Research Article

The role of some agricultural crops and weeds on decline of potato cyst nematode *Globodera rostochiensis* and their possible use as trap crops

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Abstract: The potato cyst nematode *Globodera rostochiensis* (PCN) causes severe damage to potato production worldwide. The stimulatory effects of root diffusates of 9 plant families on hatch of second stage juveniles (J2) of PCN and their possible use as trap crops were studied. In the laboratory, cysts were placed in leachates from roots and hatched J2s were collected weekly; in addition, the selected plants were grown in soil for 3 months and the rates of decline of eggs from inoculated cysts, were determined in two experiments with various plant species. Species of Solanaceae, such as the susceptible potato cultivar Marfona, tomato and the resistant potato cultivar Agria, induced 84%, 75% and 65% hatch respectively. Moreover, considerable hatching (49%-70%) was caused by exposure to root exudates of weeds of *Solanum nigrum* and *S. villosum*. Among non-host and non-Solanaceae plants, 11-32% of J2s hatched in root exudates of treatments of wheat, corn and barley in soil. There were differences in the rates of decline of J2 populations caused by the different plant species examined. In *S. sisymbriifolium* (a non-host species of Solanaceae) treatment, the density of encysted eggs declined by 62%. The potential of aforementioned plant species, the resistant cv Agria and some other tested plant species as suitable trap crops are further discussed; and in conclusion, the use of the trap crops could be an important component of PCN management program in Iran.

Keywords: Decline rate, golden potato cyst nematode, non–Solanaceae plants, *Solanum sisymbrifolium*

Introduction

Potato cyst nematodes (PCNs) *Globodera pallida* (Stone) and *G. rostochiensis* Wollenweber are among the most important pests of Solanaceous crops such as potatoes, tomatoes, and eggplants (Jones *et al*., 2013).

The golden PCN (*G. rostochiensis*) passes through a golden-yellow phase before the maturing female body turns brown, whereas *G. pallida* females pass from white directly to brown. Females are fertilized by males, embryos develop within egg to form second stage juveniles which is the dormant stage. Each swollen female may contain up to 300-500 eggs containing second-stage juveniles (J2), and its body wall hardens as it dies to form a cyst. The developed juveniles inside the cysts, enter into a dormancy known as diapause,

which is a strategy by cyst nematodes to overcome unfavorable seasonal conditions. Diapause is a stage during which the PCN juveniles, regardless of environmental conditions, cannot be stimulated to hatch until the diapause ends. They may remain viable for over 20 years. Root exudates from growing potato roots could induce more than 80% hatch. Spontaneous hatch occurs when host plants are not grown, and may reach 30% per annum (Oostenbrink, 1950; Kerry *et al.*, 2009). At least ten chemical compounds, referred to as hatching factors, have been identified as to play a role in PCN hatch (Devine *et al.*, 1996). Infestation by PCNs occurs immediately after hatching when J2s invade roots of host plants, whereby they retard root development. The reduction of root growth reduces water and nutrient uptake by the plant and decreases the yield (Fatemy and Evans, 1986; Trudgill *et al.*, 1998). In Europe, a 9% loss in total potato yield is attributed to PCNs (Evans and Rowe, 1998). Crop rotation, leads to slow decline of nematode populations, and growing resistant cultivars are the most frequently employed management strategies after prohibition of most environmentally-hazardous nematicides in many European countries (Chitwood, 2003).

One potential non-chemical control method is the use of trap crops. These are crops that trigger the hatch of PCN in soil but prevent the completion of the pest's life cycle. The use of trap crops dates back to 1939 (Carroll and McMahon, 1939). Early research focused on the use of potatoes as trap, allowing PCN juveniles to invade the roots, but fail to complete their life cycle by destroying or ploughing the plants (Halford *et al.*, 1999). Research on trap crops has also included other *Solanum* species. Host range studies by Stelter and Engel (1975), Roberts and Stone (1981) and Stelter (1987) showed that several plant species from Solanaceae family have a high degree of resistance to PCN and could stimulate its hatching.

In Iran, *G. rostochiensis* pathotype *Ro*1 is a quarantine pest currently threatening potato production in Hamadan Province. The behavior of Iranian PCN populations in response to locally growing plant species in infested fields has rarely been examined thoroughly.

Therefore, the present study was undertaken to investigate if the plant species commonly grown in rotation with potato by local growers in Hamadan i) have stimulatory effect on PCN hatching, ii) could contribute to PCN decline, and iii) whether they could be recommended as trap crops in PCN management programs.

Materials and Methods

Nematode and potato root diffusate preparation
Globodera rostochiensis cysts were collected from a naturally infested field in Hamadan province. The soil was dried and the cysts were recovered using the Fenwick can extraction procedure (Fenwick, 1940) and kept at 4 °C for at least 4 months until used.

To obtain live J2s, the egg-containing cysts were pre-soaked in distilled water for 48 h before they were transferred to a glass dish containing potato root diffusates (PRD) and collected following the protocol described by Turner *et al.* (2009). Potato tubers of a susceptible cultivar (cv) were planted in one kg pots filled with sterile potting soil. After one month, soil was saturated with distilled water, followed by adding further 100ml of distilled water. The solution draining from the pot was collected and passed through the pot three times before filtering and storage at + 4 °C until used.

***In vitro* hatching test**
Certified potato seeds were obtained from Agriculture and Natural Resources Research and Education Center of Hamadan and Botany Department, Iranian Research Institute of Plant Protection, Tehran. To collect root diffusates from other plant species, five seeds of each plant species listed in Table 1 were planted separately in pots containing 1 kg of sterile potting soil; non-planted pots were also included to represent fallow (Turner *et al.*, 2009). After plants reached the appropriate size, individual pots were watered with 100ml distilled water each week and the solution

draining from each pot was collected, filtered with Whatman No 1 filter paper and kept in the dark at + 4 ℃ before being used. This procedure was repeated weekly for 6 weeks. Root diffusates collected each week was used for corresponding test in the same week.

Table 1 Plant species used for the experiments.

Common name	Latin name	Family
Alfalfa cv Hamadani	Medicago sativa L.	Fabaceae
Barley cv Makoi	Hordeum vulgare L.	Poaceae
Barley cv Bahman	Hordeum vulgare L.	Poaceae
Black nightshade	Solanum nigrum L.	Solanaceae
Cucumber	Cucumis sativus L.	Cucurbitaceae
Canola cv Hyola	Brassica napus L.	Brassicaceae
Coriander	Coriandrum sativum L.	Apiaceae
Corn cv KSC301	Zea mays L.	Poaceae
Clover	Trifolium pratense L.	Fabaceae
Edible amaranth	Amaranthus tricolor L.	Amaranthaceae
Garlic cv Hamadani	Allium sativum L.	Amaryllidaceae
Lamb's quarters (Salmetare)	Chenopodium album L.	Amaranthaceae
Oil radish	Raphanus sativus var. oleifera L	Brassicaceae
Pumpkin cv Kado - Ajili	Cucurbita pepo sp fraternal var. styriaca	Cucurbitaceae
Potato cv Marfona	Solanum tuberosum L.	Solanaceae
Potato cv Agria	Solanum tuberosum L.	Solanaceae
Red Onion	Allium cepa L.	Amaryllidaceae
Red nightshade	Solanum villosum Mill. (formerly luteum)	Solanaceae
Saffron	Crocus sativus L.	Iridaceae
Sticky nightshade	Solanum sisymbriifolium Lam.	Solanaceae
Tomato cv Early Urbana	S. lycopersicom L.	Solanaceae
Wheat cv Sardari	Triticum aestivum L.	Poaceae
White onion	Allium cepa L.	Amaryllidaceae

cv: cultivar.

For experiments, full cysts were disinfected with 0.5% NaClO for one minute and rinsed with plenty of tap water; 20 cysts were placed in wells of tissue culture plates containing 2ml of each plant species/cultivar exudates, and the distilled water and leachates from fallow pots were used as controls. Three replicates were used for each treatment and plates were held at

20 ℃. Each week, the hatched J2 were removed, counted and fresh root exudates corresponding to appropriate week of the test were added (exudate collected at week 1 was used for the first week of the test and so on); this practice was repeated for 6 weeks (Turner *et al*., 2009). At the end of the experiment, cysts in each well were crushed, the numbers of the remaining eggs and the hatched J2s were counted and the hatching percent was calculated.

Decline rate experiment
Seeds of plant species given in Table 1 were planted in pots of 1 l capacity; filled with sterile loamy soil (with 40-40-20% sand-silt-clay; pH 7.3). The required number of cysts (40 cysts/ 100g soil) to represent an initial density (Pi) of 26 eggs and J2/g soil were added to each pot and mixed well into the soil.

A similar method was used for Solanaceae species, except that cysts were enclosed in mesh bags before being added to each pot (Fatemy, 2018), and potato cvs were planted as single sprouts attached to a piece of tuber. Unplanted pots were regarded as the fallow treatment and pots of each replicate (three replicates for each treatment) were arranged in a randomized complete block design, on a glasshouse bench with a 27/18 ℃ temperature regime. Pots were irrigated as required and fed with liquid fertilizer containing macro- and micro-elements. After 3 months, plants were uprooted and a 100-g subsample of soil was taken from each pot, air-dried and processed for cyst extraction; the number of cysts and final density of eggs and J2 in each pot were determined as described previously (Fatemy and Aghazadeh, 2016). The cysts recovered from bags were crushed and their numbers were estimated. For both types of inoculums, the hatching percent was calculated by subtracting the number of eggs/ cysts at planting from final eggs/ cysts multiplied by 100. Oostenbrink's reproduction factor (R = final nematode population/initial nematode population) is used to measure the

reproductive capacity of nematodes (Ebadi *et al.*, 2018).

Statistical analysis

Comparison between effects of different plant species on nematode hatch, survival and decline rates were made by analysis of variance (ANOVA), and means were statistically compared by Duncan's multiple range test at the 5% level. The normality and homogeneity of variance were assessed according to the Shapiro-Wilk method (SPSS, Package for the Social Sciences, SPSS Inc., Chicago IL) version 18.

Results

Under laboratory conditions, significant differences in hatch percent was observed between the various plant species ($P \leq 0.05$) (Fig. 1). The least J2s hatched in root diffusates of barley cv Makoii and saffron, followed by 13 other species such as wheat, garlic, white onion, cucumber etc., for which the hatch rate ranged between 0.5 and 5%. The rate of J2 emergence from cysts was much greater in diffusates from species of Solanaceae, ranging between 28 and 45%.
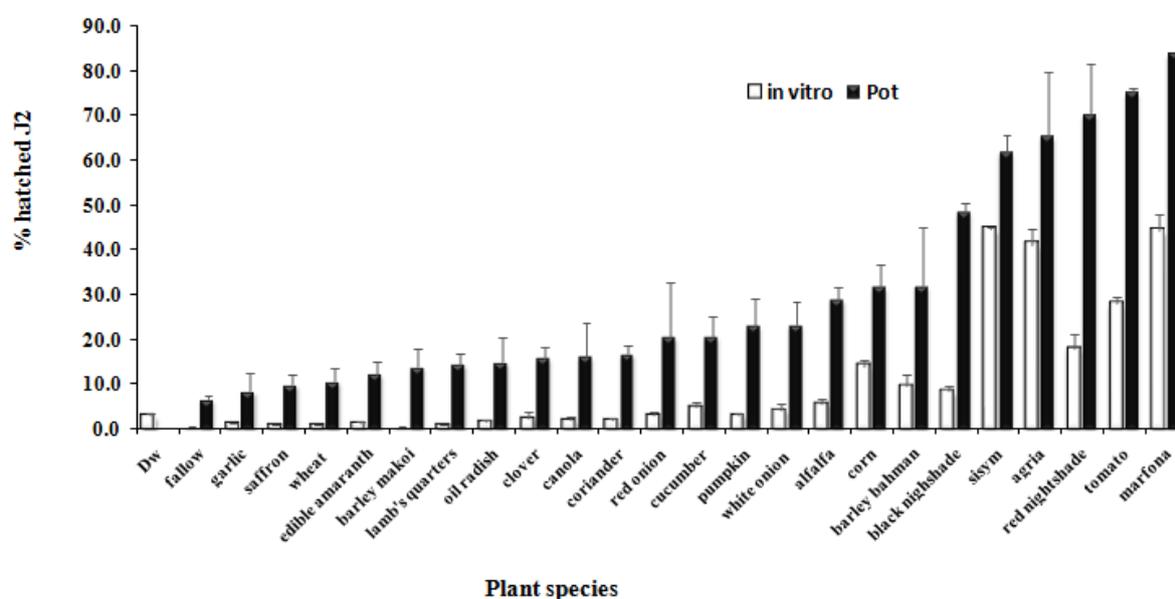

Figure 1 Hatching percentage of *Globodera rostochiensis* in response to root diffusates of various plants tested in laboratory and soil conditions. Bars are standard errors of the means. J2: second stage juvenile

The rate of emergence of J2 was higher in soil compared to *in vitro* test for all plant species Fig. 1). However, the ranking of plants based on their effect on J2 emergence from cysts was almost identical in both laboratory and soil conditions. Root exudates of alfalfa, barley cv Bahman, Cucurbitaceae (cucumber and pumpkin), corn and onions stimulated 21 to 32% of the J2s to hatch in soil. More than 49% of the J2s were stimulated to hatch by diffusates from some of the Solanaceous plants (such as black and red nightshade, Agria, tomato and Marfona). *Solanum sisymbrifollium*, a non-host species, induced nearly 62% of the juveniles to hatch in soil. Final infestation levels of eggs/g soil and reproduction rates (final/initial density) were reduced by all plants except for black and red nightshades, tomato and susceptible potato cultivar (Table 2).

Table 2 Final number of eggs/g soil and reproduction factor (R: final/initial population) of *Globodera rostochiensis* in response to stimulants from different plant species grown in soil for 3 months. Fallow: unplanted soil.

Plant species	Eggs / g soil	R
Pumpkin cv Kado-Ajili	18.2 ± 0.69 bc	0.70 ± 0.03 bc
Barley cv Makoi	21.2 ± 1.40 bcde	0.81 ± 0.05 bcdef
Wheat cv Sardari	22.4 ± 1.70 bcde	0.86 ± 0.06 bcdef
Barley cv Bahman	17.5 ± 1.20 b	0.67 ± 0.05 b
Corn	17.4 ± 0.81 b	0.67 ± 0.03 b
Alfalfa cv Hamadani	19.1 ± 0.30 bcd	0.73 ± 0.01 bcde
Cucumber	19.8 ± 0.23 bcde	0.76 ± 0.01 bcde
Canola cv Hyola	20.8 ± 0.61 bcde	0.80 ± 0.02 bcdef
Coriander	20.8 ± 0.91 bcde	0.80 ± 0.04 bcdef
Oil radish	22.0 ± 1.50 bcde	0.85 ± 0.06 bcdef
Clover	22.1 ± 1.30 bcde	0.85 ± 0.05 bcdef
Saffron	23.5 ± 1.80 cde	0.90 ± 0.07 cdef
Fallow	24.4 ± 0.00 de	0.94 ± 0.00 def
Garlic cv Hamadani	25.2 ± 2.10 e	0.97 ± 0.08 ef
Onion white	20.2 ± 0.58 bcde	0.78 ± 0.02 bcde
Onion red	21.0 ± 1.70 bcde	0.81 ± 0.07 bcdef
Lamb's quarters	23.1 ± 2.40 cde	0.89 ± 0.09 cdef
Edible amaranth	25.0 ± 2.10 e	0.96 ± 0.08 ef
Black nightshade	64.9 ± 3.10 f	2.50 ± 0.12 g
Red nightshade	135.9 ± 2.50 g	5.20 ± 0.10 k
Tomato cv Early Urbana	209. 5 ± 0.50 h	8.10 ± 0.03 n
Potato cv Marfona	242.6 ± 3.20 k	9.30 ± 0.12 s
Potato cv Agria	4.8 ± 0.60 a	0.18 ± 0.02 a
S. sisymbriifolium	6.1 ± 0.32 a	0.20 ± 0.01 g

Numbers followed by same letter(s) are not significantly different at 5% level according to Duncan`s test. Mean ± Standard Error.

Discussion

As one of the mechanisms, a trap crop stimulates hatch of juveniles by its root diffusates. Potato was the first species studied as a potential PCN trap crop. Several researchers have shown that between 73 to 87% of PCN populations have been reduced in commercial fields growing potato as trap crop irrespective of their soil types (Roosjen and Veninga, 1989; Whitehead, 1992; Hancock, 1996; Molendijk, 1996; Halford *et al.*, 1999; Lane and Trudgill, 1999) In a similar manner,

trap crops were successfully applied and are used in controlling programs of beet cyst nematode (*Heterodera schachtii* and *H. trifolii* fsp. *betae*) (Heijbroek, 1994; Koch and Gray, 1997; Hafez and Sundararaj, 1998).

Plants that were examined in this study for their suitability as trap plants, belonged to nine families. Within non-Solanaceae and non-host plants, there were marked differences in hatch estimaulation between genera, and within similar genera belonging to the same family and cultivars of the same species. Nearly 11% of the J2s hatched in response to stimulants from roots of wheat cv Sardari, compared to 32% by corn and barley cv Bahman. On the contrary, only 14% of J2s were hatched by barley cv Makoi exudates. Furthermore, saffron and alfalfa cv Hamadani (belong to Iridaceae and Fabaceae, respectively), caused 10% and 29% hatching respectively. Differences in hatch between cvs of the same species and between genera have been reported. Evans (1983) found large variations in hatching activities of root leachates obtained from different potato cultivars. Dissimilarity found in our results could partly be attributed to variation in hatch-promoting properties of these plants, although we did not analyze their chemical properties. Under field condition in Hamadan, alfalfa cv Hamadani and saffron grown continuously for 5 years, caused a reduction *G. rostochiensis* infestations by 86% and 77% respectively in a rotation trial (Gitty, 2015). However, a 5-year rotation of cucumber, garlic cv Hamadani, pumpkin cv Kado- Ajili and wheat, reduced infestation by 96% (Gitty, 2015), which suggests that other factors besides hatching stimulant properties may contribute to PCN decline rates. Rates of decline were slow in uncultivated land infested with PCN, whereas regular sowing of infested land with grass appeared to accelerate it (Turner, 1996).

Marfona is a susceptible host and was a widely grown cultivar in Hamadan until recently. This cultivar and tomato stimulated hatching by 84% and 75% respectively, and they caused cyst multiplication by almost 8.5 times. In addition, Agria, a resistant cv, induced

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65% hatching and there was virtually no nematode reproduction on its roots. This cultivar is resistant to *G. rostochiensis* pathotype *Ro*1, the presently prevalent pathotype in Hamadan region. The high hatching stimulation ability of Agria makes it a good candidate for use as a trap crop as well as a good alternative for susceptible Marfona, and seems could help reduce PCN density in Hamadan region.

Besides some natural host crops, non-host plants are also preferable for use as trap crops. Screening non-tuber bearing Solanaceae for their potential as trap crops in The Netherlands resulted in selection of *S. sisymbriifolium* (sticky nightshade) and two varieties of *S. nigrum* (black nightshade) for this purpose (Scholte, 2000b). *Solanum sisymbriifolium* is native to South America, but found throughout many parts of the world. We found that 62% of J2s hatched in response to *S. sisymbriifolium* root leachates while *G. rostochiensis* did not reproduce on the roots. These results are in agreement with other findings showing *S. sisymbriifolium* has complete resistance to both PCN species. It can reduce the population of PCN up to 80% (Scholte, 2000b; Scholte and Vos, 2000). It has been used as an effective trap crop in the UK and The Netherlands. The nematodes invade its roots but do not complete their life cycle (Sasaki-Crawley *et al.*, 2010). Promising results from the use of this species has resulted in commercial production of *S. sisymbriifolium* (Branston, 2013) as a trap plant.

To our knowledge, this is the first report on using *S. sisymbriifolium* in a PCN controlling experiment in Iran. The species has recently been identified in Iran (Eslami and Naqinezhad, 2011) growing in sand dunes along coastline in the northern provinces of Gilan and Mazandaran. The seeds used in present experiments were provided by some colleagues outside the country, and the putative effect of the native varieties of this plant on PCN is an open field of study.

Solanum nigrum and *S. villosum* (red nightshade, formerly *S. luteum*) are common weeds in potato fields in Iran, and we did not

find any published record regarding their status as host to PCN. *Solanum nigrum*, a non-tuber bearing species of the Solanaceae, has been known as a potential PCN trap crop species and has shown a greater PCN reduction trap crop than a non-host crop control treatment (Scholte, 2000a). Stimulation of juvenile hatch by root diffusates of *S. nigrum* has been reported by Russel *et al*. (I949) and Doncaster (1957). Root exudates from *S. nigrum* also cause juveniles of *Globodera tabacum tabacum* to hatch (LaMondia, 1995). We showed that leachates from *S. nigrum* and *S. villosum* induced 49% and 70% of the J2 to hatch respectively and, contrary to some reports, *G. rostochiensis* multiplied on both species, more on red nightshade than on black nightshade. In a study by Rott *et al*. (2011), isolates of Pathotype *Ro*1 of *G. rostochiensis* displayed differences in their ability to reproduce on *S. villosum*, the species being resistant to one isolate and susceptible to another. Furthermore, most of the *S. nigrum* accessions examined by Rott *et al*. (2011), were hosts to isolates of *G. rostochiensis*. There are conflicting results in the literature concerning host status of *S. nigrum* and *S. villosum* to *G. rostochiensis* (Sullivan *et al*., 2007). *Solanum nigrum* is a common annual plant which is a complex species (Dehmer and Hammer, 2004), with a variety of populations each with a potentially different effect on PCN populations. Prummel (1958) concluded that conflicting results in the literature concerning the suitability of *S. nigrum* as a host of PCN could be attributed to varietal differences within this plant species.

Despite the fact that PCN is a fairly new pest for Iran, it has successfully survived, established and adapted to the new environment in a very short period, yet has become a serious pest on potato in Hamadan. Consequently, measures that growers could exploit to reduce the levels of PCN infestation in their fields with concomitant avoiding long rotation periods, are needed. The findings in this study suggest that *S. sisymbrifolium* and resistant cvs like Agria could contribute to higher decline rate of PCN and could be used in an integrated management

approach without concerns. The relationship between PCN and common weeds like *Solanum nigrum* and *S. villosum* needs further studies under field conditions.

Acknowledgments

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کاربرد بعضی گیاهان زراعی و علف‌های هرز به‌عنوان گیاه تله در کاهش آلودگی نماتد سیست سیب‌زمینی *Globodera rostochiensis*

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چکیده: نماتد سیست سیب‌زمینی *Globodera rostochiensis* از عوامل ایجاد خسارت شدید به سیب‌زمینی در سطح جهانی به‌شمار می‌رود. تأثیر ترشحات ریشه گیاهان متعلق به ۱۰ خانواده روی تحریک لارو سن دو (J2) نماتد به تفریخ و احتمال کاربرد گیاهان به‌عنوان تله مورد بررسی قرار گرفت. در شرایط آزمایشگاهی، سیست‌ها در ترشحات ریشه گیاهان قرار داده شدند و J2 تفریخ شده به‌صورت هفتگی جمع‌آوری گردید. گیاهان انتخابی در دو آزمایش جداگانه در شرایط خاک کاشته شدند و میزان کاهش تخم‌های درون سیست‌های تلقیح شده پس از ۳ ماه تعیین گردید. بعضی از گونه‌های سولاناسه ۸۴٪، ۷۵٪ و ۶۵٪ لاروهایی را که در معرض مارفونا رقم حساس سیب‌زمینی، گوجه‌فرنگی و رقم مقاوم اگریا قرار گرفته بودند تفریخ نمودند. از طرف دیگر ترشحات علف‌های هرز *Solanum nigrum* و *S. villosum* میزان قابل‌توجهی (۷۰٪-۴۹٪) از J2ها را تفریخ نمود. هم‌چنین ۱۱٪-۳۲٪ J2ها در اثر ترشحات گندم، ذرت و جو (گیاهان غیرمیزبان و غیرسولاناسه) تفریخ گردیدند. میزان کاهش جمعیت J2 در بین گیاهان مختلف متفاوت بود. در خاک *S. sisymbriifolium* (گیاه غیرمیزبان متعلق به سولاناسه) ۶۲٪ لاروها تفریخ شده بودند. قابلیت استفاده گیاهان نامبرده، رقم مقاوم اگریا و بعضی دیگر گونه‌های گیاهی به‌عنوان گیاه تله مورد بحث بیش‌تر قرار خواهد گرفت، کاربرد گیاهان تله می‌تواند جایگاه ویژه‌ای در برنامه‌های مدیریت نماتد سیست سیب‌زمینی داشته باشد.

واژگان کلیدی: گیاهان غیرسولاناسه، نرخ کاهش، نماتد سیست سیب‌زمینی، *Solanum sisymbrifolium*