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Article

Observations on predation of *Rhizoglyphus robini* (Acari: Acaridae) on the alfalfa stem nematode, *Ditylenchus dipsaci* (Nematoda)

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Abstract

The alfalfa stem nematode, *Ditylenchus dipsaci* (Kühn) causes substantial damage to alfalfa crops in Iran every year. *Rhizoglyphus robini* Claparède is a polyphagous pest of bulbs, corms and tubers, but can be beneficial in some situations. This study examined the effect of *R. robini* as a predator of *D. dipsaci* in greenhouse conditions. Pot experiments showed that *R. robini* caused high mortality of *D. dipsaci*. The effect of *R. robini* density on mortality of *D. dipsaci* was mathematically modeled using the response surface methodology (RSM). These observations suggest that *R. robini* may contribute to regulate populations of this nematode.

Key words: *Rhizoglyphus robini*; biological control; *Ditylenchus dipsaci*; alfalfa stem nematode, predator.

Introduction

Alfalfa is an economically important fodder plant in Iran, and is grown in more than 600,000 hectares of agricultural lands (Sharafeh 1987). The alfalfa stem nematode, *Ditylenchus dipsaci* (Kühn) (Nematoda: Anguinidae) was introduced into Iran in 1966 (Abivardi and Sharafeh 1973; Barooti 1991), and now causes substantial damage to alfalfa production there (Sharafeh 1987). The alfalfa stem nematode can feed on at least 450 plant species, and occurs in many countries including Germany (also many countries in Europe), USA, India, Japan, South Africa and Australia (Evans *et al.* 1993). It occurs as many different biological races that have different ranges of host plants. The resting stage of this species can survive in both dry and moist soil for a year or more (Hooper 1972), which makes it very difficult to control. Control of this pest using synthetic nematicides is expensive, environmentally harmful, and not always successful. Biological control has potential as an alternative or supplementary method for nematode control (Sayre and Walter 1991). Many species of predatory mites found in agricultural soils feed on harmful nematodes such as *Longidorus* spp., *Heterodera* spp., and *Ditylenchus* spp., and may play an important role in controlling their populations (Karg 1993; Gerson *et al.* 2003). It has been shown that some species of mites in the family Acaridae can prey on nematodes (Sturhan and Hampel 1977; Walter *et al*. 1986, 1988; Epsky *et al.* 1988; Sell 1988; Walter and Ikonen 1989), despite the fact that they are usually considered to be pests. The bulb mite, *Rhizoglyphus robini* Claparède (Astigmata: Acaridae), appears to be virtually cosmopolitan (Fan and Zhang 2004). Not only it is a serious pest of edible tubers sand bulbs, such as potatoes and onions, but also attacks a variety of ornamental bulbs, both in the growing crop and during post-harvest storage. Despite its importance as a pest, it may also be beneficial in some circumstances (Gerson *et al.* 2003). The purpose of this study was to determine whether *R. robini* can act as a predator of the alfalfa stem nematode in glasshouse conditions. The role of *R. robini* was investigated using response surface methodology (RSM), and the best treatment conditions for maximizing mortality of *D. dipsaci* were determined.

Material and methods

Collecting and rearing of R. robini

Potatoes were obtained from a market in Tehran, and examined under a dissecting microscope. Ten males and 10 females of *R. robini* were removed to start a laboratory culture on potato dextrose agar in a Petri dish in germinator at a temperature of 27 ± 3 °C, 75 ± 5% R.H. and 12:12 (L:D). Mites were transferred into a clean culture dish every four weeks. Bacterial and fungal contamination was controlled using 10% potassium permanganate and streptomycin.

Producing and extracting nematodes

Thirty nematode-infested alfalfa plants were collected from a field in Hamedan in May 2006. Plants with natural field soil attached were transferred into 25 cm diameter pots in the glasshouse. Nematodes were extracted from plants using the method described by Coolen and D'Herde (1970) followed by the method of Whitehead and Hemming (1965), and from soil as described by Jenkins (1964). Nematodes in suspension were sterilized with 1% streptomycin sulphate for 24 hours. Nematodes in a 1 cc sample of suspension were counted on a counting slide (repeated three times), and the required amount of suspension for 1000 nematodes was added to each pot (40 cc). Nematodes were identified by their body shape, movement, oesophogeal overlap and tail shape, using a standard series of morphometric measurements (Hooper 1972).

Design of Experiment

Experiments were carried out in a glasshouse at 27 ± 3 °C and 85 ± 5% RH. Each Experimental pot contained 500 grams of sterilized soil mixture collected from the field. Hamedan alfalfa seeds were collected from the field and washed in 10% sodium hypochlorite for 15 minutes. Four germinated alfalfa coleoptiles were used in each experimental pot. A central composite design with two variables was used for the experimental plan. These variables were the numbers of *R. robini* and *D. dipsaci*. The ranges of these variables were as follows: *R. robini* (10–100) and *D. dipsaci* (500–1000). Details of the central composite design for predation tests with *R. robini* in the presence of *D. dipsaci* are presented in Table 1 (Treatment 1–23). Control pots contained no mites and no nematodes.

Statistical analysis

The influence of the variable on the results Y (mortality of *D. dipsaci*) was adjusted using the following third order polynominal function:

$$Y = b_0 + \sum b_i X_i + \sum b_{ij} X_i X_j + \sum c_i X_i^2 \quad (i \geq j,\ i, j = 1,2,3) \qquad (1)$$

In this equation, b_0 is an independent term according to the mean value of the experimental plan, b_i is a regression coefficient that explains the influence of the variables in their linear form, b_{ij} is a regression coefficient of the interaction terms between variables, and c_i is the coefficient of the quadratic form of the variables.

Results

After 90 days the nematodes in each pot were counted. Results are presented in Table 1.

Table 1. Central composite design for mortality of *D. dipsaci* with different numbers of *R. robini* and *D. dipsaci*

Treatment number	Factors		Response
	A: No. of *R. robini*	B: No. of *D. dipsaci*	mortality of *D. dipsaci*
1	10	1000	178
2	100	1000	907
3	100	500	405
4	100	750	662
5	33	750	558
6	10	500	126
7	10	500	128
8	55	500	236
9	100	1000	918
10	33	875	594
11	10	1000	179
12	10	750	150
13	10	1000	177
14	100	500	406
15	55	625	331
16	10	500	130
17	33	625	370
18	78	625	495
19	55	1000	523
20	100	1000	907
21	78	875	693
22	55	750	386
23	100	500	407

This study was conducted according to response surface methodology (RSM) and central composite design (CCD). In total, 23 experimental CCD designed treatments were conducted according to Table 1. In this model, the impacts of independent variables including numbers of *R. robini* and *D. dipsaci* on response surface were assessed. Based on the results of relation between surface response and independent factors, the mathematical model was obtained, in which the response was a function of the independent variables. For mortality of *D. dipsaci*, the analysis of variance (ANOVA) is given in Table 2. According to the ANOVA results, the fitted model of mortality of *D. dipsaci* by using Design-Expert software is given in Equation (2).

Mortality of *D. dipsaci* $= +2025.53 + 18.06 \times [R.\ robini] - 9.87 \times [D.\ dipsaci] + 0.01 \times [R.\ robini \times D.\ dipsaci] - 0.45 \times [R.\ robini^2] + 0.01 \times [D.\ dipsaci^2] - 1.57E{-}005 \times [R.\ robini^2 \times D.\ dipsaci] + 9.08E{-}007 \times [R.\ robini \times D.\ dipsaci^2] + 2.71E{-}003 \times [R.\ robini^3] - 7.16E{-}006 \times [D.\ dipsaci^3]$ (2)

Table 2. ANOVA results for response surface cubic model of mortality of *D. dipsaci*. R-squared: 0.9596, adjusted R-squared: 0.9316, CV%: 15.76.

Source	Sum of squares	Df	Mean Square	F Value	P-value Prob. > F
Model	1.410E+006	9	1.567E+005	34.29	<0.0001 significant
A [No. of *R. robini*]	10.04	1	10.04	2.197E-003	0.9633
B [No. of *D. dipsaci*]	45166.38	1	45166.38	9.88	0.0078
AB	1.504E+005	1	1.504E+005	32.91	<0.0001
A^2	2407.43	1	2407.43	0.53	0.4808
B^2	15465.48	1	15465.48	3.38	0.0888
A^2B	109.23	1	109.23	0.024	0.8795
AB^2	11.25	1	11.25	2.463E-003	0.9612
A^3	30077.29	1	30077.29	6.58	0.0235
B^3	6171.25	1	6171.25	1.35	0.2661
Residual	59404.80	13	4569.60		
Lack of fit	59312.13	5	11862.43	1024.09	<0.0001 significant
Pure error	92.67	8	11.58		
Cor Total	1.470E+006	22			

Figure 1 shows the response surface of the model for mortality of *D. dipsaci*. By using the Design of Expert software, the optimum design point was obtained for mortality of *D. dipsaci* = 912, with desirability of 0.993, for a treatment containing 100 *R. robini* and 963 *D. dipsaci*.

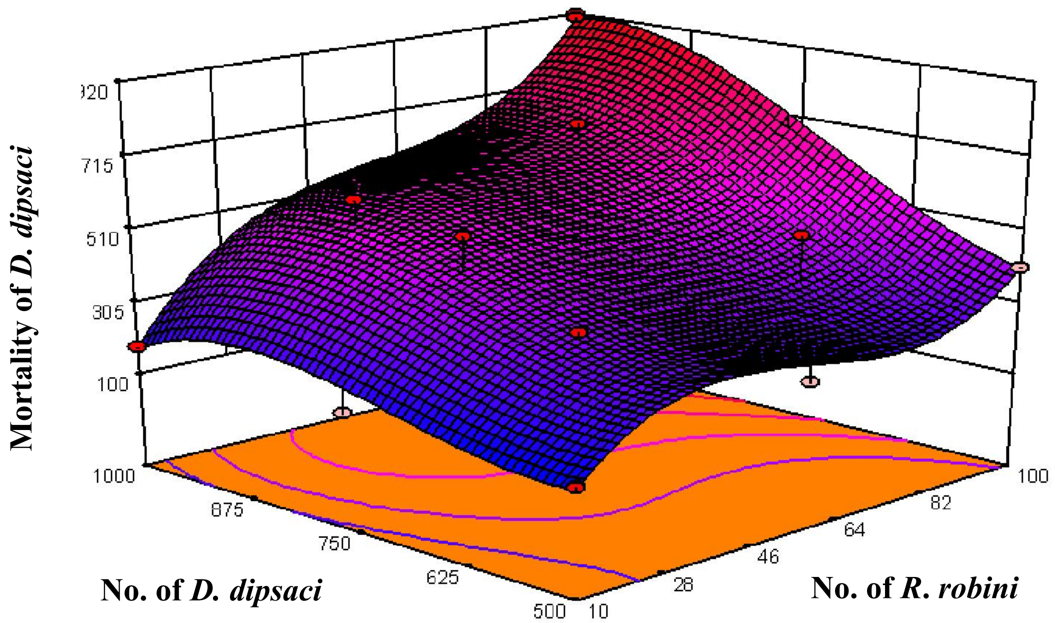

Figure 1. Response surface for mortality of *D. dipsaci* with different numbers of *R. robini* and *D. dipsaci*.

The integrated management of pest nematodes such as *D.dipsaci* is likely to include a contribution by soil-dwelling predators, especially mites. *Rhizoglyphus robini* is an important pest of edible and ornamental bulbs, both in the field and in post-harvest storage. However, the results of this small-scale glasshouse study show that the presence of this mite in agricultural soils could have beneficial effects in helping to reduce populations of pest nematodes such as *D. dipsaci*, and could be a useful component of an integrated biological control program for this pest.

Discussion

Some authors have reported that some species of *Rhizoglyphus* are predators of plant-parasitic nematodes but this has not been studied thoroughly. The very limited information that is available shows that they can prey on some plant-parasitic nematodes and therefore may have potential as biological control agents.

According to Gerson *et al.* (2003) plant-parasitic nematodes, including *Ditylenchus*, *Heterodera* and *Longidorus*, were consumed by the bulb mite, *Rhizoglyphus echinopus*. Small nematodes were completely devoured, whereas larger ones were cut into pieces and sucked out; nematode cysts were attacked only after some initial hesitation.

Another acarid, *Sancassania ultima* Samšiňak, is a rapacious feeder on all stages of gall-making *Meloidogyne* spp. (Sell 1988). The mite was strongly attracted to undamaged roots, where it searched for nematodes, an attribute that adds to its biological control potential. *Sancassania* spp., as well as *Rhizoglyphus* spp., are voracious consumers of eggs of *Ascaris* (roundworms parasitic in humans, primates and domestic animals). Five adult mites placed on 0.5 g roundworm eggs consumed about 75% of the eggs in 32 days (Lysek 1963).

Our observations in this small-scale glasshouse study are consistent with the above very limited studies. Further larger-scale studies are required to determine whether this predator is also effective under field conditions. One of the important factors that must be considered in future studies is the effect of alternative food on *R. robini*, which was not provided in the present study.

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مشاهداتی در زمینهٔ شـکارگری (Acari: Acaridae) *Rhizoglyphus robini* روی نماتد ساقهٔ یونجه، (Nematoda) *Ditylenchus dipsaci*

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چکیده

نماتود ساقه یونجه *Ditylenchus dipsaci* (Kühn) هرساله باعث خسارات شدیدی به گیاه یونجه می‌شود. گونهٔ *Rhizoglyphus robini* Claparède آفتی همه چیزخوار روی پیاز، ساقه‌های زیرزمینی و غده‌هاست اما می‌تواند در شرایطی مفید باشد. این مطالعه به بررسی اثر *R. robini* به عنوان شکارگر نماتود *D. dipsaci* در شرایط گلخانه پرداخته است. مطالعات گلدانی نشان داد *R. robini* باعث مرگ و میر زیادی در *D. dipsaci* شد. تاثیر انبوهی *R. robini* بر مرگ ومیر *D. dipsaci* با استفاده از روش صفحه پاسخ (RSM) به صورت مدل ریاضی ارایه شد. این مشاهدات نشان می‌دهد که احتمالا *R. robini* می‌تواند در تنظیم جمعیت این نماتد نقش ایفا کند.

واژگان کلیدی: *Rhizoglyphus robini*؛ کنترل بیولوژیک؛ *Ditylenchus dipsaci*؛ نماتود ساقهٔ یونجه؛ شکارگر.

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