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Comparison between Biological and Chemical Management of Sugar Beet Nematode, *Heterodera schachtii*

Manzoor Hussain*, Miloslav Zouhar and Pavel Ryšánek
Department of Plant Protection, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences, Prague, Czech Republic

ABSTRACT

This investigation was designed to compare biological management through nematophagous fungus *Lecanicillium muscarium* with chemical nematicides, Vydate, Basamid (G) and fertilizer, Calcium cyanamide through soil application to control sugar beet cyst nematodes *Heterodera schachtii*. *Lecanicillium muscarium* decreased nematode population significantly (P=0.05 and caused reductions in final numbers of cysts per 250 g of soil, eggs and juveniles per cyst. Nematicides effects were not quite significant among each other but had tremendous effects in reducing nematode population compared to that of untreated control. A significant (P=0.05) reduction in numbers of eggs per cyst warrants that nematicides were able to penetrate into cysts. Reproduction rate (Pf/Pi) of *L. muscarium* treated plants was documented minimum as compared to that of control but slightly higher compared to that of treated with nematicides. More interestingly, plant growth parameters including shoot and root were tremendously improved in *L. muscarium* treated plants than that of other treatments.

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MH designed experiment, collected and analyzed the data, MZ and PR advised the study and proofread article.

Key words
Nematophagous fungus, *Lecanicillium muscarium*, *Heterodera schachtii*, Nematicides, Reproduction rate

INTRODUCTION

Sugar beet is one of the major crops grown in Czech Republic and other European countries which is being deteriorated by sugar beet nematode, *Heterodera schachtii*. *H. schachtii* is of quarantine importance with biosecurity risks in European countries (Ravichandra, 2014). The sugar beet nematode causes overall losses of 25 to 50% or more (Agrios, 2005) and fabricates a main hindrance in production of sugar beet in central Europe, where it is responsible for economic losses estimated to be 90 million Euros annually (Müller, 1999). Higher population level of nematodes in soil creates potential losses of sugar beet yield (Heijbroek *et al*., 2002; Heinrichs, 2011; Kenter *et al*., 2014; Hauer *et al*., 2016) which is an alarming situation for the whole globe (Kiymaza and Ertek, 2015). In Czech Republic crop damages varied yearly, with yield losses up to 60% (Chod and Chodová, 2000). The losses appear in the form of reduced root weight, dead young plants or stunted growth. The amount of the pest is also accelerated with consecutive cultivation of sugar beet resulted in "soil fatigue" (Baudyš, 1935). The imperishable management of this soil dwelling nematode is challenging as it survives and lives in protective cyst for most of its life span (Renčo and Kováčik, 2015). The infective second stage juveniles (J2) hatching from the eggs is facilitated by triggering root exudates. The J2s emerge from the cyst and invade roots of host plants in search of food and to maintain their life. While becoming sedentary, they develop feeding sites called syncytia to mature and produce new offspring. The J2s are more vulnerable to be attacked by antagonistic parasites or chemical substances in soil while seeking out the host plant roots. This is a target phase of non-fumigant nematicides which might interrupt activity of the J2s. Disruption of the early J2 infection of sugar beet root is critical because it determines the yield effects of the nematode populations present in an infested field (Xing and Westphal, 2009; Westphal, 2013).

Management approaches include annual crop rotation and resistant cover crops to suppress population of sugar beet nematode densities (Muller and Steudel, 1983; Steudel *et al*., 1989; Koch and Gray, 1997; Meinecke and Westphal, 2014). In Europe and North America, integrated pest management (IPM) strategies are adopted to suppress the nematode below economic threshold levels under restrictions of nematicidal compounds (Roberts *et al*., 1980; Steudel *et al*., 1981). Organic amendments including cattle manures and composts have also been extensively studied and proven excellent in reducing plant parasitic nematodes (Renčo *et al*., 2007, Renčo and Kováčik, 2012, 2015). Moreover, alternative strategies including soil solarization, organic amendments, biofumigation, mycorrhization, fallow land, crop rotation, and host plant resistances are being used (Renčo *et al*., 2012) but these measures either do not eliminate nematodes as expected or cost effective. Continuous growing of sugar beet in same growing fields brings a risk of buildup of population massiveness of *H. schachtii* (Meinecke and Westphal,

* Corresponding author: manzoor.sahi@gmail.com
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2014), where weeds play a vital role for multiplication of nematodes in absence of sugar beet season (Meinecke and Westphal, 2014) and also resulted in "soil fatigue" (Baudyš, 1935). Trap crops are also cultivated in Europe especially in Germany before sugar beet crop to suppress the nematode population (Buhre *et al*., 2004).

In Germany, the first resistant variety of sugar beet to this nematode was released in 1998, and the first tolerant variety in 2005 (Bundessortenamt, 2013). The tolerant varieties originated from *Beta maritima*, but they may not recede nematode population density (Daub and Westphal, 2012; Niere, 2009b). Commonly, nematophagous fungi are found in agricultural soils but their information is quite limited. The nematophagous fungi develop a successful saprophytic relationship with soil dwelling nematodes (Persmark and Jasson, 1997). Therefore, studies of ecology play a key role in understanding the relationships between nematophagous fungi and phytonematodes. Due to high toxicity and under restriction of chemical nematicides, scientists are struggling to find alternative strategies to diminish the population of plant parasitic nematodes (Persmark and Jasson, 1997; Renčo *et al*., 2012). *Lecanicillium muscarium* has been proven to be potential candidate against larvae of insect pests and plant parasitic nematodes (Shinya *et al*., 2008; Goettel *et al*., 2008). The objective of this study was to quantify the comparative effects of nematicides and fertilizer (Calcium cyanamide) with nematophagous fungus on population buildup of *H. schachtii* and plant growth. We hypothesized that *L. muscarium* might be replaced with application of lethal nematicides in hard infected soil. Moreover, fungi may also serve as food to fungal feeding nematodes which are beneficial to regulate soil food web.

MATERIALS AND METHODS

Nematode culture

Sugar beet nematodes, *H. schachtii* infested soil was collected from farmer's fields of Semice and Litol in Czech Republic and brought into lab. Cysts were extracted from infested soil using a Fenwick flotation can method and their identification was performed based on morphological characters under microscope and at molecular level through polymerase chain reaction (PCR) (Caswell and Thomason, 1985). The cysts were used to inoculate the sugar beet susceptible variety "Alpaca" to multipy its population and allowed to grow for fourteen weeks approximately. At the end of growing period, plants were uprooted and mature brown cysts were collected as above. The cysts were ruptured between slides and J2 were used for inoculation.

Fungal culture

Nematophagous fungus, *L. muscarium* was cultured on Potato Dextrose Broth (PDB) loaded in 250 ml flasks in laboratory to prepare the culture filtrate. Mycelia of fungus were harvested from PDB, weighed and standard solution (W/V) was prepared in distilled water. 20 ml of the 30% (W/V) solution of fungus was pipetted on top of soil around root zone at two leaves stage of sugar beet plants.

Nematicides applications

Two chemicals including Vydate and Basamid (G) while one fertilizer Calcium cyanamide were used at the rate of 4.85g/L, 2g/L and 5g/L, respectively. The chemicals mixed soil was transferred into pots and covered with plastic sheet for one day for Vydate, two weeks for Basamid (G) and three weeks for Calcium cyanamide to avoid phytotoxicity.

Experimental protocol

The experiment was installed under controlled conditions in greenhouse at temperature range of 25-27˚C. Two seeds of sugar beet susceptible cultivar "Alpaca" were sown in plastic pots of volume 500 cm^3 containing sterilized soil. There were three treatments of nematicides and one treatment of fungus with five replicates of each one. The experiment was repeated once. The control plants included alone with fungus inoculation or nematicides or with nematodes. The treated plants were placed on the greenhouse bench in a complete randomized design. After two days of fungus inoculation, 1000 fresh J2 were introduced into each pot. The plants with nematodes only served as control for comparison. Experimental plants were allowed to grow for almost fourteen weeks.

Data collection

After eight weeks, sugar beet plants were carefully uprooted from the pots and their roots were clipped from the shoots. The roots were gently washed and blotted dry. Fresh root shoot weights and lengths were noted. The nematode population size, number of eggs per cyst from soil, J2 population from root system and soil was estimated. J2s were extracted from root systems and from the soil of each individual plant (Hussey and Barker, 1973, Whitehead and Hemming, 1965). The total numbers of eggs and nematodes in the soil were counted and comprised the total nematode population. The reproduction factor was calculated by dividing the final population by 1000 J2, the initial inoculum level.

Data analysis

Experiments were repeated once each with five replicates. Data from all experiments were pooled and

Table I.- Effects of *Lecanicillium muscarium*, nematicides and fertilizer on reproduction of *Heterodera schachtii* on sugar beet at Pi= 1000 j2 per plant.

Treatments	Cysts/250g of soil	Eggs/250g of soil	J2/root system	Eggs/cyst	J2/250g of soil	*Pf/Pi
L. muscarium+ *H. schachtii*	7.4 ± 1.1 a	234.4 ± 3.6 a	1002 ± 28 a	180 ± 8 a	133.8 ± 4.1 a	3.93 ± 0.36 a
Vydate + *H. schachtii*	5.8 ± 0.8 b	261 ± 4.5 b	918.8 ± 7 bc	134 ± 10 b	119.2 ± 5.8 b	2.71 ± 0.29b
Basamid (G)+*H. schachtii*	6 ± 0.7 b	268.6 ± 2.3 c	915.8 ± 2 b	134.8 ± 4b	108.4 ± 1.1 c	2.74 ± 0.15b
Calcium cyanamide + *H. schachtii*	6.2 ± 0.8 ab	229.6 ± 12.2 a	927.6 ± 7 c	136.6 ± 7b	108.4 ± 3.7 c	2.84 ± 0.22b
Hetrodera schachtii	13.2 ± 1.3 c	502.2 ± 9.0 d	2144 ± 10 d	227.4 ± 5c	455.4 ± 4.1 d	9.06 ± 0.64 c

Data is presented as mean ± standard deviation of ten replications.
Values within a column followed by the same letter are not significantly different according to T-Test at $P = 0.05$.
*RF, Reproduction factor whereas "Pf" is final nematode population density divided by initial nematode population density (Pi).

subjected to ANOVA test; means were partitioned by the Duncan Multiple Range Test (DMRT) by using software Statistics 8.1.

RESULTS

Results of our experiments showed that all treatments (chemical, biological) affected development and thus reproduction and population of *Heterodera schachtii* (Table I). All nematological parameters (number of cysts/250g soil; eggs/250g soil; J2/root; eggs/cysts; J2/250 soil) as well nematode reproduction factor were significantly lower in comparison to untreated control (*P=0.05*). Maximum reduction in nematode population was occurred in the pots treated with Basamid (G) which was significantly (*P*=0.05) similar to that of Vydate but different (*P*=0.05) from other treatments in the experiment. Whereas better reduction in nematode population occurred when plants were treated with fungi which was comparable to Calcium cyanamide.

Minimum number of cysts were found in case of Vydate and Basamid (G) as compared to that of control. Moreover, number of cysts found in case of Calcium cyanamide were not significantly (*P*=0.05) different from *L. muscarium*. Moreover, significant reduction of eggs within cysts occurred in case of nematicides as well as in *L. muscarium* as compared to that of control. The cysts were found colonized by *L. muscarium* (data not shown). Furthermore, *L. muscarium* alone did not have any negative effect on plant growth while some chlorotic spots were observed in the plants treated with Dazomet (Fig. 1). Nematode reproduction rate (Pf/Pi) was lowered up to three times in case of *L. muscarium* than that of control but relatively higher than nematicides. As regard plant growth parameters, *L. muscarium* had excellent effects on improving plant growth (Fig. 2). Apparently, the plants treated with *L. muscarium* were found healthier with more green pigment compared to nematicidal treatments. The excellent sugar beet yield was obtained in case of *L. muscarium* treated plants. Nematicides certainly reduced the nematode population in soil but their effects on plant

Fig. 1. Phytotoxic effects of nematicide, Basamid (G) on leaves of sugar beet. Chlorotic spots are shown with the help of arrows.

Fig. 2. Effects of *Lecanicillium muscarium* on *Heterodera schachtii* and growth of sugar beet, (A) in comparison of control (with nematodes treated) plants (B)

growth were not exceptional (Fig. 3). Maximum foliage growth was observed with *L. muscarium* treated plants but rest of growth parameters were almost similar ($P = 0.05$) in all treatments.

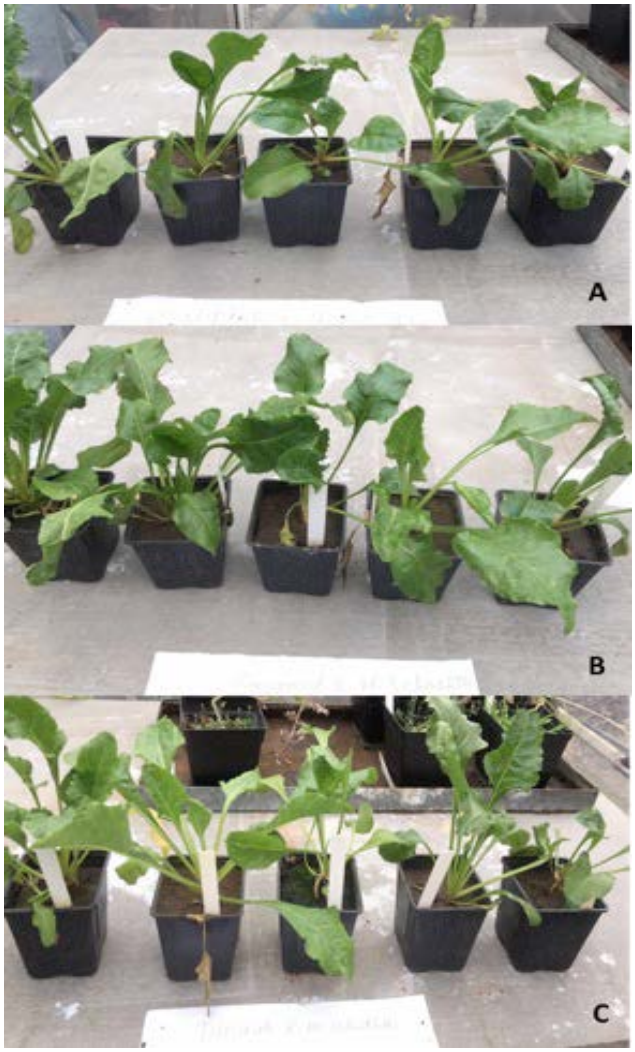

Fig. 3. Effects of nematicides, Vydate (A), Basamid (B), and calcium cyanamide (C) on *Heterodera schachtii* and growth of sugar beet.

DISCUSSION

All three nematicides and *L. muscarium* had remarkable impact on reduction of nematode population density. Two months after seeding, nematicides as well as fungus produced excellent results by receding the nematode population and increasing plants vigor. In case of *L. muscarium* application, plant roots were found colonized with fungal mycelium by developing a protecting sheet.

The reduction of nematode population is based upon the concentration of nematicides, their dispersion and distribution of concentration time product (CT) (Seinhorst, 1973). Nematicides are deposited in a small soil volume on application from which they can translocate more rapidly into soil to kill nematodes. Nematicide, Vydate (Oxamyl) has capacity to move quickly from site of application to the target sites. It also has advantage to be broad spectrum systemic nematicides which can kill wide range of parasites (McGarvey *et al*., 1984). Therefore, in our investigation Vydate demonstrated excellent results in reducing number of cysts and juveniles in soil. Moreover, the active ingredient of Oxamyl has been known to be effective in controlling root knot nematode juvenile invasion of roots (Radewald *et al*., 1970). The Basamid (Dazomet) ranked second which also showed better results but proved to be relatively less efficient. Dazomet is powerful multi-fumigant degraded into dithiocarbamic acid which in turn decomposes to methyl isothiocyanate, formaldehyde, hydrogen sulfide and methylamine (Roberts and Hutson, 1999). The less efficiency of Dazomet could be related to non-uniform distribution among soil particles or its complex mechanism of action. Although all nematicides were able to control nematode infestation in soil but we observe phytotoxic effects on plant growth especially on leaves. Oxamyl or carbamate nematicides have also been proved to affect the movement and orientation of nematodes towards host roots, rather than killing them even at low concentration (Wright, 1981). But concentration, time of dispersal, soil depth and texture are rather important to get successful control of nematodes. Calcium cyanamide was also found efficient in controlling plant parasitic nematodes population which resulted in reduction nematode reproduction rate. Our study validates the results of Dickson (1998) and Giannakou and Karpouzas (2003). Our results suggest that no nematicide, apart from oxamyl could efficiently diffuse within roots and kill nematodes living within cysts. This is probably one of the reasons why oxamyl has been the most efficient nematicide for the last several years. As regard to *L. muscarium,* it was able to reduce the number of cysts, J2 in soil and eggs in root systems. *Lecanicillium muscarium* also efficiently reduced the rate of nematode reproduction three times less than that of control (Table II). Improved growth with healthy leaves and high sugar beet yield were observed which indicates that the fungi did not had any phytotoxic effect on plant growth (Fig. 2). The roots were found colonized by fungus, which provide a barrier against nematodes to penetrate into root system (McGarvey *et al*., 1984). Efficiency of *L. muscarium* could be related to its growth and parasitism activity on production of infection propagules (conidia) and enzymes even at wide range of temperature (5-30°C) with an optimum at 25°C (Fenice *et al*., 1996, 1997).

Table II.- Effects of *Lecanicillium muscarium*, nematicides and fertilizer on plant growth of sugar beet Pi= 1000 j2 per plant.

Treatments	Fresh shoot wt. (g)	Fresh root wt. (g)	Fresh root length (cm)	Fresh shoot length (cm)
L. muscarium + *H. schachtii*	35.2 ± 3.7 a	27.8 ± 2.3 a	5.94 ± 0.69 a	25.2 ± 2.7 a
Vydate + *H. schachtii*	23.4 ± 1.5 b	25.6 ± 2.7 a	5.90 ± 0.53 a	22.2 ± 1.3 a
Basamid (G)+ *H. schachtii*	22.6 ± 1.5 b	24.2 ± 3.4 ba	5.89 ± 0.32 a	24.4 ± 2.6 a
Calcium cyanamide+*H. schachtii*	22 ± 1.4 b	23.8 ± 3.7 ba	5.87 ± 0.44 a	23.0 ± 2.0 a
L. muscarium	39.2 ± 2.7 a	29.8 ± 3.3 a	5.99 ± 0.61 a	27.2 ± 2.5 a
Vydate	23.2 ± 1.3 b	23.6 ± 2.5 ba	5.85 ± 0.23 a	22.4 ± 1.2 a
Basamid (G)	22.4 ± 1.2 b	24 ± 2.4 ba	5.83 ± 0.28 a	24.3 ± 2.2 a
Calcium cyanamide	23 ± 1.4 b	23.4 ± 3.3 ba	5.81 ± 0.44 a	24.0 ± 2.0 a
Hetrodera schachtii	21.6 ± 1.8 b	20 ± 3.2 b	5.74 ± 0.38 a	16.6 ± 2.07 b

Data is presented as mean ± standard deviation of ten replications.
Values within a column followed by the same letter are not significantly different according to T- Test at $P = 0.05$.

The studies also have proved that nematodes are attracted to fungi (Jasson and Norbring- Hertz, 1979; Jasson, 1982). This attraction may enhance the parasitism of fungi by diverting nematode attention. *Lecanicillium muscarium* have also been proved to elicit systemic resistance on endophytic colonization of roots (Hirano *et al*., 2008).

CONCLUSION

Lecanicillium muscarium along with nematicides and fertilizer had potentially reduced nematode density in soil. Moreover, it also showed remarkable effects on plant growth which further need to be investigated. In case of nematicide, Basamid (Dazomet) some phytotoxic effects were observed in the form of small chlorotic spots on leaves which also be consider into account (Fig. 1).

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Conflict of interest statement

The authors have declared no conflict of interest

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