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Research Article

Effect of entomopathogenic nematodes on acorn weevil, *Curculio glandium* (Marsham) (Coleoptera: Curculionidae) in field conditions

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ABSTRACT: The acorn weevil, *Curculio glandium* (Marsham) (Coleoptera: Curculionidae), is a major forest pest that affecting oak trees. The pest disturbs regeneration of host trees by feeding on their immature acorns.The susceptibility of C. glandiumlastinstar grubs to two species of indigenous entomopathogenic nematodes (*Steinernema carpocapsae* IRAZ9 and *Heterorhabditis bacteriophora* IRAZ5) was examined under field conditions in Arasbaran forest. Our results suggested that the pest grubs were more susceptible to *S. carpocapsae* than *H. bacteriophora*. The mean overall mortality by two species of nematodes was calculated 66.75% and 45.65% respectively. *Steinernema carpocapsae* caused higher grub mortality comparing to *H. bacteriophora* at all concentrations and therefore it can be introduced as appropriate candidate for biological control of *C. glandium* grubs. Regression analysis showed a direct relationship between concentrations increasing and mortality in both nematode species. LC_{50} values for *H. bacteriophora* and *S. carpocapsae* were calculated 1258 and 1584 IJs/ ml, respectively.

KEY WORDS: Acorn weevil, field evaluation, *Steinernema carpocapsae, Heterorhabditis bacteriophora*

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INTRODUCTION

Acorn weevils of the genus *Curculio* (Coleoptera: Curculionidae) damage mature acorns with potential impacts on the fitness of oak trees (*Quercus* spp.) in North America, Europe (Udaka and Sinclair 2014) and Asia.*Curculiog landium* (Marsham), is a widespread pest of acorns in oak forests of Iran. This beetle is a major pest of oak trees in Arasbaran forests (East Azarbaijan province, north-west Iran) and eliminate regeneration of the host trees. *C. glandium* may cause up to 80% regeneration loss of oak trees. Female weevils lay eggs in young acorns and grubs feed and develop within the acorns. After the ripe acorns fall to the ground, fully developed grubs cease feeding and burrow into the soil to overwinter some *C. glandium* grubs might experience two winters in the soil by developing to the adult stage (Pelisson *et al*. 2012).

Entomopathogenic nematodes (EPNs) belonging to the genera *Steinernema* and *Heterorhabditis,* are biological control agents that kill their arthropod hosts, because their infective juveniles are mutualistically associated with a specific kind of symbiotic bacteria, which are pathogenic to a variety of arthropods (Grewal *et al*., 2005). The nematodes complete their development and live for two or three generations inside their host. When food is depleted, infective juveniles exit from host cadaver searching for new hosts (Grewal and Georgis, 1999). Infective juveniles (IJs), the only free-living stage of the nematodes, enter hosts through natural openings (mouth, anus, and spiracles), or in some cases, through the cuticle. After entering the host's hemocoel, nematodes release their symbiotic bacteria, which are primarily responsible for killing the host, defending against secondary invaders, and providing the nematodes with nutrition (Dowds and Peters, 2002). These nematodes are particularly suited to controlling soil pests (Klein, 1990). They have potential for use in augmentative and/or inundative biological control (Parkman and Smart, 1996). They can be mass produced *in vitro* (Ehlers 2001) and have a high biocontrol potential when applied to manage control weevils (Curculionidae) in nurseries (Van and Raupp, 2006) and forestry (Torr *et al.,* 2007). Based on previous studies, useful effect of EPNs has been shown in controlling weevils. For example, hazelnut weevil, *Curculio nucum* L.(Batalla-Carrera *et al.,* 2013), black vine weevil, *Otiorhynchussulcatus* Fabricius (Lola-Luz *et al.,* 2005), root weevil, *Diaprepes abreviatus* (L.) (Shapiro and Mc-

Coy, 2000), chestnut weevil, *Curculio elephas* (Gyllenhal) (Kepenekci *et al.* 2004) and pecan weevil, *Curculio caryae* Horn (Smith *et al.* 1993) are controlled by the nematodes successfully.

Given the necessity to control *C. glandium* and limitations of chemical pesticide application in natural resources as well as failure of chemical control of *C. glandium* due to the cryptic habitat of grubs, alternative control methods are needed. Therefore, to determine the potential impact of entomopathogenic nematodes, in the present study indigenous EPNs, *Heterorhabditis* IRAZ5 and *S.carpocapsae* IRAZ9 were tested upon the last larval instar of *C. glandium*, the life stage of the pest spent in the soil, under field conditions in Arasbaran forest.

MATERIALS AND METHODS

Site Description

The Arasbaran Protected Area covers an area of 72,465 ha in NW Iran (latitude 38º 41'-39º 07' and longitude 46º 42'- 46º 58'). At its lowest altitude (350 m above sea level) the mean annual temperatures is 14ºC whereas at its highest altitude (over 2840 m) the average is 5ºC. Annual precipitation is between 316 mm in the uplands and 686 mm in the afforested lowlands (Jalili *et al.* 2003). Dominant plant species in the Arasbaran forests are hornbeam and oak with 51 and 37% coverage, respectively, but there are three main types including pure oak, pure hornbeam, and mixed oak/ hornbeam forests. Some coniferous species occur in a few mixed forests (Nikdel and Sadaghian, 2002).

Source of Insect and Nematodes

Acorn weevil mature grubs were collected from oak forests of the Arasbaran Protected Area in the northeast portion of the Azarbaijan province. Insects were maintained in boxes filled with autoclaved soil at 22˚C for 15 days. Diseased individuals were removed before being used. Native EPNs, *S. carpocapsae* IRAZ9 and *H. bacteriophora* IRAZ5 were reared at 22±2˚C in last instar grubs of *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) according to the method of Woodring and Kaya (1998). IJs that emerged from cadavers were recovered using White traps (White, 1927). After storage at 7˚C for 10 days, the nematodes (IJs) viability was checked by observation of movement under a stereomicroscope and were acclimatized at field temperature for 5 hours prior to the experiments.

Nematode Field Assay

The study was conducted in early autumn 2013 at a range of temperature from 18 to 23˚C and with a relative humidity between 55 and 58%. Meteorological data were obtained from a weather station located in the Research station in Kaleibar town (3 km away from the experiment site). The experimental plots were plastic boxes (70 cm length, 50 cm width and 30 cm depth) with a screen bottom for water drainage. The boxes were arranged in completely randomized design. After digging a chamber at the canopy of selected oak trees, the boxes were arranged in a completely randomized design in the chambers so that, their top surface was flush with the surface of land. Then, inside each box was filled with local moist soil. The soil was a loamy sand (78% sand, 16% silt, 6% clay; 1.8-2.36% organic matter), with a pH range of 6.13 -7.28 and soil moisture levels were 19.5-22.6% at the start of experiment. Eight different treatments, corresponding to two EPN species and four different concentrations of each species were used to assess their efficacy. Nematodes were used at different concentrations (0.750, 1500 and 3000 IJs per 1ml of sterile distilled water) with a volume of 80ml in each plot (plastic boxes). Nematode suspensions were applied with 240-ml spray bottles on the soil surface in each plot. Application was at dusk to reduce the adverse effects of high temperatures and UV. One day after nematode application, 40 last instar grub were placed on the soil surface of plots and allowed to naturally burrow into the soil and in one day before release of *C. glandium* grubs, plots were covered with white plastic to inhibit soil moisture loss, and cloth to provide shading. There were 12 replications per treatment (totally 96 applications) and the trial was repeated twice.

Three days after the start of experiment, four blocks were randomly chosen and number of dead and alive grubs were recorded. So that with soil depletion of boxes, the number of dead and live insects were counted. Due to the disturbance of the soil in the examined blocks, the assessed plots were then discarded. In the sixth and ninth days of the experiment, the same procedure was repeated in other blocks. Thus, the mortality rates were noted in the mentioned days. To ensure the death of grubs by nematode, carcases were incubated on White traps and examined under a dissecting microscope to confirm the presence of nematodes.

Statistical Analysis

To determine the effect of nematode treatments, mortalities were converted to percentages and adjusted for control mortality, using Abbott's correction formula (Abbott 1925). All data were analysed using SPSS-PC v.16.0 (SPSS 2007). To evaluate the effectiveness of EPNs against *C. glandium* grub percentage mortalities were square-root transformed before analysis. The level at which all analyses were considered significant was $P<0.05$. The quantity (concentration) of IJ per ml of distilled water was transformed logarithmically. Lethal concentration (LC50) values were

estimated for each nematode using Probit analysis (SAS Institute, 1996).

RESULTS AND DISCUSSION

Data analysis indicated significant differences between untreated control and treatments of *S. carpocapsae* (F = 30.67; P<.0001) and *H. bacteriophora* (F = 9.096; P<.0001).The results revealed that the last instar grub of *C. glandium* were susceptible to two nematode species tested. When a dose of 3000 IJs/ml was applied mortality of grubs reached 78.0% with *S. carpocapsae* and 72.7% with *H. bacteriophora* (Fig. 1). The overall mean larval mortality caused by *S. carpocapsae* was 66.25% compared to 45.65% for *H. bacteriophora.* Thus, *S. carpocapsae* caused higher mortality compared to *H. bacteriophora* in all concentrations.

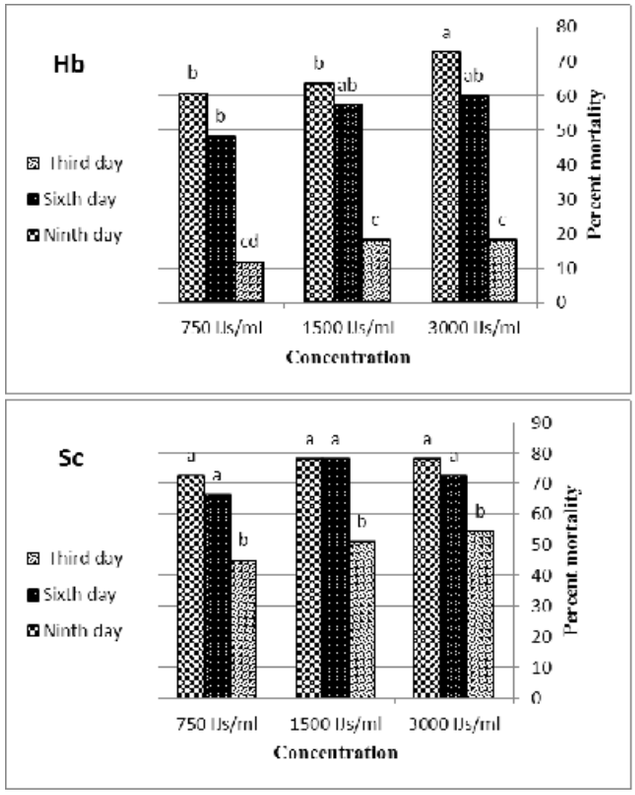

Fig. 1. Percent mortality of last instargrubs of *Curculio glandium* at different concentrations of the entomopathogenic nematodes *Heterorhabditis bacteriophora* (Hb) and *Steinernema carpocapsae* (Sc) at 3th, 6th and 9th days after treatment. Bars with the same letters are not significantly different (*P* < 0.05).

In third day after application the mortality recorded was 45.0%, 51.0% and 54.5% for *S. carpocapsae* IRAZ9 and the above mentioned values for *H. bacteriophora* IRAZ5 was 11.8%, 18.2% and 18.2%. In sixth day after application the mortality was 66.4%, 78.0% and 72.7% in plots treated with *S. carpocapsae* and 48.2%, 57.3% and 60.0% with *H. bacteriophora*. In ninth day after application the treatments showed efficacies of 72.7%, 78.0% and 78.0% for *S. carpocapsae* and 60.9%, 63.6% and 72.7% for *H. bacteriophora*. In all above cases, the percent values are listed in order of increasing concentration (750, 1500 and 3000 IJs/ml).

The statistical differences between applied doses and nematode species were indicated above bars of mortality percentage in Fig. 1. Significant differences were found between nematode species (t = -2.334, P<0.033) and applied doses for *H. bacteriophora* (t = -2.333, P<0.023) but differences between concentrations of *S. carpocapsae* were not significant (t = 3.17, *P*>0.05). In other words, with increasing concentrations of *H. bacteriophora* grub mortality was increased accordingly, but this did not happen in the other species of nematode. Significant differences were observed between times of after experiment (3, 6 and 9 day) for *H. bacteriophora* (t = -3.121, *P*<0.05) but for *S. carpocapsae* these differences was significant only between the 3 and 6 days after application.

Based on probit analysis, LC_{50}±SE of *S. carpocapsae* and *H. bacteriophora* against *C. glandium* were determined as 1584±45 IJs/ml and 1258±39 IJs/ml, in addition, 95% confidence intervals of the mentioned values were 1523-1786 and 1211-1299, respectively. Regression analysis of the dose-mortality response showed significant relationships for both nematodes (Fig. 2).

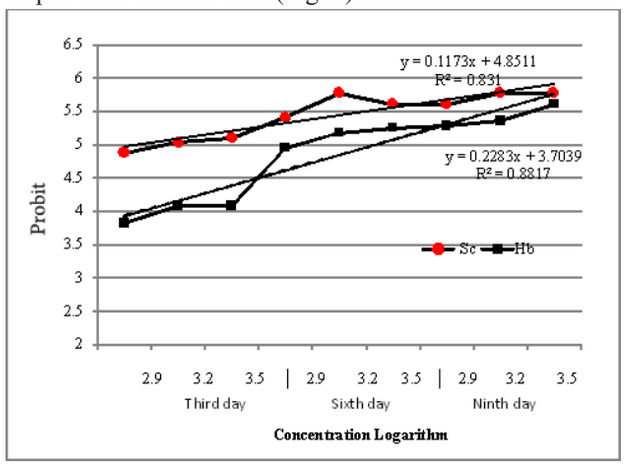

Fig. 2. Dose-mortality response of *Curculio glandium* grubs exposed to different concentrations of *Heterorhabditis bacteriophora* and *Steinernema carpocapsae*, at 3th, 6th and 9th days after treatment.

The results of the experiment showed that both nematode species should be effective in controlling acorn weevil grubs in field conditions, while *S. carpocapsae* caused higher grub mortality compared to *H. bacteriophora* in all

concentrations and can be suggested as a candidate for applied control of the pest. Although, there are no documented reports on the infectivity of EPNs upon *C. glandium* grubs to be compared with the results of our study, in similar studies EPNs have been found highly infective against a number of other curculionids. Shapiro and McCoy (2000) tested nine entomopathogenic nematodes against the citrus weevil, *Diaprepes abbreviates*, at 20, 24, and 29°C and found that *S. riobrave* produced abbreviatus the greatest mortality at all tested temperatures. Smith *et al.* (1993) tested *S. carpocapsae*, *S. feltiae*, and *H. bacteriophora* on the pecan weevil and reported that *S. carpocapsae* was the most virulent species among the tested nematodes. *Heterorhabditis marelatus* was the most virulent species assayed against black vine weevil grubs, *Otiorhynchus* spp. (Berry *et al.,* 1997). In the latest study, *S. feltiae* strain D114, *Steinernema* sp. strain D122 and *H. bacteriophora* strain DG46 were used on the hazelnut weevil, *Curculio nucum* and reduced the pest population, ranging from 32% to 88% efficacy.The results demonstrate that *S. carpocapsae* is more virulent to *C. glandium* grubs than *H. bacteriophora*. In addition, this nematode causes mortality in less time. It is likely that the different host-finding strategies exhibited by the ambusher *H. bacteriophora* and the cruiser *S. carpocapsae* is one of the most important reasons for the observed differences. *Steinernema carpocapsae*, having cruiser behaviour, has more contact with the host as compared to *H. bacteriophora*. Precisely, this was observed in testing the same nematodes against fifth instar grubs of brown-tail moth, *Euproctis chrysorrhoea* L. (Lepidoptera: Lymantriidae) in previous laboratory studies. So that, mean mortalities for *H. bacteriophora* and *S. carpocapsae* on the fifth larval stages at over all rates were 51.1 and 67.8, respectively (Nikdel *et al.* 2010).

Although the pest grubs live in the soil at least six months of year, we prefer to do study in early autumn,when the mature grubs emerge from the oak fruits and burrow into the ground. Contact between nematode and grubs, in this approach will be more likely.

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