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EVALUATION OF THE EFFECTIVENESS OF IRON PHOSPHATE AND THE PARASITIC NEMATODE *PHASMARHABDITIS HERMAPHRODITA* IN REDUCING PLANT DAMAGE CAUSED BY THE SLUG *ARION VULGARIS* MOQUIN-TANDON, 1885

Jan Kozłowski[1], Monika Jaskulska[1], Maria Kozłowska[2]

[1] Department of Zoology, Institute of Plant Protection – National Research Institute, Władysława Węgorka 20, 60-318 Poznań, Poland (e-mail: j.kozlowski@iorpib.poznan.pl)

[2] Department of Mathematical and Statistical Methods, Poznań University of Life Sciences, Wojska Polskiego 28, 60-637 Poznań, Poland

Abstract: *Arion vulgaris* is one of the most important slug pests of crop plants, and is extremely hard to control. We assessed the effectiveness of a slug pellets containing iron phosphate, compared with methiocarb- and metaldehyde-based molluscicides, and of the parasitic nematode *P. hermaphrodita* contained in the commercial bioproduct Nemaslug. The effect of various doses of these substances and the nematode in reducing slug damage to Chinese cabbage plants was evaluated in laboratory experiments. A reduction in slug numbers and plant damage was obtained following application of the molluscicides, and a reduction in plant damage following application of *P. hermaphrodita* (immersion of plant roots in the nematode-containing liquid). The results are of great practical significance, because they show that it is possible to reduce the recommended dose of iron phosphate (5 g/m^2) by one half. Another important finding is that application of the nematode in the form of root immersion is more effective than spraying. The study demonstrates the usefulness of the nematode and of the iron phosphate molluscicide in protecting plants from *A. vulgaris*; this is of particular importance for crops on which the application of traditional molluscicides is not possible.

Key words: *A. vulgaris*, iron phosphate, *P. hermaphrodita,* pest control

INTRODUCTION

The most common methods of pest slug control involve the use of chemical bait pellets containing metaldehyde or carbamates (methiocarb, thiodicarb) (Henderson & Triebskorn 2002). The use of molluscicides containing these active substances causes numerous problems relating to their application and their adverse impact on the environment. Both substances are toxic to vertebrates (Fletcher et al. 1994), and methiocarb is also toxic to useful invertebrates such as ground beetles (Purves & Bannon 1992). A molluscicide containing iron phosphate (Ferramol, Neudorff GmbH, Germany), which has recently been registered in certain European countries (Speiser & Kistler 2002, Rae et al. 2009), is safer for the environment. It shows very low toxicity to mammals, and occurs naturally in various forms (Clark 1993). When used against slugs, it inhibits grazing and causes their death. Laboratory and field experiments have demonstrated a high effectiveness of iron phosphate in protection of various plant species, although there are differences in the level of protection achieved against different slug species (Koch et al. 2000, Iglesias & Speiser 2001, Iglesias et al. 2001a, Speiser & Kistler 2002, Rae et al. 2009). The recommended dose of the iron phosphate molluscicide 1% (Ferramol) is high (2.5 to 5 g/m^2), which makes its use expensive (Speiser & Kistler 2002, Rae et al. 2009). On the other hand, the absence of any adverse impact on the environment speaks in favour of its wider use.

Great hopes have been placed in the parasitic nematode *Phasmarhabditis hermaphrodita* as a means of plant protection. The nematode carries the bacterium *Moraxella osloensis*, which is toxic to slugs, causing their death and/or inhibiting their grazing. Several authors report that *P. hermaphrodita* may protect plants against certain slugs (Wilson et al. 1993, 1994, 2000, Ester & Geelen 1996, Glen et al. 1996, 2000, Iglesias et al. 2001a, b, Iglesias & Speiser 2001, Speiser et al. 2001, Grewal et al. 2003, Rae et al. 2009). It is used in the formulation of a biological product called Nemaslug (Becker Underwood, UK), and is recommended for use on soil in a dose of 3 × 10^9 infectious young nematodes per hectare. *P. hermaphrodita* infects various slug and snail species, although not all of them are killed at a similar rate, which indicates their different sensitivity to the nematode (Wilson et al. 1993, Glen et al. 1996, Grewal et al. 2003). For example, in large species of the family Arionidae, the sensitivity to *P. hermaphrodita* decreases with increasing body size (Glen et al. 1996, Speiser et al. 2001, Grimm 2002, Grewal et al. 2003). Some authors report that *P. hermaphrodita* causes significant reduction in the plant damage caused by slugs by inhibiting their grazing, without causing their death (Ester & Geelen 1996, Glen et al. 1996, 2000, Wilson et al. 1999, Iglesias et al. 2001a, b, Grimm 2002, Rae et al. 2009). In recent years, research on *P. hermaphrodita* has focused on determining appropriate doses and techniques for its use against particular slug species in various age categories, so as to reduce the costs of slug control. Laboratory and small field experiments have been carried out to investigate the effectiveness of multiple applications of *P. hermaphrodita* in reduced doses (Ester et al. 2003a, b, Rae et al. 2006, 2009).

Developing environmentally safe methods of reducing the damage done by *Arion vulgaris* Moquin-Tandon, 1885, formerly misidentified as *Arion lusitanicus* Mabille, 1868 (Welter-Schultes 2012) is a priority at present. In central and northern Europe this slug poses a serious risk to many species of crop plants, and it is difficult to control. It causes severe damage particularly in gardens and in certain field crops (Godan 1979, South 1992, Frank 1998, Grimm 2001, Glen & Moens 2002, Moens & Glen 2002, Port & Ester 2002, Kozłowski 2007, 2012).

The aim of this study was to investigate the possibility of reducing the applied dose of iron phosphate and to compare the effectiveness of two methods of application of the nematode *P. hermaphrodita* in reducing the damage done to Chinese cabbage plants by *A. vulgaris*.

MATERIAL AND METHODS

The specimens of *A. vulgaris* used in the experiments came from a population in Łańcut near Rzeszów. After hatching from collected eggs, the slugs were kept on clay soil with humus in semi-transparent plastic containers (26 × 26 × 14 cm) with ventilation holes. Their diet consisted of cabbage and oilseed rape leaves, potato tubers, wheat bran, powdered milk and calcium carbonate, which were replaced three times a week. They were kept in a climate chamber at a temperature of 17°C, RH 70% ± 3%, with a 12/12 hour photoperiod. Experiments to determine the effect of the molluscicides and of *P. hermaphrodita* were carried out in identical containers in the same conditions.

In the tests of the effectiveness of iron phosphate, the commercial molluscicide Anti-Limaces Ferramol (0.99% a.s., Scotts France SAS, recommended dose – 5 g/m^2) was applied in three different doses (1.0, 2.5 and 5.0 g/m^2), while the two molluscicides used for comparison, methiocarb (Mesuol Alimax 02 RB, 2% a.s.) and metaldehyde (Snacol 05 GB, 5% a.s.) were applied only in the recommended doses (0.5 g/m^2 and 0.4 g/m^2, respectively). No-choice tests were done on the Hilton variety of Chinese cabbage (3–4 leaf stage) growing in a 5 cm layer of soil inside containers. To each container with six plants, molluscicide granules were introduced in the appropriate dose, along with two slugs (average weight 1.97 g) which had been starved for the previous 48 hours. Containers with plants and slugs but without molluscicides were used as control. Five replicates were performed for each molluscicide and each control.

In tests of the nematode effectiveness, invasive larvae of *P. hermaphrodita* were used, obtained from the commercial bioproduct Nemaslug (Becker Underwood, UK), on plants of the Hilton variety of Chinese cabbage. *P. hermaphrodita* was applied with two methods: 1) spraying plants and soil; 2) immersion of plant roots. The spraying was performed using two different doses of the nematode, which were applied three times, after 1, 6 and 12 days (3 × 5 and 3 × 10 nematodes per cm^2 of soil surface area in the container). Root immersion was carried out using two different single doses: 4,800 and 9,600 nematodes per plant. The roots of each plant were immersed in 30 ml of water with nematodes and the plants were watered with the remnants of the solution when planting. To increase the adhesion of the nematodes to the plant roots, CMC (carboxymethyl cellulose) was added in the concentration of 0.5% (PETERS et al. 2002). Plants at the 4–5 leaf stage were planted in a 5 cm layer of clay soil with humus, with six plants in each container. In the test in which the nematode was applied as spray, the soil and the plants in the containers were sprayed with the first two doses of the nematode, and after 24 hours two slugs (average weight 2.31 g) were placed in each container. The remaining two sprayings with the same doses of the nematode were performed after 6 and 12 days. In the control containers the soil and plants were sprayed with water. In the test in which the nematode was applied as immersion, the roots and lower parts of the plants were placed in 100 ml of the nematode-containing liquid in the appropriate dose, or in water in the case of control, and were then planted in the containers with soil. After 24 hours, two slugs (average weight 2.14 g) were placed in each container. Five replicates were performed for each experimental variant.

In both experiments, the vitality of the slugs and the degree of plant damage (five-point scale: 0 – no damage, 25, 50, 75 and 100% damaged plant surface) were assessed every two days. In the experiment with *P. hermaphrodita*, symptoms of infection of the slugs by the nematode (reduced mobility and grazing, slackening of the body, cessation of movement and grazing, bloating of the body around the mantle, slug death) were monitored during one month. The weighted mean values of the amount of plant damage caused by the slugs for the particular experimental variant in each experiment were subject to statistical analysis. Analysis of covariance (ANCOVA) was applied, taking account of the variation in the weight of the slugs; adjusted means were determined; the contrasts of the adjusted means were estimated; and hypotheses concerning the comparisons were tested using Student's *t*-test (STATISTICA ver. 10, significance level $\alpha = 0.05$).

RESULTS

EFFECTIVENESS OF IRON PHOSPHATE

The tested molluscicides caused paralysis and subsequent death of some of the slugs. The percentage of slugs killed following the applied doses was as follows: iron phosphate, dose 1.0 g/m^2 – 20% (2 dead 7 days after application), dose 2.5 g/m^2 – 50% (4 dead after 9 days and 1 after 11 days), and dose 5.0 g/m^2 – 80% (2 dead after 5 days, 4 after 7 days and 2 after 9 days); methiocarb, dose 0.5 g/m^2 – 90% (7 dead after 7 days, 1 after 9 days and 1 after 11 days); metaldehyde, dose 0.4 g/m^2 – 30% (3 dead after 7 days).

The applied molluscicides were found to reduce the damage done to the plants by *A. vulgaris* after just 24 hours from their application (F_{1d}=7.3, d.f.=5; 28, p<0.001) (Fig. 1). By that time, iron phosphate in doses of 1.0, 2.5 and 5.0 g/m^2, and metaldehyde in a dose of 0.4 g/m^2, had caused significant reduction in the plant damage, compared with the untreated (control) plants (the respective *p* values for the contrasts between test treatments and control were 0.016, 0.002, 0.0001 and <0.0001). However, damage to the plants treated with methiocarb did not differ significantly from the damage to the control plants (p=0.439).

On subsequent days of observation, significant differences were found for the compared objects in the amount of damage done to the plants by *A. vulgaris* (F_{3d} = 7.5, d.f. = 5; 28, p < 0.001; F_{5d} = 10.3, d.f. = 5; 28, p < 0.001; F_{7d} = 17.8, d.f. = 5; 28, p < 0.001; F_{9d} = 15.4, d.f. = 5; 28, p < 0.001; F_{11d} = 20.5, d.f. = 5; 28, p < 0.001; F_{13d} = 15.9, d.f. = 5; 28, p < 0.001 and F_{15d} = 28.7, d.f. = 5; 28, p < 0.001). From the third day onwards, all of the molluscicides produced a significant reduction in plant damage compared to the control. On the third day, the largest contrast with the control was obtained for metaldehyde (c_{3d} = −17.67, p < 0.001) and for iron phosphate in the dose of 5.0 g/m^2 (c_{3d} = −17.66, p < 0.001), while the smallest was obtained for methiocarb (c_{3d} = −9.16, p = 0.025). There were no significant differences in effectiveness among the tested molluscicides on the third and fifth days of observation. On the seventh day after application, significant differences were found for the contrasts between iron phosphate in the doses of 2.5 and 5.0 g/m^2 and metaldehyde in the dose of 0.4 g/m^2 (c_{7d} = −22.6, p=0.009 and c_{7d} = −23.3, p=0.008, respectively). Differences between iron phosphate in those two doses and metaldehyde persisted till the last day of observation. On the 15th day the value obtained for the first contrast was c_{15d} = −50.4 (p < 0.001), and that of the second contrast was c_{15d} = −58.5 (p < 0.001). It should also be noted that the effectiveness of iron phosphate in the doses of 2.5 and 5.0 g/m^2 was similar: the results obtained for successive days of observation were: c_{1d} = 2.6, p = 0.41; c_{3d} = 0.93, p = 0.85; c_{5d} = 2.2, p = 0.76; c_{7d} = 0.7, p = 0.93; c_{9d} = −1.7, p = 0.88; c_{11d} = 1.3, p = 0.91; c_{13d} = 2.0, p = 0.88; c_{15d} = 8.1, p = 0.50.

In the course of the entire experiment, the largest reductions in plant damage, relative to the control, were obtained following the application of iron phos-

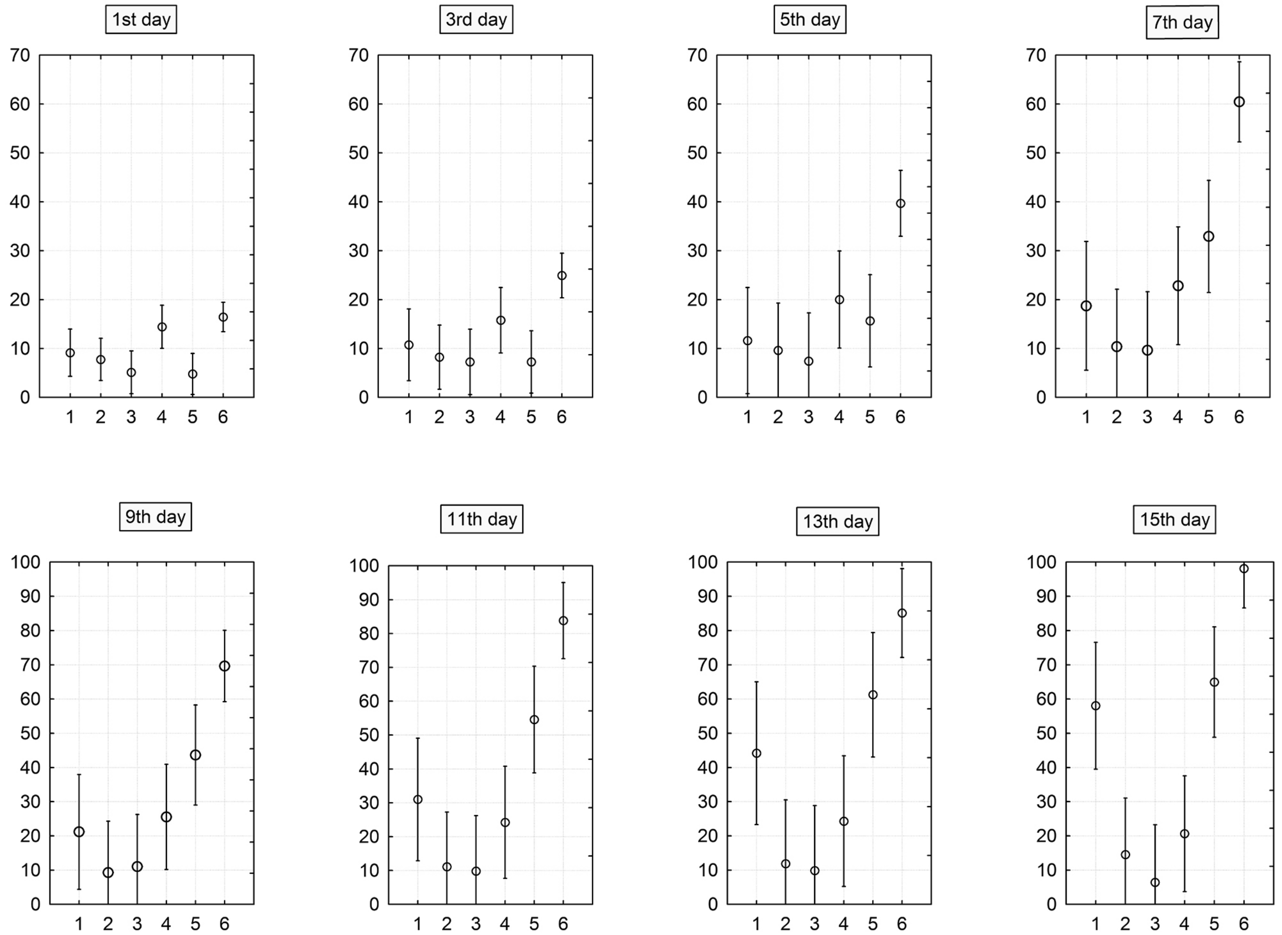

Fig. 1. Adjusted means of damage to seedlings of Chinese cabbage caused by *Arion vulgaris* after treatment with various compounds on consecutive days: axis Y – means in % from ANCOVA and confidence intervals; axis X – 1 denotes iron phosphate 0.99%, dose 1.0 g/m²; 2 – iron phosphate 0.99%, dose 2.5 g/m²; 3 – iron phosphate 0.99%, dose 5.0 g/m²; 4 – methiocarb 2%, dose 0.5 g/m²; 5 – metaldehyde 5%, dose 0.4 g/m²; 6 – control treatment

phate in the doses of 5.0 and 2.5 g/m², and amounted to 93.4% and 85.2%, respectively.

EFFECTIVENESS OF *P. HERMAPHRODITA*

On the 9th day of observation, following application of the nematode as spraying, with the dose of 2 × 5 nematodes/cm², one slug was found to have symptoms of infection (body slackening and reduced mobility). Following the third spraying with the dose of 5 nematodes/cm², symptoms were observed in another two slugs (17th and 19th days of observation). Similar symptoms occurred in one slug on the 13th day, following application of the nematode in the dose of 3 × 10/cm². Following application of the nematode as root immersion, in the dose of 4,800 nematodes per plant, one dead slug was found (4th day after application) as well as five slugs with symptoms of infection as described above (two on the 2nd day, one on the 5th day, and two more on the 17th day). Four of these later died (3, 23, 29 and 31 days after application of the nematode). The use of the increased dose of 9,600 nematodes per plant did not cause an increase in the number of slugs with symptoms of infection or in the number of dead slugs. At that dose, over the entire observation period, symptoms of infection were observed in five slugs (one on the 1st day, two on the 15th day, and another two on the 17th day), one of which died 25 days after application of the nematode. The total mortality of *A. vulgaris* was 5% in the first two weeks following application of the nematode, and 10% in the two subsequent weeks of observation.

The amount of damage done by the slugs to the cabbage plants treated with *P. hermaphrodita*, up to the 5th day following application, was similar to that done to untreated plants (F_{1d} = 1.3, d.f. = 5; 23, p = 0.29; F_{3d} = 1.6, d.f. = 5; 23, p = 0.21; F_{5d} = 2.1, d.f. = 5; 23, p = 0.10) (Fig. 2). The first significant differences were observed on the 7th day of observation, i.e. seven days after application of the nematode (F_{7d} = 2.7, d.f. = 5; 23, p = 0.45). The plants immersed in the nematode-containing liquid, in

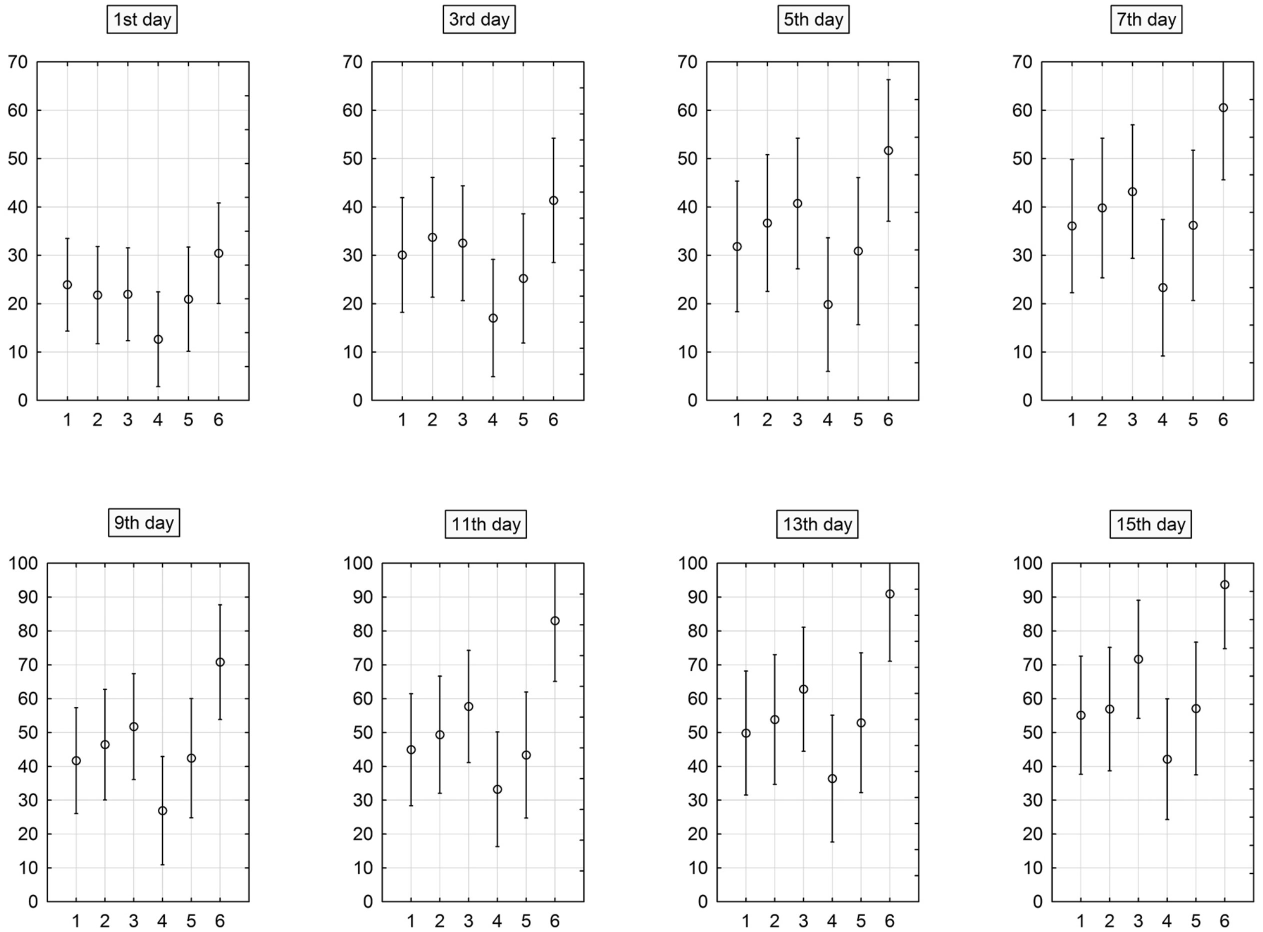

Fig. 2. Adjusted means of damage to seedlings of Chinese cabbage caused by *Arion vulgaris* after treatment with different dose and form of application *Phasmarhabditis hermaphrodita* on consecutive days: axis Y – means in % from ANCOVA and confidence intervals; axis X – 1 denotes spraying *P. hermaphrodita,* dose 3 × 5 pcs/cm²; 2 – spraying *P. hermaphrodita,* dose 3 × 10 pcs/cm²; 3 – spraying control 3 × H_2O; 4 – dipping *P. hermaphrodita,* dose 4,800 pcs/plant; 5 – dipping *P. hermaphrodita,* dose 9,600 pcs/plant; 6 – dipping control H_2O

both doses (4,800 and 9,600 nematodes per plant), were significantly less damaged than the untreated plants (for group contrast represented by vector [0,0,0,1,1,–2] we obtained $c_{7d} = -61.6$, $p = 0.004$, and for each dose separately: $c_{7d} = -37.3$, $p = 0.002$ and $c_{7d} = -24.4$, $p = 0.043$, respectively). Similar differences were obtained 9, 11, 13, and 15 days after the nematode application ($F_{9d} = 3.0$, d.f. = 5; 23, $p = 0.033$; $F_{11d} = 3.7$, d.f. = 5; 23, $p = 0.014$; $F_{13d} = 3.6$, d.f. = 5; 23, $p = 0.015$, and $F_{15d} = 3.8$, d.f. = 5; 23, $p = 0.012$). On those days, damage to the nematode-immersed plants was significantly smaller than to the untreated plants; the results for the 9th, 11th, 13th and 15th days of observation were: for group contrast represented by vector [0,0,0,1,1,–2]: $c_{9d} = -72.3$, $p = 0.003$; $c_{11d} = -89.5$, $p < 0.001$; $c_{13d} = -92.6$, $p = 0.002$; $c_{15d} = -88.2$, $p = 0.002$, and for each dose separately: $c_{9d} = -43.9$, $p = 0.001$ and $c_{9d} = -28.4$, $p = 0.038$; $c_{11d} = -49.8$, $p < 0.001$ and $c_{11d} = -39.7$, $p = 0.008$; $c_{13d} = -54.6$, $p < 0.001$ and $c_{13d} = -38.1$, $p = 0.019$; $c_{15d} = -51.6$, $p < 0.001$ and $c_{15d} = -36.6$, $p = 0.018$. No differences were found between the amount of plant damage following application of the nematode spray and the damage found in the control, irrespective of whether the dose was 3 × 5 or 3 × 10 nematodes/cm².

After 15 days the reduction in the damage to the nematode-immersed plants, compared to the control, was 55.0% for the dose of 4,800 nematodes per plant, and 39.1% for the dose of 9,600 nematodes per plant. It was observed that nematodes applied as root immersion caused a similar reduction in slug damage for both doses ($c_{15d} = -15.0$, $p = 0.23$). Following application of the nematode spray, in the doses of 3 × 5 and 3 × 10 nematodes/cm², after 15 days the reduction in plant damage, compared to the control, was 23.1% and 20.5% respectively, but was not statistically significant.

DISCUSSION

The study of the effectiveness of the molluscicide containing iron phosphate compared with those containing methiocarb and metaldehyde showed that the substances had lethal effects on slugs, causing a reduction in their grazing, and consequently in the damage done to plants. The first slugs died after five days, the highest rate of mortality being observed seven days after application of the molluscicides. As a result of the decrease in the number of slugs, there was a marked reduction in plant damage, compared to the control. This indicates that the molluscicides used protected the plants from damage caused by *A. vulgaris*, the most effective being the molluscicide with iron phosphate applied in the dose of 5 g/m^2 (recommended in Poland), with a slightly decreased effectiveness when the dose was 2.5 g/m^2, similar to that of the molluscicide with methiocarb applied in the dose of 0.5 g/m^2. This suggests that the effectiveness of the reduced dose of 2.5 g/m^2 of the iron phosphate molluscicide is comparable to that of molluscicides containing methiocarb and metaldehyde. This is of great practical significance, as it means that the recommended dose of iron phosphate (5 g/m^2) can be reduced to one half of that value, which will significantly reduce the costs of using that molluscicide. This applies chiefly to crops with a smaller slug population, where a reduced dose of iron phosphate will probably be sufficient to protect the plants from the slugs.

A high effectiveness of iron phosphate pellets was also observed by other authors. Rae et al. (2009) found that, in vegetable patches, iron phosphate pellets and metaldehyde provided very good protection for lettuce plants against *Arion ater* (Linnaeus) and *Deroceras reticulatum* (O. F. Müller), and that their effectiveness was similar. Iron phosphate was also found to be effective in protecting lettuce, beetroot, oilseed rape and certain ornamental plants against *D. reticulatum*, *A. lusitanicus* Mabille, *A. ater* and *A. hortensis* (Férussac) (Koch et al. 2000, Iglesias & Speiser 2001, Iglesias et al. 2001a, b, Speiser & Kistler 2002, Rae et al. 2009). It should be noted that iron phosphate is markedly superior to the presently used molluscicides with methiocarb and metaldehyde in terms of the absence of adverse effects on other animals (Clark 1993, Speiser & Kistler 2002). The substance occurs naturally in mineral form, and is widely used in human foods and as an ingredient in artificial fertilizers. Consequently it is safe for the environment and can be used in organic cultivation.

The study of effectiveness of *P. hermaphrodita* used in the form of spray applied to plants and soil, or as root immersion, showed that the nematode had a weakly lethal effect on *A. vulgaris* (15% mortality). The nematode-infected slugs had very mild symptoms in the form of body slackening and partial loss of mobility, or did not display any visible external symptoms of infection. The characteristic symptoms of swelling of the body around the mantle, described by Wilson et al. (1993) in the case of *D. reticulatum*, were not observed. Nonetheless, *P. hermaphrodita* applied as root immersion to Chinese cabbage plants brought a significant reduction in the damage done by *A. vulgaris*, the reduction reaching 61% for the dose of 4,800 nematodes per plant. This suggests that the infection of some of the slugs with the nematode led to inhibition of their grazing activity. Doubling of the nematode dose did not cause any further increase in the number of infected or dead slugs or reduction in plant damage.

Some authors reported that *P. hermaphrodita* caused a reduction in plant damage caused by slugs, but without having a significant effect on their numbers (Wilson et al. 1994, Ester & Geelen 1996, Glen et al. 1996, 2000, Iglesias et al. 2001a, Rae et al. 2009). This applied particularly to large species of the genus *Arion*, such as *A. lusitanicus* Mabille (Glen et al. 2000) and *A. ater* (Linnaeus) (Iglesias et al. 2001b). *P. hermaphrodita* applied as spray (3 × 5 and 3 × 10 nematodes/cm^2) did not bring a statistically significant reduction in the damage done to Chinese cabbage by *A. vulgaris*. According to Rae et al. (2006), a triple application of one half of the recommended dose of *P. hermaphrodita* (3 × 5 nematodes/cm^2) provided significant protection against damage to Chinese cabbage caused by *D. reticulatum*. Ester et al. (2003b) reported that several applications of low doses of nematodes provided significant protection of asparagus against slugs. However in other studies, using adult specimens of *A. lusitanicus*, no infection of the slugs by the nematode was observed following its application as spray (Koch et al. 2000), similarly as in the present study.

This study confirmed the high effectiveness of iron phosphate pellets in reducing the damage to plants caused by *A. vulgaris*, comparable to the effectiveness of the presently used molluscicides containing methiocarb and m`1etaldehyde. It was also shown that a reduced dose of one half of the recommended level of iron phosphate could protect plants from slug damage. In the experiment on the effect of *P. hermaphrodita*, the damage caused by *A. vulgaris* was found to be reduced only when the nematode was applied as root immersion. This method can be recommended when certain species of plants, such as brassicas, are planted out in areas inhabited by slugs. Some authors recommend combining the application of low doses of iron phosphate pellets and of the nematode *P. hermaphrodita* (Rae et al. 2009). Such a solution may be very useful in modern integrated systems for protecting plants against pest slugs.

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