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Brief Report

Foliar Application of Entomopathogenic Nematodes against Cereal Leaf Beetle *Oulema melanopus* L. (Coleoptera: Chrysomelidae) on Wheat

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Abstract: Cereal monocultures are very susceptible to many pests, especially to those living on leaves, which largely affects yield by decreasing its quality. The most dangerous of them is the cereal leaf beetle (*Oulema melanopus* L.). In cases of heavy infestation by its larvae, the surface of plants may be reduced by 50%, and sometimes even by 80%, with a main yield loss of 10–25%. The aim of the presented study was to assess the efficiency of a native isolate of *Steinernema feltiae* (Filipjev, 1934) and commercial preparation Larvanem (*Heterorhabditis bacteriophora* (Poinar, 1975)) in controlling the larvae of *O. melanopus*, and to reduce crops damage in the field. Nematodes were applied in a dosage of 2 million IJs/m^2 as a suspension of 11 litres per square metre. A hand sprinkler with field lance and flat-stream nozzles was used for applications at the lowest working pressure of 3000 hPa. The effectiveness of both nematode species was moderate: 47.8% for *S. feltiae* isolate and 49.5% for *H. bacteriophora*. The biggest reduction in leaf damage was found in crops treated with the commercial preparation, where the index of leaf infection was 32%, being more than twofold smaller than that for the control.

Keywords: biological control; *Oulema melanopus*; Larvanem; *Steinernema feltiae*

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1. Introduction

Cereal production is one of the main branches of agriculture in Poland. Poland is the second-largest country in the EU with respect to the surface area of cereal crops (after France) and the third with respect to harvest (after France and Germany) [1].

Cereal monocultures are very susceptible to many pests, especially those feeding on leaves, which substantially affect the yield and quality of the harvest. There are two species of cereal leaf beetles in Poland: *Oulema melanopus* (L.) and *Oulema gallaeciana* Heyden both belonging to the Chrysomelidae family. The first is more common in the central and northern parts of the country, while the second dominates in south-eastern Poland [2]. *O. melanopus* is also the most important wheat pest in many other countries [3,4], ranging from central Siberia through Sweden, Great Britain, Spain, western Africa [5,6] to the United States and Canada [5,7].

When feeding in spring, the imago of the beetle damages leaves by making many characteristic narrow and elongated holes that it gnaws along the leaf veins. In the case of the mass appearance of beetles, such damage may disturb the proper growth and development of affected plants. If the crops' structure includes both winter and spring cereals, the earliest hatching beetles start feeding on winter rye but rarely lay eggs on it.

Later on, they transfer onto other winter and spring cereal species [8]. The feeding and egg-laying period is prolonged and may last 2–3 weeks, depending on the weather conditions. In favourable atmospheric conditions (warm, dry and sunny weather), one female is able to lay 200 to 300 eggs. At the end of May and at the beginning of June, the larvae start feeding on the leaves, causing further damage. The larvae scrape off the upper epidermis of leaves, eat parenchymal tissue along veins but do not damage lower skin. After some time, the lower skin dries out and turns white. The larvae of cereal leaf beetles may decrease the assimilation surface area of the flag and sub-flag leaves by 50 or sometimes even by 80 percent [9–11]. Moreover, feeding larvae contaminate cereal leaves with viscous substances and faeces. All these factors decrease the assimilating surface area of leaves and, consequently, the yield of crops.

Cereal leaf beetles are usually controlled when the larvae hatched from the earliest laid eggs reach a size of 4 mm, and at the same time, large numbers of hatching larvae numbers are observed [8]. Chemical agents are used most commonly to limit the number of cereal leaf beetles (both larvae and imagines) [12]. Biological control of cereal leaf beetles is based on the introduction of natural enemies of adult insects, which is the most effective strategy for controlling adult forms of the pest. Since the 1980′s, studies have been carried out in Western Europe and in Poland on the use of bacteria, entomopathogenic fungi, parasitoids and predators for this purpose. Natural enemies of cereal leaf beetles include predatory insects of the family Carabidae (Cleoptera) [13,14]. Parasitoids of cereal beetle larvae used in biological control include species such as: *Tetrastichus julis* Yang, *Diaparsis carinifer* (Thomson) and *Lemophagus curtis* (Townes) (Hymenoptera: Ichneumonidae). Parasitoids of eggs are: *Anaphes flavipes* (Foester) (Hymenoptera: Mymaridae) and *Trichogramma* sp. [7,15], while parasitoids of pupae are species of the order Hymenopterous [16,17]. Entomopathogenic pathogens that might limit the growth and spreading of beetles on cereal plants are fungi: *Alternaria alternata* (Fr.) Keissl. and bacteria *Bacillus thuringiensis* Berliner, 1915 [18].

Recently, more attention has been focused on the possibility of using entomopathogenic nematodes (EPNs) to limit populations of many harmful insect species. In particular, two genera of entomopathogenic nematodes were demonstrated to be useful for the biological control of many harmful insect species: *Steinernema* (Steinernematidae) living in symbiosis with bacteria of the genus *Xenorhabdus* (Thomas and Poinar, 1979) and *Heterorhabditis* (Heterorhabditidae) with symbiotic bacteria of the genus *Photorhabdus* [19–22]. About 250 insect species representing 10 orders were found to be sensitive to entomopathogenic nematodes [21].

Our field studies were preceded by laboratory tests to assess the sensitivity of beetles and larvae of *O. melanopus* to native isolates of entomopathogenic nematodes represented by two species: *S. feltiae* (four isolates) and *Heterorhabditis megidis* (Poinar, Jackson and Klein, 1987) (one isolate). The isolate of *S. feltiae* ZAG 15 was the most effective, causing 100% mortality in both larvae and adult insects [2], and was selected for field studies.

The aim of this study was to assess the effectiveness of the native strain of *S. feltiae* Zag 15 in controlling *O. melanopus* larvae and leaf damage in field conditions. For comparative purposes, we used a commercial preparation Larvanem (*H. bacteriophora*) designed to control beetles' larvae.

2. Materials and Methods

2.1. Entomopathogenic Nematodes

Isolate of *S. feltiae* Zag 15. The strain was isolated in 2010 from meadow soil in the Zagożdżonka River valley (N 51°23′10.4820″, E 21°33′15.54120″) and is maintained in a laboratory culture at Department of Animal Environment Biology, Institute of Animal Sciences, Warsaw University of Life Sciences, Warsaw, Poland [2].

Commercial preparation Larvanem produced by Koppert/Holland with infective juveniles (IJs) of *H. bacteriophora* (1 pack contains 50 million IJs).

Cereal leaf beetle. The first assessment of the number of naturally occurring adults, eggs and larvae of the cereal leaf beetle in experimental plots was made on 23 April 2019. Then the development of larvae was assessed at weekly intervals and every 2–3 days from the tillering phase of winter wheat BBCH 20 (Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie scale-the scale used to identify the phenological development stages of plants. BBCH-scales has developed for a range of crop species where similar growth stages of each plant are given the same code) to the end of earing phase BBCH 59.

2.2. Experimental Plots

The study was carried out on experimental plots of the Field Experimental Station in Winna Góra (N 52°12′21.5″; E 17°26′51.2″83), where the variety of winter wheat Bogatka was sown on 20 September 2018. Before sowing, the soil was prepared with a two-furrow plough and tiller. Mineral fertilisation per hectare amounted to N—100 kg (60 kg after the start of vegetation (phase BBCH 29) and 40 kg in internode phase 3 (BBCH 33), P—9.0 kg and K—130 kg. Phosphorus and potassium fertilisers were applied once before sowing. During the plant growth, only herbicides were applied without any preparations against pests. The experiment was carried out on podzolic soil of the IIIb and IVa quality class.

Meteorological data during the experiment (25 May to 2 June) were acquired from the weather station at the Field Experimental Station in Winna Góra and measured between 6 a.m. and 6 p.m. The average mean daily temperature during the observation period was 21.8 °C (max. 24.0; min. 17.8); the mean daily relative humidity was 66.8% (max. 85.8; min. 48.3). No rain was registered during this period.

Three experimental areas were selected, each with four 2 m × 2 m plots: the first area with plots H1–H4 was treated with commercial preparation Larvanem, the second with plots P1–P4 was treated with the application of isolate of *S. feltiae* Zag 15 and the third with control plots K1–K4 was sprinkled with water with adjuvant.

2.3. Application of Nematodes

Application of nematodes was performed on 26 May when most larvae (≥90%) achieved the size of 3–4 mm during a count on the previous day. Nematodes at a dosage of 2 million IJs/m^2 were applied in the form of 11 litres of suspension per m^2. From the literature data, it is known that the critical period for IJs larval survival is often minutes or hours after application, even if carried out in soil. UV radiation and rapid drying off of larvae following the application are responsible for the dramatic decline in the number of IJs larvae (40–80%) in that phase [23]. Therefore, we used a dosage that was two times bigger than that recommended by the producer for soil applications. Water suspensions of IJs larvae of isolate *S. feltiae* Zag 15 and of preparation Larvanem were prepared directly before application. A hand sprinkler with a field lance and flat-stream nozzles was used for application at the lowest working pressure of 3000 hPa. To ensure better spreading and maintaining suspension in a drop and to protect IJs larvae from UV radiation, an adjuvant AtpolanBio 80 EC was used at a dosage of 9.6 mL per m^2. Nematodes were applied once during plant growth on 26 May 2019.

The Assessment of Treatment Effectiveness

We assessed the number of larvae feeding on plants on the day before applying the nematodes and 2, 4 and 7 days after the application of nematodes. Larvae were counted on 50 leaves (on the flag leaf and the second and third leaf from the top) randomly collected from 25 plants growing in the middle of each plot. The effectiveness of treatment (*Sk*) was calculated using Abbott's formula [24]:

$$Sk = \left(1 - \frac{C1 \times A2}{C2 \times A1}\right) \times 100 \quad (1)$$

where:

$C1$—the number of larvae on control plants before treatment;

$C2$—the number of larvae on control plants after treatment;
$A1$—the number of larvae on protected plants before treatment;
$A2$—the number of larvae on protected plants after treatment.

The criteria of the efficiency of pest control by nematodes were adopted from the directive of the Ministry of Agriculture and Rural Development issued 4/8/2004 (Journal of Laws No. 183 position 1890) where at least 80% efficiency is termed "control", 60–80% is termed "mean control" and 40–60% efficiency is termed "limited control".

Damage to the wheat caused by cereal leaf beetles was assessed 14 days after treatment (when larvae started to transfer to the soil) by estimating the surface infection of the two top leaves: the flag leaf and the second leaf on 100 shoots from every plot in the phase of milk maturity (BBCH 73–77). The degree of damage was ascribed on the following scale: 1°—minor, 2°—moderate and 3°—severe damage [9]. Obtained results were transformed into the index of infection (IP) according to Townsend–Heurberger's [25] formula:

$$\mathrm{IP} = \frac{\sum_0^i (n \times v)}{i \times N} \times 100\ [\%] \tag{2}$$

where:
n—the number of plants of a given degree of infection,
v—degree of infection (from 0 to i),
i—the highest degree of infection,
N—total number of analysed plants.

2.4. Statistical Analysis

One-way analysis of variance was carried out to determine the main effect of treatment on the variability of the number of larvae in four terms. Mean values were calculated for all terms. The Tukey's honestly significant differences (HSDs) were used to determine differences across treatments for all terms. Chi-square (χ^2) statistics were used to analyse the independence of distribution of the number of plants for three studied treatments: P, H and K.

3. Results

The first adults of the cereal leaf beetle were observed on 23 April 2019. Appropriate meteorological conditions in this period, i.e., temperature above 24 °C and moderate precipitation facilitated the development of the insects. The first egg clutches were observed on 14 May and after 5–7 days, larvae started to hatch (19 to 21 May). The weather was moderately warm (the average/maximum daily temperature was 17 °C) with low precipitation. These conditions were also favourable for the development of the pest. Until the day of application, the temperature varied from 16.3 °C to 18.5 °C and there was neither high sun exposure nor precipitation.

Performed studies showed significant differences between control and test plots treated with nematodes on every day of the assessment of their effectiveness. The highest effectiveness of applied treatment was found seven days after the application of EPNs. Slightly higher effectiveness was noted for Larvanem preparation (Tables 1 and 2).

The application of isolate *S. feltiae* Zag 15 and of commercial preparation Larvanem resulted in a significant decrease in the number of damaged leaves compared with the control (χ^2 = 136.44, $p < 0.001$). The least damage of leaves was found on plots treated with the commercial preparation. For plants from these plots, IP equalled 32% and was two times lower than that in the control (Table 3).

Table 1. The effectiveness of performed treatment based on the number of *O. melanopus* larvae.

Plots	Number of Larvae				Effectiveness acc. to Abott
	Before Treatment 25 May 2021	After Treatment 28 May 2021	After Treatment 30 May 2021	After Treatment 2 June 2021	
H1 *	19	12	7	6	49.5%
H2	19	9	5	11	
H3	13	17	13	11	
H4	18	14	8	4	
P1	15	12	7	5	47.8%
P2	18	16	8	7	
P3	12	18	12	11	
P4	15	12	7	4	
K1	18	14	11	5	-
K2	13	11	9	5	
K3	14	12	8	6	
K4	16	12	8	7	

* H1–H4, plots treated with Larvanem preparation. P1–P4, plots treated with *S. feltiae* Zag 15. K1–K4, control plots.

Table 2. The mean number of *O. melanopus* larvae for three treatments.

Treatment	25 May 2021	28 May 2021	30 May 2021	2 June 2021
H	17.25 ^{b}	13.00 ^{b}	8.25 ^{a}	8.00 ^{c}
K	15.25 ^{a}	12.25 ^{a}	9.00 ^{b}	5.75 ^{a}
P	15.00 ^{a}	14.50 ^{c}	8.50 ^{ab}	6.75 ^{b}

^{a,b,c} Means in columns followed by the same letters are not significantly different.

Table 3. The damage of wheat leaves caused by feeding larvae of the cereal leaf beetle.

Plots	Number of Healthy Plants	Number of Plants Damaged in Degrees			Index of Infection (IP)
		1° *	2°	3°	
P	40 ^{b}	84 ^{c}	44 ^{a}	32 ^{b}	44.7%
H	80 ^{a}	60 ^{b}	48 ^{ab}	12 ^{a}	32.0%
K	12 ^{c}	32 ^{a}	96 ^{c}	60 ^{c}	67.3%

* The degree of damage to wheat leaves on the scale: 1—weakly infected plants; 2—moderately infected plants; 3—strongly infected plants.
^{a,b,c} Means in columns followed by the same letters are not significantly different.

4. Discussion

The aim of the presented study was to assess the efficiency of a native isolate of *S. feltiae* and commercial preparation Larvanem (*H. bacteriophora*) in controlling the larvae of *O. melanopus* and reducing crop damage in the field. The commercial preparation Larvanem, to our knowledge, has not been used before to control *O. melanopus* though it has been shown to be effective against other beetles [26]. Our study has shown that both native isolate of *S. feltiae* and a commercial preparation Larvanem had a significant effect on reducing the leaf damage with IP (infection index) of 44.7% and 32%, respectively. Our hypothesis was that the a native isolate of nematodes might be more effective against *O. melanopus* than a commercial product developed for and tested against other pests. The complexity of host–nematode–bacteria interplay may mean that *O. melanopus* is more able to resist native isolates it co-evolved with, however, given a sufficient level of pest control using native isolates, it is an attractive choice from the ecological perspective. Taking into account the possibilities of finetuning the dosage, the number of applications, adjuvants and time of day of treatment, or targeting different development stages of larvae of *O. melanopus*, both treatments are interesting candidates for further research in field studies. The results of this study are even more satisfactory in that we used only a single treatment applied on plants of small leaf area. According to Trdan et al. [27], leaf area might be one of the factors affecting success in nematode application.

To our surprise, we have observed only moderate effectiveness in controlling larvae, with an Abbot index of 47.8% for *S. feltiae* isolate and 49.5% for *H. bacteriophora*. Considering the clear impact on leaf health, a question arises if the Abbot index is an appropriate technique for assessing the impact of nematodes on *O. melanopus*. If in the control field the larvae were migrating to the soil to undergo further developmental stages while nematode-treated larvae were more stationary, staying on the leaves but not able to feed, could explain similar numbers of larvae on nematode-treated and control fields. It is also possible that in the field condition, the nematodes have more long-term impact on the pest than in the laboratory conditions and though infected and not-feeding, the larvae stay attached to the leaves. Thirdly, because of the way *O. melanopus* are attached to the leaves the dead or sick larvae might remain on the plants for a prolonged period of time and a visual assessment of larvae presence might not in itself be a good indicator of larvae survival. In large scale field studies, the main success criteria are the impact on the crop fitness and the crop yield in particular, therefore, it will be interesting to investigate further if in the case of nematodes control of *O. melanopus*, it is more reliable to look at the crop yield rather than the larvae count.

Both our results and findings of Laznik et al. [22] indicate a high sensitivity of the cereal leaf beetle *O. melanopus* to entomopathogenic nematodes compared with other species of beetles such as the Colorado potato beetle (*Leptinotarsa decemlineata* (Say)) [28,29], common cockchafer [*Melolontha melolontha* (L.)], rice weevil (*Sitophilus oryzae* (L.)) [30] and *Agriotes obscurus (L.)* [31].

Studies carried out in Slovenia [32] showed that Slovenian isolate *S. carpocapsae* (C101), as well as the commercial preparation were effective means of biological control of *O. melanopus* in field conditions. An insecticide thiametoxam used in the same study showed only slightly higher effectiveness than the treatments with the use of EPNs. In studies by Kaniuczak and Siekaniec [33,34] and Siekaniec et al. [35], the application of chemical insecticides limited the infection of leaves by 72.4% on average. Apart from chemical insecticides, natural insecticide Biospin (active substance—spinosad) was used in these studies. Its effectiveness ranged from 31.8 to 55%.

Promising results of laboratory experiments pertaining to the effectiveness of EPNs against insect pests do not often translate to comparable results in field tests [36]. Authors, who compare results from laboratory tests with field results underline that too high temperatures and UV radiation are unfavourable factors in field experiments, hence, results from the field are weaker than laboratory ones. For example, Abbas et al. [37] usually obtained 100% mortality of larvae and imagines of the red palm weevil *Rhynchophorus ferrugineus* (Oliv.) when testing various isolates of *Steinernema* and *Heterorhabditis* in laboratory studies, while in the field only in one case they obtained mortality exceeding 66%. Undoubtedly, the sensitivity of entomopathogenic nematodes to extreme environmental factors is a barrier to using their full potential as biological means for controlling harmful insects. However, if key limiting factors (i.e., temperature, humidity and UV radiation) are appropriate, the application of EPNs on leaves may bring satisfactory effects. This conclusion is supported by studies of Trdan et al. [27], Williams and Walters [38], Unruch and Lacey [39]. The effectiveness of nematodes comparable to that obtained with chemical insecticides might also be achieved by using precise application techniques [40], the addition of adjuvants [41,42], the application in proper growth phase of the pest [27,38,43] and by selection of appropriate species and/or strains since they manifest different requirements for temperature and humidity [36,44].

5. Conclusions

Results obtained in our study show that isolates of S. feltiae Zag15 and commercial preparation Larvanem are effective biological means in reducing leaf damage by cereal leaf beetle in the field.

Slightly better effectiveness was demonstrated for Larvanem, where the index of infection (IP) was 32% (vs. 44.7% for *S. feltiae* Zag15). Results of the performed application of nematodes may be considered satisfactory, taking into account the single application and small leaf area of wheat. The impact on larvae survival was less clear. Nevertheless, despite the fact that the chemical control is still more effective and cheaper, nematodes have a clear potential for reducing the use of pesticides, and further research into improvements of appropriate procedures and methods of application, methods of assessment of larvae survival and impact on crops yield are highly recommended.

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