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CONTROL OF THE CARROT CYST NEMATODE *Heterodera carotae* BY TANNIN AQUEOUS SOLUTIONS

Lara MAISTRELLO[1], Nicola SASANELLI[2], Giacomo VACCARI[1], Ion TODERAS[3], Elena IURCU-STRAISTARU[3]

[1]Department of Life Sciences, University of Modena and Reggio Emilia, Pad. Besta - Street Amendola 2, 42122 Reggio Emilia, Italy
[2]Institute for Sustainable Plant Protection, CNR, 122 Amendola Street, 70126 Bari, Italy
[3]Institute of Zoology, ASM, 1 Academiei Street, 2028 Chisinau, Republic of Moldova

Corresponding author email: nicola.sasanelli@ipsp.cnr.it

Abstract

A field trial was carried out to test the nematicidal activity of chestnut tannin aqueous solutions against the carrot cyst nematode Heterodera carotae. A soil naturally infested by the cyst nematode was subdivided in 2 m x 3 m plots distributed in a randomized block design with five replications/treatment. Plots were treated with tannin aqueous solutions at rates of 25 or 45 g/m^2 in 4 l water/m^2 applied in pre-emergence, 25 or 45 g/m^2 in 4 l water/m^2 applied in pre-emergence and 30 days after carrot emergence. Untreated soil and fenamiphos (60 l c.p./ha) treated plots were used as controls. Number and weight of marketable tap-roots from the central square metre of each plot were recorded at harvest. Cysts and number of eggs and juveniles/100 g soil were also determined. Cysts were extracted from soil samples, collected in each plot, by the Fenwick can. Data were statistically analysed and means compared by LSD's test. On the base of results, the use of tannin should be favourably considered for plant protection against phytoparasitic nematodes although some aspects remain to be investigated.

Key words*: carrot cyst nematode, Chestnut tannin, Heterodera carotae, nematode control.*

INTRODUCTION

The carrot cyst nematode *Heterodera carotae* Jones causes considerable yield losses to the most important European carrot growing areas. The amount of yield losses is related to the soil nematode population density at sowing (Sasanelli, 1994). The use of fumigant (Dimethyl disulphide or 1.3 Dichloropropene, liquid - oxamyl - or granular - fenamiphos) formulations of nematicides can successfully control the nematode (Bealir, 1984; Lamberti et al. 2001; Colombo et al. 2004; Curto et al., 2014). However, the recent European Legislations such as the directive on the sustainable use of pesticides (Directive 2009/128/EC) have deeply revised and restricted the use of pesticides on agricultural crops focusing the attention on environmental safety, human and animal health. Plant protection from phytoparasitic nematodes should therefore rely on alternative control strategies that are both environmentally sound and economically sustainable. Among the alternatives, the more promising ones include the use of soil amendments (D'Addabbo, Sasanelli, 1996), soil solarization alone or in combination with fumigants (Greco et al., 1990, 1992), and more recently the use of microorganism exametabolites or plant-derived formulations (Toderas et al., 2016; D'Addabbo et al., 2008).

Compounds at low environmental impact with nematicidal activity have been reported from many botanical families (Chitwood, 2002; Caboni et al., 2012; Cavoski et al., 2012; Renčo et al., 2014). Plant extract of quillay (*Quillaya saponaria* Molina), neem (*Azadirachta indica* Juss), tagetes (*Tagetes erecta* L.) and sesame (*Sesamum indicum* L.) are already available as commercial formulations. Among the natural products extracted from plants, tannins have been reported in the literature to possess antihelmintic properties especially for gastrointestinal nematodes in ruminants both *in vivo* and *in vitro* experiments (Hoste et al., 2006) and a nematicidal activity against the root-knot nematode *Meloidogyne javanica* (Treub) Chitw. and the potato cyst nematode *Globodera rostochiensis* (Woll.) Barhens

(Maistrello et al., 2010; Renčo et al., 2012). Although plant parasitic nematodes can cause severe yield losses to many agricultural crops, few information is available on the effect of tannins extracted from chestnut plants (*Castanea sativa* Mill.) on the carrot cyst nematode *H. carotae*. Therefore, to verify the possibility to use tannins also against the carrot cyst nematode a trial was carried out on a carrot crop in a field naturally infested by the nematode.

MATERIALS AND METHODS

The field trial was carried out in 2016 at Zapponeta (Province of Foggia, Apulia Region, Southern Italy) (41°.45'N, 15°.96'E) in a sandy soil homogenously and heavily infested by the carrot cyst nematode *H. carotae* (Pi=13.5 eggs and juveniles/g soil) (Figure 1). The field was deeply ploughed, rotavated and subdivided in 2 m x 3 m plots, spaced 0.5 m each other, distributed in a randomized block design with five replications for each treatment (Figure 2).

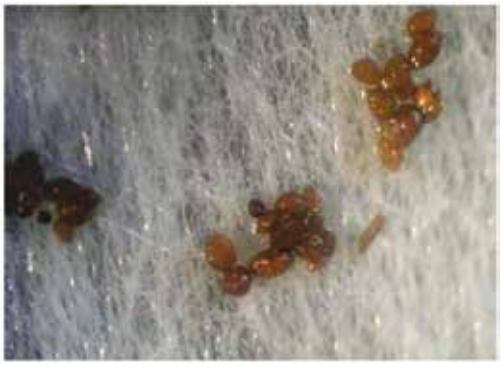

Figure 1. Cysts of *Heterodera carotae*

Figure 2. Experimental field subdivided in 6 m^2 plots

Carrot cv. Presto was sown by a seed drill at the density of 300 seeds/m^2 ten days before chestnut aqueous solution treatments.

The tannins were extracted by vapour from chestnut wood, without chemical solvents, in powder form after dehydration (Saviotan®, Radicofani, Siena Province, Central Italy). Chestnut aqueous solution treatments were: a) 25 g/m^2 in 4 l water/m^2 applied in pre-emergence; b) 25 g/m^2 in 4 l water/m^2 applied in pre-emergence and 30 days later; c) 45 g/m^2 in 4 l water/m^2 applied in pre-emergence; d) 45 g/m^2 in 4 l water/m^2 applied in pre-emergence and 30 days later (Figures 3 and 4). Tannin aqueous solutions were uniformly distributed on plots using a watering-can. Untreated soil and the nematicide fenamiphos (60 L c.p./ha) were used as controls.

Figure 3. Preparation of aqueous tannin solutions

Figure 4. Plots treated with aqueous tannin solutions

During the crop cycle the crop received all the necessary maintenance (irrigation, fertilization, weed control etc.).

At harvest, number and weight of marketable tap-roots from the central square meter of each plot were recorded (Figure 5). Soil samples, each a composite of 20 cores, were collected in the same central area of each plot (Figure 6). Cysts from a 100 g dried sub sample were extracted with a Fenwick can and crushed to count eggs and juveniles (Figure 7).

Figure 5. Square meter representative of the entire plot

Figure 6. Central area useful to collect soil samples

Figure 7. Apparatus of Fenwick for cysts extraction

Data from the experiment were subjected to analysis of variance (ANOVA) and means compared by Least Significant Difference's Test. All statistical analysis were performed using the PlotIT program V. 3.2.

RESULTS AND DISCUSSIONS

In the trial, carrot marketable yield ranged between 22.1 t/ha (untreated control) and 69.2 t/ha (fenamiphos control). Treatments with tannin solutions at 45 g/m^2 applied in pre or in pre and post-emergence significantly increased carrot marketable yield in comparison to the untreated control (Table 1). Among tannin treatments applied at different rates (25 and 45 g/m^2) and application time (in pre and in pre and post-emergence) no significant differences were observed in carrot marketable yield. The highest carrot marketable yield was recorded in fenamiphos treated plots and it was significantly different (P=0.05) from those recorded in all other treatments with the exception of tannin applied in pre-emergence at 45 g/m^2 rate (Table 1).

All treatments significantly increased the average weight of carrots compared to the untreated control excluding the lowest dose of tannin applied before emergence (Table 1). The average weight of carrot, for both applied rates, was not affected by the application time. No significant differences were observed between application in pre-emergence and in pre-emergence and 30 days later (Table 1).

No significant differences were observed in the number of cysts/100 g soil among the different treatments including the fenamiphos and the untreated control (Table 1).

The final nematode population density observed in the untreated control was significantly higher than those observed in all other treatments in which no differences were observed (Table 1).

The highest reproduction rate (ratio between final and initial nematode population density Pf/Pi) was observed in the untreated control (3.1) and it was significantly higher than those in the other treatments, which ranged between 1.2 and 1.6.

On the base of our results it is possible conclude that is not useful to repeat tannin treatments two times in pre emergence and 30

days later because no statistical differences were observed between the two application times.

The highest per cent increase in the marketable yield in comparison to the untreated control was observed in the treatment in which the tannin was applied in pre emergence at the rate 45 g/m^2 (Figure 8).

Figure 8. Untreated and tannin treated (45 g/m^2) plots

A similar trend was observed for the same treatment for the percent reduction of the soil nematode population density (62%) in comparison to the untreated control (Table 2).

The high initial nematode population density (13.5 eggs and juveniles/g soil), higher than the tolerance limit of carrot to *H. carotae* (T= 0.80 eggs and juveniles/g soil), could have masked the possible increase effect on marketable production by the tannin treatments.

Post emergence treatments do not seem to affect the protection of the crop, probably because the most delicate moment is the emission of small roots from the seeds, when the roots are immediately attacked by the nematode juveniles in the soil.

On the mechanisms of action of the tannin on the reduction of *H. carotae* population density in the soil, compared to control, it is possible to formulate hypotheses such as the negative influence of this substance (polyphenols) in the mechanisms of egg embryogenesis or that tannin can act as a larvae repellent or disrupt the juveniles chemoreception towards the carrot radical exudates.

Table 1. Effect of different chestnut aqueous solution treatments on carrot yield and on the soil population density of *Heterodera carotae*

Treatment (aqueous solutions)	Dose (g or ml/m^2)	Application time	Marketable yield (t/ha)		Average weight (g/carrot)		No. cysts/100 g soil		Eggs and J2/g soil		Pf/Pi	
Tannin	25	Pre-emergence	36.8[1]	ab[2]	50.7	ab	85	a	18	a	1.3	a
		Pre-emergence + 30 days later	43.3	ab	61.1	bc	109	a	19	a	1.4	a
Tannin	45	Pre-emergence	52.9	bc	58.4	bc	98	a	16	a	1.2	a
		Pre-emergence + 30 days later	46.6	b	58.5	bc	108	a	20	a	1.5	a
Fenamiphos	6	Pre-emergence	69.2	c	71.8	c	98	a	22	a	1.6	a
Untreated control	---	---	22.1	a	41.3	a	121	a	42	b	3.1	b

[1]Each value is an average of five replications.

[2]Data flanked in each column by the same letter are not statistically different according to Least Significant Difference's test (P=0.05).

Table 2. Effect of aqueous tannin solution treatments, at different doses and application time, on the percent increase of carrot marketable yield and per cent decrease of *Heterodera carotae* soil population density compared to the untreated control

Treatment (aqueous solutions)	Dose (g or ml/m^2)	Application time	% Increase of marketable yield[1]	Significance (P=0.01)	% Decrease of soil nematode population density[1]	Significance (P=0.01)
Tannin	25	Pre-emergence	67		57	*
		Pre-emergence + 30 days later	96		55	*
Tannin	45	Pre-emergence	139	*[2]	62	*
		Pre-emergence + 30 days later	111	*	52	*
Fenamiphos	6	Pre-emergence	213	*	48	*
Untreated Control	---	---	---		---	

[1]In comparison to control.
[2]*Significant at P=0.01.

CONCLUSIONS

The use of tannins offers promising perspectives of practical application in plant protection because it could represent a valid alternative to the use of synthetic nematicides. It could be considered in the context of nematicidal strategies at low environmental impact, not oriented to the eradication of the pest but to a progressive reduction of the level of nematode soil infestation below the damage threshold of the crop, with a lower impact on plant-soil balance compared to synthetic products.

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