos and fosthiazate has little effect on non-target organisms like bacteria and fungi. The same findings were documented relating to their effect on natural soil nematode community (Wada et al., 2011). Thus, being non-fumigants and their selectivity to act against plant parasitic nematodes could make them better alternatives for the control of cyst nematodes. Fosthiazate is already used in the UK for control of potato cyst nematode (Woods et al., 1999). Efficacy of fosthiazate at higher application rates is comparable to soil fumigation using effective fumigants (Pullen and Fortnum, 1999). Therefore, fosthiazate could be one of the best alternatives to the environmentally harmful fumigants which are being phased out. This study, therefore, demonstrated an immediate potential control strategy which can be employed for the control of *H. schachtii* on Chinese cabbage. However, it is important to note that the findings were based on initial tests carried out under controlled laboratory conditions; thus, detailed

comprehensive field investigations are required to test the efficacy and potential of these nematicidal compounds for control of SBCN.

Acknowledgements

This research was carried out with the support of the "Research Program for Agriculture Science and Technology Development (Project No. PJ010774)", Rural Development of Administration, Republic of Korea.

Literature Cited

Akhtar, M. and M. Irshad (1993) Potentiality of phytochemicals in nematode control: a review. Bioresour Technol. 48:189-201.

Chang, H. W., K. H. Kim, Y. D. Nam, S. W. Roh, M. S. Kim, C. O. Jeon and J. W. Bae (2008) Analysis of yeast and archaeal population dynamics in kimchi using denaturing gradient gel electrophoresis. Int J. Food Microbiol. 126:159-166.

Cone, M. (2010) Insecticide to be banned-Three decades after tainted melons sickened 2,000 people. Environmental Health News. Available: http://www. environmentalhealthnews. org/ehs/news/aldicarb-phaseout. Accessed 27 July 2016.

Dang, Q. L., C. H. Lim and J. C. Kim (2012) Current status of botanical pesticides for crop protection. Res. Plant Dis. 18(3):175-185.

EPPO (2013) PM 7/119 (1) Nematode extraction. EPPO Bulletin 43:471-495, doi:10.111/eppo.12077

FAOstat (2013) http://faostat3.fao.org. Accessed 27 July 2016.

Faske, T. R. and J. L. Starr (2006) Sensitivity of *Meloidogyne incognita* and *Rotylenchulus reniformis* to abamectin. J. Nematol. 38:240.

Faske, T. R. and K. Hurd (2015) Sensitivity of *Meloidogyne incognita* and *Rotylenchulus reniformis* to Fluopyram. J. Nematol. 47:316.

Gray, F. A., G. D. Franc and E. D. Kerr (1992) Sugar beet nematode. Cooperative Extension Service and Department of Plant, Soil and Insect Sciences, College of Agriculture, University of Wyoming.

Griffin, G. D. (1987) Efficacy of using split and post plant applications of aldicarb for control of *Heterodera schachtii* on sugarbeet. J. Nematol. 19 (Annals 1):119.

Hafez, S. L. and M. M. Seyedbagheri (1997) Sugarbeet cyst nematode: impact on sugarbeet production in Idaho and Eastern Oregon. University of Idaho, College of Agriculture, Cooperative Extension System, Agricultural Experiment Station.

Holden, P. W. (1986) Pesticides and groundwater quality: Issues and problems in four states. National Academy Press. Washington, USA. 124pp.

Kabir M. F., J. H. Shin, O. G. Kweon and D. W. Lee (2015) Temperature and root extract effect on hatching and development of *Heterodera schachtii.* Korean J. Soil Zool. 19:22-27.

Kang, J. S., Y. S. Moon and S. H. Lee (2012) Inhibition properties of three acetylcholinesterases of the pinewood nematode *Bursaphelenchus xylophilus* by organophosphates and carbamates. Pest Biochem Physiol. 104:157-162.

Kim, H. H., Y. H. Jung, D. H. Kim, T. K. Ha, J. B. Yoon, C. G. Park and H. Y. Choo (2015) Control effects of imicyafos GR against two species of the root-knot nematodes (*Meloidogyne incognita* and *Meloidogyne hapla*). Korean J. Pestic Sci. 19:101-105. (In Korean)

Kim, H. I., C. P. Hong, S. Im, S. R. Choi and Y. P. Lim (2014) Development of molecular markers and application for breeding in Chinese cabbage. Korean J. Hortic. Sci. Technol. 32(6):745-752. (In Korean)

Kim, M. J., C. K. Shim, Y. K. Kim, S. J. Hong, J. H. Park, E. J. Han and H. J. Jee (2012) Screening of resistance cultivar to clubroot caused by *Plasmodiophora brassicae* for organic cultivation of Chinese cabbage. Res. Plant Dis. 18:123-128.

Korea Crop Protection Association (KCPA). (2016) 2016 pesticide manual. Korea Crop Protection Association. Seoul, Korea. (In Korean)

Kweon, O. G., J. H. Shin, F. M. Kabir, J. K. Lee and D. W. Lee (2016) Dispersal of sugar beet cyst nematode (*Heterodera schachtii*) by water and soil in highland Chinese cabbage fields. Korean J. Hortic. Sci. Technol. 34:195-205. (In Korean)

Lee, J. K., B. Y. Park and M. R. Cho (2013) Sugar beet cyst nematode occurrence and prevention of diffusion, 2013 Annual meeting on the Korean Society of Pesticide Science. P.60 (Abstr).

Park, K. Y., J. K. Jeong, Y. E. Lee and J. W. Daily III (2014) Health benefits of kimchi (Korean fermented vegetables) as a probiotic food. J. Med Food. 17:6-20.

Pullen, M. P. and B. A. Fortnum (1999) Fosthiazate controls *Meloidogyne arenaria* and *M. incognita* in flue-cured tobacco. J. Nematol. 31:694.

Richard D. O. B. (1960) Toxic phosphorus esters: chemistry, metabolism, and biological effects. Academic Press, UK. 434pp.

Ritter, W. F. (1990) Pesticide contamination of ground water in the United States-A review. J. Environ Sci Heal B. 25:1-29.

Shin, J. H., O. G. Kwon, C. M. Lee, S. M. Lee, Y. H. Choi, J. H. Kim, Y. S. Kim and D. W. Lee (2016) Nematicidal activity of *Eclipta prostrata* extract and terthiophene against pine wood nematode, *Bursaphelenchus xylophilus*. Korean J. Pestic. Sci. 20:56-65.

Turner, S. J. and J. A. Rowe (2006) Cyst nematodes. In Plant Nematology; Roland N. P. and M. Maurice; CABI, Wallingford, Oxfordshire, UK: pp. 91-122.

Urwin, P. E., A. Levesley, M. J. McPherson and H. J. Atkinson

(2000) Transgenic resistance to the nematode *Rotylenchulus reniformis* conferred by *Arabidopsis thaliana* plants expressing proteinase inhibitors. Mol Breed. 6:257-264.

Wada, S. and K. Toyota (2008) Effect of three organophosphorous nematicides on non-target nematodes and soil microbial community. Microbes Environ. 23:331-336.

Wada, S., K. Toyota and A. Takada (2011) Effects of the nematicide imicyafos on soil nematode community structure and damage to radish caused by *Pratylenchus penetrans*. J. Nematol. 43:1.

Woods, S. R., P. P. J. Haydock and C. Edmunds (1999) Mode of action of fosthiazate used for the control of the potato cyst nematode *Globodera pallida*. Ann Appl Biol. 135:409-415.

Yu, C. C., C. W. Kearns and R. L. Metcalf (1972) Acetylcholinesterase inhibition by substituted phenyl N-alkyl carbamates. J. Agric Food Chem. 20:537-540.

Zarina, B., S. S. Shaukat and I. A. Siddiqui (2003) Suppression of *Meloidogyne javanica* by *Conyza canadensis*, *Blumea obliqua*, *Amaranthus viridis* and *Eclipta prostrata*. Plant Pathol J. 2:174-180.

Zhang, T. Z., Y. Zeng, H. LI, G. J. Chen, J. S. Su and Q. F. Weng (2010) Photo activated toxicity of extracts from 14 Compositae plants against *Bursaphelenchus xylophilus* and *Culex pipiens pallens*. Acta Botanica Boreali-Occidentalia Sinica, 4:35.