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ORIGINAL ARTICLE

Management of foliar nematode *Aphelenchoides ritzemabosi* on *Anemone hupehensis* using plant extracts and pesticides

Aneta Chałańska[1] · Aleksandra Bogumił[1] · Gabriel Łabanowski[1]

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Abstract In 2013 and 2014, the effects of different products and plant extracts in the control of *Aphelenchoides ritzemabosi* (Schwartz, 1911) Steiner and Buhrer, 1932 on *Anemone hupehensis* plants variety Prinz Heinrich were estimated. Experiments were carried out under containerized cultivation. Evaluation of the effectiveness of nematicides was conducted using two common methods based on the number of nematodes per leaf and the percentage of damaged leaves. In both experiments oxamyl and abamectin, with aqueous extract of *Allium sativum*, were the most effective (efficacy about 40%). The aqueous bulb extracts of *A. sativum*, solution of extracts of *Quillaja saponaria* and solution of spirotetramat in combination with aqueous extract of *A. sativum* were ineffective. In both experimental years, the significant correlation between the number of nematodes in leaves and the sampling date was recorded. Based on Spearman rank correlation test and regression, it was shown that counting of nematodes in leaves is still the most reliable method for diagnosis of the damage caused by foliar nematodes.

Keywords Anemone · *Aphelenchoides ritzemabosi* · Control · Nematicides · Plant extracts

✉ Aneta Chałańska
aneta.chalanska@inhort.pl

[1] Department of Vegetable and Ornamental Plant Protection, Research Institute of Horticulture, Skierniewice, Poland

Introduction

Foliar nematode (*Aphelenchoides ritzemabosi*) (Schwarz 1911) Steiner and Buhrer is a common plant-parasite infecting more than 200 plant species. The characteristic symptoms of infection are concerned with lesions bounded by the major veins in leaves. Leaves parasitized by these nematodes loose color, turn brown and fall off (de Waele 2002). Because of the broad spectrum of hosts and the life cycle of these nematodes that takes place inside the leaf, there is a necessity to use suitable strategies to prevent the spread of *A. ritzemabosi* and to select corresponding nematicide that effectively penetrate the leaf tissue.

Among the active ingredients of pesticides used for plant protection against different nematodes, the most popular are: oxamyl (Philis 1994; Tu et al. 1996), spirotetramat (Smiley et al. 2011, 2012), diazinon (La Mondia 1999; Jagdale and Grewal 2002), carbofuran (Adegbite and Agbaje 2007; Jada et al. 2011). Despite the efficacy of chemical substances, nematicides are being increasingly replaced by various eco-friendly biological products. At present, there are no registered nematicides available for the control of foliar nematodes on ornamental plants, neither in Poland, nor in a number of other countries. This situation forces the search for alternative solutions. Many phytochemicals are known from their nematicidal activity. Plant oils may include some of the long list of compounds but not all of them. Most plant oils studied for nematode control are essential oils containing large proportions of volatile terpenes and other compounds. These compounds involved in plants may act as repellents, attractants, hatching stimulants or inhibitors as well as nematotoxicants (Chitwood 2002). Plant extracts and microorganisms antagonistic to nematodes or producing antibiotics are also successfully used in biological controls in laboratory tests (Nguyen et al. 2009), glasshouse trials (Van Damme et al. 2005) and field experiments (Rao et al. 2004; Okeniyi et al. 2013). Among biological products, the nematicidal properties were revealed by an aqueous bulb extract of *Allium sativum* L. (Tibugari et al. 2012; El-Nagdi and Youssef 2013), extracts of the soapbark tree—*Quillaja saponaria* Molina (Insunza et al. 2001; San Martin and Magunacelaya 2005), extracts of *Mentha* sp. L. (Caboni et al. 2013), oils of *Syzygium aromaticum* (L.) Merrill and Perry and *Thymus vulgaris* L. (Elbadri et al. 2008), abamectin—produced by an actinomycete bacterium *Streptomyces avermitilis* (ex Burg et al. 1979) Kim and Goodfellow 2002 (James et al. 2006; Korayem et al. 2008) and azadirachtin isolated from the neem tree *Azadirachta indica* A. Juss. (Lynn et al. 2010; Khan et al. 2012). Studies conducted by Jagdale and Grewal (2002) showed that some products are effective only in in vitro tests, whereas they are not suitable in the case of infected leaves. Research conducted by Tibugari et al. (2012), also El-Nagdi and Youssef (2013) with extracts of garlic, as well as by San Martin and Magunacelaya (2005) with extracts of the soapbark tree, showed that these components have nematicidal activity not only in laboratory tests, but also in greenhouse experiments. As it has been shown, an active compound against plant parasitic nematodes in *A. sativum* is allicin (Gupta and Sharmaj 1993) and the active compound in *Q. saponaria* against these pests are saponins (Zasada et al. 2010) or whole extracts containing saponin and non-saponin fractions (San Martin and Magunacelaya 2005). Most of the available information is concerned with the management of the root lesion or root-knot nematodes (Ingham et al. 2000; Agbenin et al. 2005; Zasada et al. 2010; Giannakou 2011). Protection against foliar nematodes is mainly based on chemical products, and only a few articles describe other methods of control (La Mondia 1999; Jagdale and Grewal 2002). From the economic point of view, it is important to find solutions for limiting foliar nematodes in container cultivation of ornamental plants conducted on a commercial scale. Evaluation of the effectiveness of nematicides is most often carried out on the basis of the number of nematodes isolated from soil (El-Nagdi and Youssef 2013; Giannakou, 2011; Ingham et al. 2000; Lynn et al. 2010) or plant material (Cabrera et al. 2009; Rao et al. 2004). In the case of foliar nematodes, extraction of specimens is associated with leaf destruction. Therefore, on each sampling date, the nematodes are isolated from the subsequent leaf, hence the initial population variant. The easiest way to estimate the effectiveness of pesticide is the evaluation of the number or percentage of damaged leaves (Hesling and Wallace 1960; Szczygieł 1969; Kohl et al. 2010). This kind of assessment is a non-invasive method although not completely foolproof. Nematodes could also be extracted from asymptomatic leaf tissue. Riedel and Powell (1974) isolated *A. fragariae* from leaves without visible symptoms, during the control of foliar nematodes in begonia with oxamyl. Kohl et al. (2010) and Kohl (2011) also found foliar nematodes in asymptomatic lantana leaves. It is crucial to evaluate the usefulness of this method in the estimation of nematicide efficacy in comparison with the standard procedure based on counting nematodes in leaves.

The objectives of the present study were: (i) the estimation of the efficacy of the products in the management of *A. ritzemabosi* on *Anemone hupehensis* (Lemoine) Lemoine in conditions of the container cultivation, typical for the production in nurseries, (ii) the comparison of two assessment methods of efficacy of tested pesticides and biocontrol products.

Materials and methods

Experimental procedure

Specimens of *A. ritzemabosi* were collected from infected plants in commercial nurseries located in Central Poland. Species identification was carried out based on morphology and morphometric parameters according to available identification keys and species descriptions (Siddiqi 1974a, b; Baranovskaya 1981; Andrássy 2007). Healthy plants were infested by spraying a water solution of living nematodes. Two analogical container trials using infected plants were conducted in 2013 and 2014 at the experimental field of the Research Institute of Horticulture in Skierniewice. The first research was established in June, the second one in September. Tests on plants of *A. hupehensis* (Lemoine) of the variety Prinz Heinrich were carried out for 4 consecutive weeks.

The following treatments were used:

1. Untreated control,
2. Vydate 10 G (oxamyl 100 g/kg, DuPont) at a dose 0.05 g/l of soil; one soil application,
3. 2.5% aqueous extract of *A. sativum* L. (prepared according to Dąbrowski and Seredyńska (2007)); 4 foliar applications at 7-day intervals.
4. 10% solution of Quillaja extract (2.2% water-glycolic extract of bark *Q. saponaria* Molina, Provital S.A.); 4 foliar applications at 7-day intervals.
5. 0.15% solution of Movento 100 SC (spirotetramat 100 g/l, Bayer CropScience) + 2.5% aqueous extract of *A. sativum* (prepared as above); 2 foliar applications of garlic extract at 1-week intervals alternately with 2 applications of Movento at 2-weeks intervals.
6. 0.075% solution of Vertimec 018 EC (abamectin 18 g/l, Syngenta Crop Protection) + 2.5% aqueous extract

of *A. sativum* (prepared as above); 2 foliar applications of garlic extract at 1-week interval alternately with 2 applications of Vertimec at 2-weeks intervals.

Spraying was carried out at a temperature of 16–20 °C and at a minimum humidity of 63%. In both experiments, 40 plants per combination were used (four repetitions for combination, ten plants in each replication). The estimation of the efficacy of products was based on the number of isolated nematodes and on the percentage of damaged leaves. Nematodes were extracted from two leaves of each plant by the Baermann funnel method and counted under the light microscope at 32× magnification. Nematodes and the percentage of damaged leaves were counted twice: before treatment (PRE-T) and at the end of the experiment (T + 28).

Statistical analysis

The results were statistically analyzed using the analysis of variance (ANOVA) using the transformed number of nematodes per leaf according to function: $y = \log(x + 1)$ and % of damaged leaves according to Bliss transformation ($y = \arcsin\sqrt{x}$). The significance of differences between means was evaluated using the multiple Duncan's test at a level of $\alpha = 0.05$. The analysis was performed in STATISTICA v. 10 (Stat Soft Inc., 2011). The average number of nematodes counted per leaf at each sampling date for each product was tested for correlation with the percentage of damaged leaves using the Spearman rank correlation test. Data were also elaborated by the regression method according to the linear function ($y = bx + a$) for the relationship between the number of nematodes per leaf and the percentage of damaged leaves counted in the successive dates of observation (y), and the number of days passing from the date of application to the successive dates of observation (x). Equation $y = bx + a$ determined the regression line, in which parameter "b" in linear relation indicated the sloping angle of line and the means speed of nematode reduction, and parameter "a" meant the initial level of nematode population on the day of treatment. The significance of the difference in parameters was estimated using Student's t test, at a level $\alpha = 0.05$. On the basis of the average number of nematodes, the effectiveness of the products was calculated, according to the Henderson–Tilton formula. To evaluate the effectiveness of the product in the control of nematodes, the criteria was used in accordance with the Regulation of the Polish Ministry of Agriculture and Rural Development, on August 4, 2004—Journal of Law No. 183, item 1890, where at least 80% = a good level of control; 60–80% = a medium level of control and 40–60% = a limited level of control.

Results

In the experiment conducted in 2013, 4 weeks after the first treatment of products, both the average number of nematodes and the percentage of damaged leaves increased in all tested products. The lowest increase of the nematode population was observed for oxamyl and abamectin with aqueous extract of garlic. The application of oxamyl also resulted in the lowest growth in the percentage of damaged leaves (Table 1). The efficacy of this nematicide was 40%. A similar effect was achieved 28 days after the application of abamectin with aqueous extract of *A. sativum*, in which the efficiency reached 42.2%. The application of other products was ineffective. The correlation between the number of nematodes in leaves and the percentage of damaged leaves was only significant in the untreated control.

The results of the second experiment revealed the efficiency of oxamyl and abamectin with aqueous extract of *A. sativum* (Table 2). The reduction in the number of nematodes was also observed in the case of other tested products, but it did not reach the limited level of pest control. The decrease in the number of nematodes was contributed to the reduction in the percentage of damaged leaves with the exception of oxamyl, despite the highest efficacy (44.4%). The Spearman rank correlation test did not prove the above observation.

In both years, in almost all treatments, the correlation between the number of nematodes in leaves and the sampling date was significant and the equation of the regression line with parameters a and b was determined (Tables 3, 4). In contrast, the correlation between the percentage of damaged leaves and the sampling date was not significant.

Discussion

Current studies showed difficulties in the control of foliar nematodes. Only two of the five tested substances (oxamyl and abamectin with aqueous extract of garlic) were effective at a limited level in both experiments. The reduction of the population of *A. ritzemabosi* in the case of other products was observed only in the second year. However, it did not reach 40%. The reaction of pesticides is different depending on environmental conditions and biological factors. Wheeler et al. (2013) observed the relationship between the irrigation rate, soil moisture and the nematicide performance. The studies of Oka et al. (2013) indicated an impact of such soil environments as soil type and organic matter. It is possible that in favorable conditions, the efficacy may increase; however, it requires further detailed studies.

Table 1 Efficacy of tested products in the control of *Aphelenchoides ritzemabosi* on *Anemone hupehensis* in 2013

	Combination	Average number of nematodes/leaf*		Efficacy (according to Henderson–Tilton formula)	Average % of damaged leaves**		Spearman ρ for number of nematodes/leaf and % of damaged leaves ($p = 0.05$)
		PRE-T	T + 28		PRE-T	T + 28	
1	Untreated	2.5 ab	31.6 d	–	10.5 cd	20.9 f	0.439***
2	Oxamyl	2.5 ab	7.9 c	*40.0*	10.1 b–d	10.8 d	0.078
3	Extract of *Allium sativum*	2.0 a	63.1 e	0.0	0.9 b	20.5 e	−0.092
4	Extract of *Quillaja saponaria*	2.0 a	63.1 e	0.0	0.2 a	10.2 b–d	0.311
5	Abamectin + extract of *Allium sativum*	2.5 ab	100.0 e	0.0	0.3 a	10.0 bc	0.111
6	Spirotetramat + Extract of *Allium sativum*	4.0 b	20.0 d	*42.2*	0.1 a	10.4 b–d	−0.004

Means in columns followed by the same letter do not differ at 5% level of significance Duncan test

* According to function: $y = \log(x + 1)$

** According to Bliss transformation

*** Values marked with a star in column are significantly different according to Spearman rank correlation

Table 2 Efficacy of tested products in the control of *Aphelenchoides ritzemabosi* on *Anemone hupehensis* in 2014

	Combination	Average number of nematodes/leaf*		Efficacy (according to Henderson–Tilton formula)	Average % of damaged leaves**		Spearman ρ for number of nematodes/leaf and % of damaged leaves ($p = 0.05$)
		PRE-T	T + 28		PRE-T	T + 28	
1	Untreated	125.9 b–d	501.2 fg	–	20.4 ab	40.3 d	0.146
2	Oxamyl	125.9 bc	31.6 a	*44.4*	30.0 bc	40.4 d	0.182
3	Extract of *Allium sativum*	398.1 f	199.5 c–e	31.2	30.7 cd	30.3 b–d	0.150
4	Extract of *Quillaja saponaria*	251.2 e	199.5 de	25.5	10.8 a	10.6 a	0.072
5	Spirotetramat + extract of *Allium sativum*	631.0 g	199.5 c–e	36.1	60.5 e	50.5 e	0.105
6	Abamectin + extract of *Allium sativum*	631.0 g	125.9 b	*41.7*	30.7 cd	30.7 cd	−0.218

Means in columns followed by the same letter do not differ at 5% level of significance Duncan test

* According to function: $y = \log(x + 1)$

** According to Bliss transformation

The ability to use oxamyl in the management of nematodes showed in this study was also verified by other authors. In Jagdale and Grewal (2002) studies, Vydate in formulation GR showed over 70% reduction of *A. fragariae* population on *Hosta* spp. Similar results were observed by Zasada et al. (2010). Their experiments concerned with the management of root lesion nematode *Pratylenchus penetrans* also revealed high nematicidal efficacy of oxamyl.

Present research demonstrated that abamectin, in combination with aqueous extract of *A. sativum*, may be effective in controlling the population of *A. ritzemabosi*. Both abamectin and garlic extract were previously tested by other researchers. Cabrera et al. (2009) and Faske and Starr (2007) obtained the efficacy of abamectin in reducing the number of *Meloidogyne* spp. In turn, James et al. (2006) reported that this substance may be useful in the protection against pine wilt disease caused by *Bursaphelenchus xylophilus*. Aqueous extracts of *A. sativum* significantly reduced root-knot infection indices on tomato with respect to *Meloidogyne incognita* (Agbenin et al. 2005, El-Nagdi and Youssef 2013) and *M. javanica* (Tibugari et al. 2012). Park et al. (2005) found that garlic oils, whose main compounds were diallyl disulfide, diallyl sulfide and diallyl trisulfide, could be useful as nematicides for the practical use against pine wood nematode. In our study, the separate application of extract of *A. sativum* did not prove to be effective. The combined use of these products is better in achieving repeated effects in the reduction of foliar nematodes.

Spirotetramat, in combination with an extract of *A. sativum*, was not effective. Data from the literature also shows that the use of spirotetramat against nematodes varies. Smiley et al. (2011) suggested that applications of this substance reduced the density of *Heterodera avenae*. In turn, the research of Zasada et al. (2010) and Smiley

Table 3 Correlation between average number of nematodes and the sampling date in 2013

	Combination (C)	Correlation coefficient	Regression parameters	
			a	*b***
1	Untreated	0.826*	0.377	0.044
2	Oxamyl	0.408*	0.554	0.015
3	Extract of *Allium sativum*	0.781*	0.455	0.054
4	Extract of *Quillaja saponaria*	0.684*	0.649	0.042
5	Spirotetramat + extract of *Allium sativum*	0.814*	0.467	0.055
6	Abamectin + extract of *Allium sativum*	0.505*	0.794	0.021

* Values marked with a star in column are significantly different according to Spearman rank correlation

** Comparison of *b* parameters according to Student's *t* test: C1 ≠ C2, C1 ≠ C6, C2 ≠ C3, C2 ≠ C4, C2 ≠ C5, C3 ≠ C6, C4 ≠ C5, C4 ≠ C6, C5 ≠ 6

Table 4 Correlation between average number of nematodes and the sampling date in 2014

	Combination (C)	Correlation coefficient	Regression parameters	
			a	*b***
1	Untreated	0.730*	2.114	0.020
2	Oxamyl	−0.580*	2.145	0.022
3	Extract of *Allium sativum*	−0.290*	2.573	0.010
4	Extract of *Quillaja saponaria*	−0.003	–	–
5	Spirotetramat + extract of *Allium sativum*	−0.573*	2.805	0.019
6	Abamectin + extract of *Allium sativum*	−0.719*	2.819	0.027

* Values marked with a star in column are significantly different according to Spearman rank correlation

** Comparison of *b* parameters according to Student's *t* test: C1 ≠ C2, C1 ≠ C3, C1 ≠ C5, C1 ≠ C6, C2 ≠ C3, C3 ≠ C6

et al. (2012) showed inefficiency of this product in the controlling of *Pratylenchus* spp. population. These differences suggest that nematode species are characterized by differential sensitivity to the nematicides. Similar observations were noted by La Mondia (1999).

The application of extract of *Q. saponaria* was also ineffective. The ability to control nematodes by this extract was reported in several previous publications (Insunza et al. 2001; Giannakou 2011; San Martin and Magunacelaya 2005). In Chałańska et al. (2013), studies on anemone plants showed that the application of soapbark tree extract was highly effective, but the concentration of *Q. saponaria* extract used in that study was five times higher and caused phytotoxicity symptoms in plants. In this present study, the application of harmless doses of this product did not reduce the population of nematodes. Conclusions drawn by the comparison of literature data and the results of this study are that in future, studies also with different doses should be tested—higher than in the present study, but still not causing phytotoxicity.

The selection of a suitable product in managing foliar nematodes is associated with many difficulties. The efficacy of nematicides depends on various factors. In our study, the impact on results could be altered by atmospheric temperature and humidity. Szczygieł (1966) and Szczygieł and Hasior (1972) drew attention to this. With respect to foliar nematodes, additional difficulties are connected with the effective penetration of nematicides into leaf tissue. Available products for nematode control could protect the plants against visible damage caused by foliar nematodes, without exterminating all specimens (Kohl 2011). The precise estimation of the population of *A. ritzemabosi* before and after pest control application is necessary in order to make a proper assessment of the effectiveness of the tested pesticide and to determine the further implementation of a plant protection plan, depending on environmental conditions and the number of nematodes. As it was shown in the present research, in spite of the counting of nematodes from another leaf at each sampling date, this method is still the most reliable diagnosis of nematode damage. Moreover, it allows to determine the number of nematodes after the treatments. It is an essential part of foliar nematode management because they are known as a highly reproductive group (La Mondia 1999) requiring constant monitoring.

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