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Efficacy of native entomopathogenic nematodes from Turkey against the alder leaf beetle, *Agelastica alni* L. (Coleoptera: Chrysomelidae), under laboratory conditions

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Abstract

The alder leaf beetle, *Agelastica alni* L. (Coleoptera: Chrysomelidae), is one of the most defoliator pests of oak and alder trees. In the present study, the efficacies of three native strains of entomopathogenic nematodes, *Heterorhabditis bacteriophora* (ZET35), *Steinernema feltiae* (ZET31), and *Steinernema websteri* (AS-1), were tested against pre-pupae and adults of *A. alni*. Experiments were conducted by four concentrations under laboratory conditions in 2015. Four different temperature regimes were tested at concentration of 1000 infective juveniles (IJs)/ml under laboratory conditions. It was observed that pre-pupae were more sensitive than adults in all tests. Based on screening tests, *S. websteri* was the most effective isolate on both pre-pupae and adults of *A. alni* at concentration of 1000 IJs/ml with 79.17 and 71.11% mortality, respectively. It caused the highest mortality values at all temperatures, except for 30 °C against pre-pupae and adults. Results of the present study suggested that *S. websteri* and *H. bacteriophora* had significant potentials against *A. alni*.

Keywords: Entomopatogenic nematodes, Biological control, *Agelastica alni*, Forest pests

Background

The alder leaf beetle, *Agelastica alni* L. (Coleoptera: Chrysomelidae), is widely distributed in the Europe, Caucasus, Siberia, North-Eastern Kazakhstan, and the USA (Sezen et al. 2004). *A. alni* feeds on variety of broad-leaf species including hazelnut (*Corylus* spp.) and alder (*Alnus* spp.) during spring and summer seasons and occasionally damages other plant species and genera such as *Betula pendula* (Fagales: Betulaceae), *Salix caprea* (Malpighiales: Salicaceae), *Populus* spp. (Malpighiales: Salicaceae), and *Tilia* spp. (Malvales: Malvaceae) (Medvedev 1983). Since the pest has high reproductive rate, it causes severe defoliation to host plants in native habitats. Adults and larvae of *A. alni* attack host plant or its products with significant commercial value and cause mortality or predispose host to infestation by other pests. They cause loss of markets due to quarantine status. The final damage of the pest is unsightly and repeated heavy defoliation and can cause growth loss in large trees and mortality of young plants. In addition, foliar injury can be unsightly in residential areas, parks, and forest recreation sites.

Pests are generally controlled by chemical pesticides that may lead to developing resistance by the target pest in addition to causing harms to human and environment (Ffrench-Constant et al. 2004). Therefore, researchers have been studying to develop alternatives for pesticides. An alternative method to chemical pesticides is biocontrol and the microbial biocontrol agents with no harmful effects on human health and environment. The common microbial biocontrol agents are viruses, bacteria, fungi, and nematodes (Vega and Kaya 2012).

Entomopathogenic nematodes (EPNs) from the families Steinernematidae and Heterorhabditidae are among such alternatives as biological control agents against insect pests, especially the ones inhabiting soil or in the cryptic habitats (Kaya and Gaugler 1993). EPNs have been tested

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successfully as potential biological control agents of insect pests in Turkey (Kepenekci and Susurluk 2006; Yilmaz et al. 2009; Gokce et al. 2013; Erbas et al. 2014; Kepenekci et al. 2015).

Up to now, control strategies applied for *A. alni* are still insufficient to prevent its damage. However, increasing interest in developing environmentally safe pest control methods has inspired us to study the potential of different biological agents against the pest.

The present study aimed to evaluate the efficacy of three EPNs isolated from Turkey against pre-pupae and adults of *A. alni* under laboratory conditions.

Materials and methods

Collection of insects

A. alni adults and larvae were collected from infested *Alnus glutinosa* trees in the vicinity of Trabzon, Turkey, between March and June, 2015. The larvae were carefully handpicked from undersides of leaves by a soft fine-tipped paintbrush, and the adults were caught by a sweep net. Insect samples were placed into plastic boxes (20 cm deep and 20 cm diameter) with ventilated lids and freshly collected plane leaves as food. Afterwards, the collection was transported to the laboratory. Healthy adults and pre-pupae were acclimated for 2 days to the laboratory conditions then healthy ones were used for bioassays.

Nematode isolates

Heterorhabditis bacteriophora (ZET35), *Steinernema feltiae* (ZET31), and *Steinernema websteri* (AS-1) isolates, used in the experiments, were maintained in the collection of the entomopathogens, Department of Biology, Faculty of Science at Karadeniz Technical University (Erbas et al. 2014; Gokce et al. 2015). Nematode cultures were maintained in last instar greater wax moth larvae, *Galleria mellonella* L. (Lepidoptera: Pyralidae) (Woodring and Kaya 1988), and infective juveniles were stored in distilled water at 10 °C. Before starting the experiments, the nematodes were kept at 25 °C.

Laboratory bioassay

Experiments were carried out for all isolates to determine their pathogenicity against *A. alni*. Plastic boxes (4 cm deep and 3.4 cm diameter) were used for the experiments. Each box was filled with 40 g sterilized sandy soil and adjusted to 7% (*w*/*v*) moisture by adding distilled water.

The efficacy of EPNs was tested at three concentrations: 200, 500, and 1000 infective juveniles (IJs) in 1 ml of water per plastic box (10, 25, and 50 IJs per individual in 50 μl of distilled water). For the control groups, only 50 μl of water was added to each box. The treated boxes were kept at room temperature for 1 h, and then a single pre-pupa or an adult were placed on the sand surface in the boxes capped with a lid. Screening tests were conducted at 25 °C, and mortality rates were assessed on 7 days after treatment. Dead insects were dissected under the stereomicroscope to ascertain that mortality resulted from nematodes' infection.

To determine the effect of increasing temperature, 1000 IJs ml^{-1} were applied to the boxes, which were then placed in incubators at 15, 20, 25, or 30 °C. Seven days after nematode treatments, the sandy soil in each box was poured out and mortality rate of the tested insects was recorded. Experiments were performed with 30 pre-pupae or adults for each nematode concentration and temperature regimes. The experiments were repeated three times on different dates.

Data analysis

Mortality data were corrected by Abbott's formula (Abbott 1925). The data were subjected to ANOVA and subsequently to Duncan multiple range tests ($p < 0.001$) to compare isolates with each other and the control group. Lethal concentrations (LC_{50}) for EPNs against

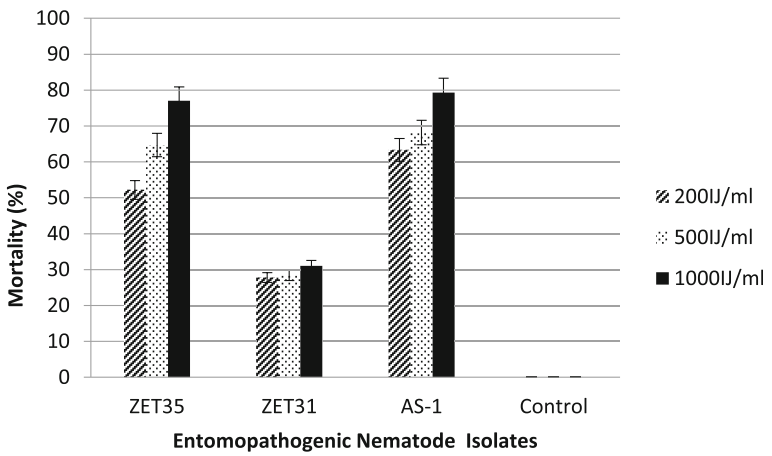

Fig. 1 Efficacy of *H. bacteriophora* (ZET35), *S. feltiae* (ZET31), and *S. websteri* (AS-1) on pre-pupae of *A. alni* at 25 °C

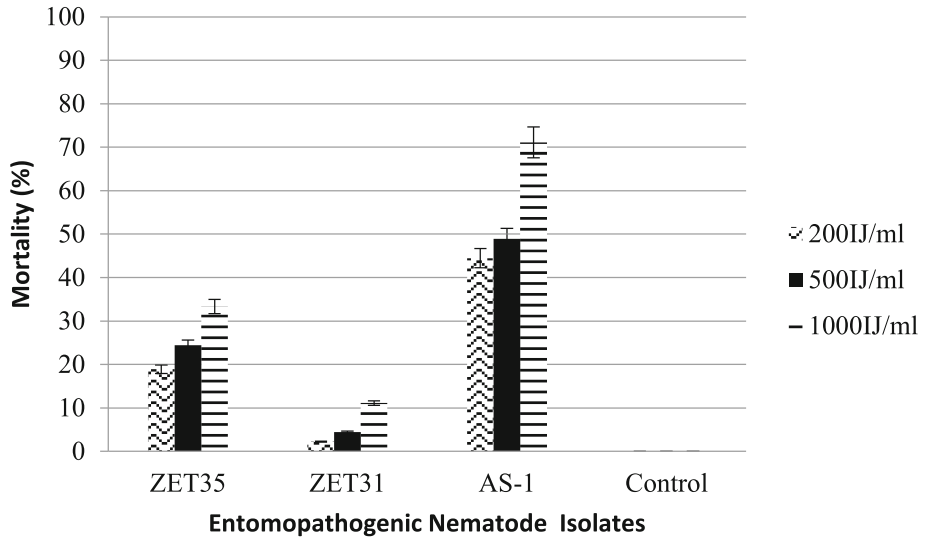

Fig. 2 Efficacy of *H. bacteriophora* (ZET35), *S. feltiae* (ZET31), and *S. websteri* (AS-1) on adults of the alder leaf, *A. alni* beetle at 25 °C

pre-pupae or adults of *A. alni* were calculated by probit analysis. All analyses were performed using SPSS 23.0 statistical software (IBM Corporation, Armonk).

Results and discussion

The virulence of the three native EPN isolates (*H. bacteriophora* (ZET35), *S. feltiae* (ZET31), and *S. websteri* (AS-1)) against pre-pupae and adults of *A. alni* at four different concentrations (0, 200, 500, or 1000 IJs ml^{-1}) and four different temperature regimes (15, 20, 25, and 30 °C) were studied under laboratory conditions. Also, the detected effects of increasing concentration of infective juveniles and temperature on virulence were determined (Figs. 1 and 2).

Results showed that pre-pupae were more sensitive than adults in all tests. Mortality rate of the pre-pupae and adults increased by increasing gradually the concentration of nematodes. The highest and fastest mortality (79.31%) in pre-pupae was obtained by *S. websteri* (AS-1) at 50 IJs/individual ($F = 3571, 86$; df = 6, 35; $p < 0.001$) (Fig. 1). Additionally, application of 25 and 50 IJs/individual of *S. websteri* during the same period resulted in 63.33 and 68.18% mortality, respectively. It was also observed that *H. bacteriophora* caused 52.22, 64.77, and 77.01% mortality at concentrations of 10, 25, and 50 IJs, respectively ($F = 1317, 40$; df = 3, 35; $p < 0.001$). Adults of *A. alni* were also found to be susceptible to *S. websteri* (AS-1), with mean percentage mortality ranging between 44.44 and 71.11% ($F = 2121, 4$; df = 3, 35; $p < 0.001$) (Fig. 2). Also, lethal concentration (LC_{50}) of each entomopathogenic nematode for *A. alni* was determined (Table 1). The isolates, *H. bacteriophora* (ZET35), S. *feltiae* (ZET31), and *S. websteri* (AS-1) killed pre-pupae at LC_{50} values of 201, 8064, and 64 IJs per pre-pupa, respectively. These results indicated that pre-pupae of the pest were more susceptible than adults. Also, LC_{50} calculations showed that *H. bacteriophora* and *S. websteri* had better values against both pre-pupae and adult of *A. alni*.

The virulence of *H. bacteriophora*, S. *feltiae*, and *S. websteri* to pre-pupae and adults of *A. alni* with 1000 IJs ml^{-1} was determined, under laboratory conditions at four different temperatures (15, 20, 25, and 30 °C). Different temperatures caused significant sensitivity rates on pre-pupae. Mortality rates with *H. bacteriophora* increased with the increasing temperature. It reached 100% at 30 °C ($F = 302, 07$; df = 3, 35; $p < 0.001$). This value was the highest mortality among all tests. The highest mortality with S. *feltiae* was 70.11% at 15 °C ($F = 1185, 7$; df = 3, 35; $p < 0.001$), and mortality rates decreased with the increasing of temperature. *S. websteri* also caused approximately the same mortality rates (from 70 to 85.56%) on pre-pupae of *A. alni* (Fig. 3).

Table 1 Calculated LC_{50} values for pre-pupae and adults of *A. alni* treated with three EPN isolates, *Heterorhabditis bacteriophora* (ZET35), *Steinernema feltiae* (ZET31), and *Steinernema websteri* (AS-1)

EPNs	Stages	LC_{50} (IJ ml^{-1})	95% limit		Slope	Intercept	X^2	df
			Lower	Upper				
ZET35	Pre-pupae	201.6	101.8	399.2	1,6	1,1	0,3	1
	Adult	6220.4	849.3	ND	0,5	2,8	0,5	1
ZET31	Pre-pupae	8604.6	429.1	ND	0,3	3,5	0,9	1
	Adult	ND	6926.7	ND	0,6	1,7	0,7	1
AS-1	Pre-pupae	64.3	13.9	297.0	0,7	3,6	0,6	1
	Adult	326.8	110.1	970.3	0,9	2,5	0,3	1

ND not determined

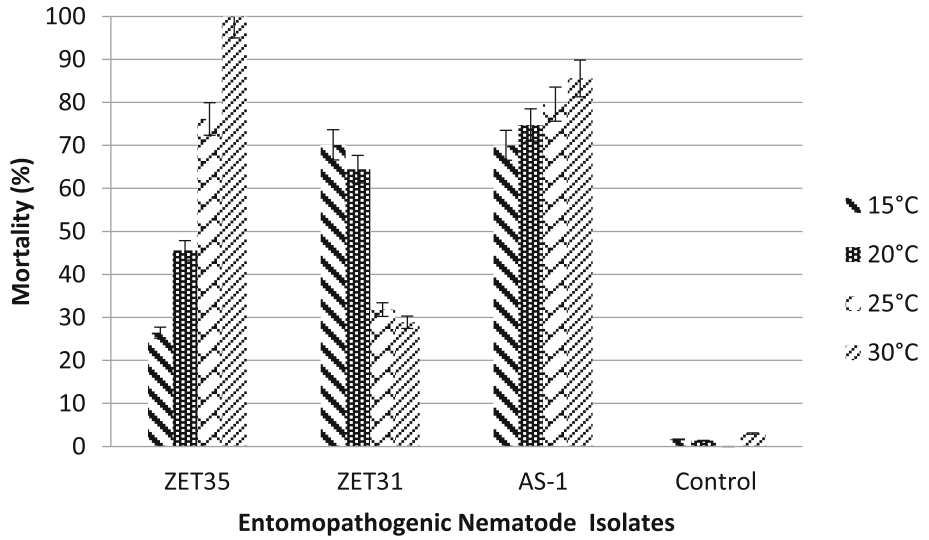

Fig. 3 Mean mortality rate of three native strains on pre-pupae of *A. alni* treated at a concentration of 1000 IJs/ml at four tested temperatures

Adults exhibited less sensitivity at all tested temperatures. Mortality ratio by *H. bacteriophopra* increased with increasing of temperature and reached 40% at 30 °C (F = 1588, 08; df = 3, 35; $p < 0.001$). As in the pre-pupae, mortality rates of adults by *S. feltiae* decreased as the temperature increased. The highest mortality by *S. websteri* was provided at 25 °C on adults (68.54%) (F = 11, 35, 10; df = 3, 35; $p < 0.001$).

The highest pathogenic effect was recorded by *S. feltiae* on pre-pupae at 15 °C, while the mortality caused by *S. feltiae* on both pre-pupae and adults decreased by increasing the temperature. This exhibits that *S. feltiae* is more active and effective at lower temperatures. An optimal biological activity of *S. feltiae* was detected in the temperature at 25 °C (Belair et al. 2003). Besides these two situations, the pathogenic effect of *S. websteri* increased to 16% on pre-pupae and changed from 70.4 to 86.66% mortalities by increasing the temperature. *S. websteri* caused high mortality of pre-pupae of *A. alni* at different temperatures. Some groups also investigated the efficacy of some EPN species/strains on *A. alni*. Doucet et al. (1996) reported that *H. bacteriophora* was found to be effective at temperature between 18 and 30 °C with an optimum range of 22–26 °C. Tomalak (2004) tested the infectivity and biocontrol potential of *H. megidis* and *S. feltiae* on *A. alni* under the laboratory and

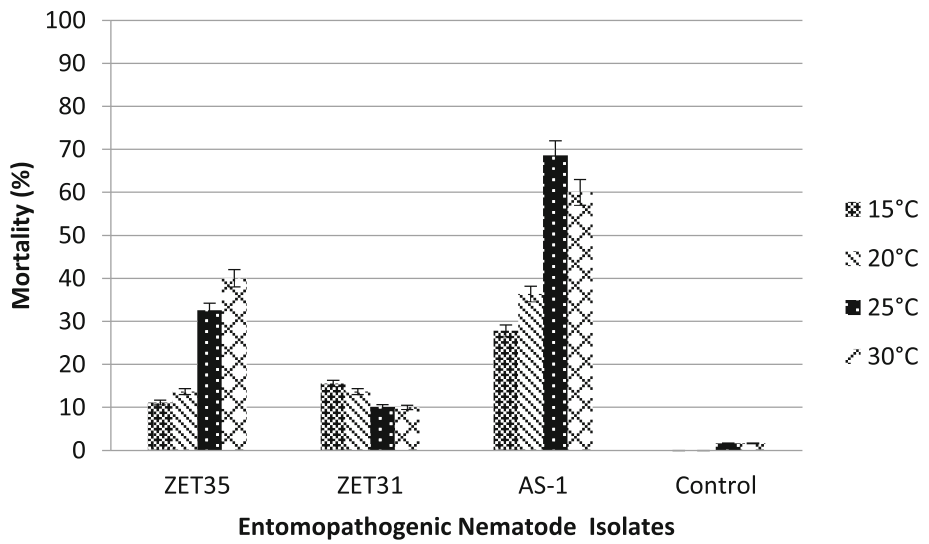

Fig. 4 Mean mortality rate of three native strains on adults of *A. alni* treated at a concentration of 1000 IJs/ml at four tested temperatures

semi-field experiment conditions and reported that *H. megidis* caused a significant mortality against last instar larvae of *A. alni*. He also demonstrated that 50 IJs of *S. feltiae* (ScP) strain against last instar larvae of *A. alni* caused 56–66% mortality. Our results reported that *S. feltiae* caused the highest mortality (69.6%) at 15 °C. Also, *H. bacteriophora* and *S. websteri* had 98.51 and 86.66% mortality on pre-pupae of *A. alni* at 30 °C at the same period, respectively. Also, Choo et al. (2002) demonstrated that *S. carpocapsae* and *H. bacteriophora* were found to be highly virulent against different larval stages (first, second, and third larval instars) of *Agelastica coerulea*, where the both isolates had high mortality rates, while *H. bacteriopohora* caused 100% mortality on all larval stages. In agreement with other groups, our results showed that 15 °C recorded lower mortality than 20 or 25 °C (Choo et al. 2002; Belair et al. 2003; Trdan et al. 2009).

Trdan et al. (2009) performed laboratory studies to determine the effectiveness of *S. feltiae*, *S. carpocapsae*, *H. bacteriophora*, and *H. megidis* on *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) which is another member of Chrysomelidae family at three different temperatures (15, 20, and 25 °C). Although his studies showed that the lowest efficacy against all stages of the insect was at 15 °C, in the present tests, *S. feltiae* (ZET31) gave 70.11 and 64.44% mortality on pre-pupae of *A. alni* at 15 and 20 °C, respectively. Also, our study showed that the highest mortality on pre-pupae of *A. alni* provided as 98.51% mortality for *H. bacteriophora* at 30 °C.

The effectiveness of EPNs in controlling *chrysomelids* is affected by biotic and abiotic conditions. One of the most important abiotic factors is temperature, which influenced the activity of the nematodes. Increasing the temperature from 15 to 30 °C caused a significant increase in pre-pupae and adult mortality rates of *A. alni* after the treatments of *S. websteri* and *H. bacteriophora* isolates. In contrast, mortality caused by *S. feltiae* ZET31 was significantly lower at 30 °C than at 15 °C against pre-pupae and adult of *A. alni* (Figs. 3 and 4).

Conclusions

Obtained results may suggest that *H. bacteriophora* (ZET35) and *S. websteri* (AS-1) can be used as biological control agents against pre-pupae and adults of *A. alni*. Future field studies are recommended with the aim of finding a better biological control agent against *A. alni* and for using these nematode isolates as biopesticides.

Authors' contributions

All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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