ACTIVITY OF CHESTNUT TANNINS AGAINST THE SOUTHERN ROOT-KNOT NEMATODE *MELOIDOGYNE INCognITA*

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Studies on the effects of tannins on plant-parasitic nematodes are few. A new formulation of a hydrolyzable tannin extracted from chestnut (SaviotaN®) was tested for efficacy in controlling *Meloidogyne incognita*. Therefore, *in vitro* and pot experiments on tomato were performed to investigate the nematicidal activity of tannin aqueous solutions at different concentrations on *M. incognita*. In the *in vitro* experiment the following concentrations of tannin at 0.30, 0.40, 0.50, 0.75, 1.00, 1.25, 1.50 g L⁻¹ were tested for their effect on the nematode. The second-stage juveniles (J2s) immobility increased with increasing concentration and exposure time. All tested tannin concentrations were effective to reduce viability from about 45 to 70% after 10 days of exposure, in comparison to the treated and untreated controls. The immobile J2s recovered their mobility over time after rinsing and transferring them in water, showing a nematostatic activity of tannins. In the pot experiment, tannins, as aqueous solutions at rates from 0.30 to 1.50 g L⁻¹, were applied to soil at three different application times (1: only at transplant; 2: at transplant, two weeks after transplant and repeated every seven days; 3: at transplant and two weeks later). The activity of tannins was compared to treated and untreated controls. Tested rates mostly repeated were effective to control nematode attack in comparison to untreated control. The height of treated plants was not significantly influenced by the different applied rates of tannins, whereas nematode population density and root galling index were affected by repeated application times. No visual symptoms of phytotoxicity were detected. The use of SaviotaN® appears promising for the control of *M. incognita* in sustainable agriculture of short-term crops and/or when nematode population densities are low and as a supplement to other chemical treatments.

**KEY WORDS:** Root-knot nematode; *Meloidogyne incognita*; Tomato; Hydrolyzable tannins; SaviotaN®

INTRODUCTION

Root-Knot Nematodes (RKN) are the most common and widespread group of nematodes in the world causing dramatic yield losses to a wide range of crops. The simultaneous presence of RKN and plant pathogenic fungi may cause synergistic damages (Ragozzino & D’Errico, 2011). The European Union has deeply restricted the use of pesticides on agricultural crops focusing the attention on environmental impacts. Various plant species or compounds extracted and exuded from plants (i.e. alkaloids, phenolic compounds, saponines, etc.) are able to control plant diseases or, at least, to shape the microbial rhizosphere in favour of beneficial microbes thus helping the plant to overcome stress conditions (Sukul., 1992; Chitwood, 2002; Soppelsa et al., 2011; Mocali et al., 2015; Lombardi et al., 2018). Many of the discovered biologically active phytochemicals are considered safer to humans and environment than conventional pesticides (Chitwood, 2002). Among them, tannins play a significant role in numerous ecological processes (Kraus et al., 2003), protect plants against herbivores (Feeny, 1976) and are toxic for numerous bacteria, fungi and yeasts (Scalbert, 1991). Additionally, tannins are a group of water-soluble polyphenolic compounds that have the ability to precipitate proteins (Bate-Smith & Swain, 1962). They are found in higher plants mainly grouped into two classes, termed condensed and hydrolyzable tannins. Both classes have been shown to possess nematicidal activity (Mian & Rodríguez-Kabana, 1982; Mohamed et al., 2000; Naz et al., 2013), and termed condensed have been shown to inhibit gastrointestinal nematodes (Butter et al., 2001, Athanasadou et al., 2001; Hoste et al., 2006). Several studies demonstrated that tannins inhibit microbial activity (Baldwin et al., 1983; Benoit & Starkey, 1968a; b; Benoit et al., 1968; Fierer et al., 2001; Harrison, 1971; Lewis & Starkey, 1968; Schimmel et al., 1996; 1998; Schulte et al., 1992), whereas much more remains to be investigated about their effects on other soil biota such as plant parasitic nematodes (Kraus et al., 2003). Hewlett et al. (1997) suggest that the behavioural response of different nematode species to tannic acid is variable. In their studies, tannic acid was attractive for *Meloidogyne arenaria* (Neal) Chitw. and *M. incognita* (Kofoid & White) Chitw., whereas it was repellant for *Radopholus similis* (Cobb) Thorne and no effects were observed on *Heterodera glycines* Ichinohe. In literature, tannins from chestnut have been reported to affect plant parasitic nematodes (Badra & Elgindi, 1979; Hewlett et al., 1997; Maistrello et al., 2010). Soil treatments with tannic acid were found to control *M. arenaria* on squash (Mian & Rodríguez-Kabana, 1982). A previous
formulation of tannins extracted from chestnut wood (brand name SaviotaN®) has been tested on *M. javanica* and on *Globodera rostochiensis* (Woll.) Skarbilovich at concentrations ranging from 0.32 to 20.48 g L⁻¹ (MAISTRELLO et al., 2010; RENÇO et al., 2012), and on eggs and juveniles of *M. incognita* at two concentrations (2 and 5 g L⁻¹) showing a nematostatic action and an inhibitory effect on eggs hatching (CARLETTI & MAISTRELLO, 2012). However, these authors have used a different formulation of SaviotaN® and higher concentrations of application therefore their results are not comparable to our data. The objective of the present work was to evaluate the effect of a new formulation of SaviotaN® nutraceutical for crops (Gruppo Mauro Saviola s.r.l., Viadana, Italy) in wet powder (WP) containing 75% of pure tannins on the RKN *Meloidogyne incognita* both in vitro and in pot experiments on tomato under controlled conditions.

**MATERIAL AND METHODS**

**CHEMICALS**

The chestnut tannin extract SaviotaN® was tested in seven doses ranging from 0.30 to 1.50 g L⁻¹ as reported in Table 1. Fosthiazate 150 g L⁻¹ (Nemathorin® 150 EC; Syngenta), abamectin 20 g L⁻¹ (Tervigo® SC 1; Syngenta) and garlic extract, *Allium sativum* (Garlic Allicin < 1 ppm (Garland®; Omex Agriculture Ltd) were used for comparison at recommended doses.

**Table 1 – Schedule treatments and codes.**

<table>
<thead>
<tr>
<th>Treatment codes</th>
<th>Active ingredient</th>
<th>Dose kg/ha⁻¹</th>
<th>Dose g L⁻¹ H₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>T30</td>
<td>Tannin WP 75%</td>
<td>6 kg</td>
<td>0.30 g</td>
</tr>
<tr>
<td>T40</td>
<td>Tannin WP 75%</td>
<td>8 kg</td>
<td>0.40 g</td>
</tr>
<tr>
<td>T50</td>
<td>Tannin WP 75%</td>
<td>10 kg</td>
<td>0.50 g</td>
</tr>
<tr>
<td>T75</td>
<td>Tannin WP 75%</td>
<td>15 kg</td>
<td>0.75 g</td>
</tr>
<tr>
<td>T10</td>
<td>Tannin WP 75%</td>
<td>20 kg</td>
<td>1.00 g</td>
</tr>
<tr>
<td>T12</td>
<td>Tannin WP 75%</td>
<td>25 kg</td>
<td>1.25 g</td>
</tr>
<tr>
<td>T15</td>
<td>Tannin WP 75%</td>
<td>30 kg</td>
<td>1.50 g</td>
</tr>
<tr>
<td>Fost</td>
<td>Fosthiazate 150 g L⁻¹</td>
<td>10.0 L</td>
<td>0.50 mL</td>
</tr>
<tr>
<td>Aba</td>
<td>Abamectin 20 g L⁻¹</td>
<td>5.0 L</td>
<td>0.25 mL</td>
</tr>
<tr>
<td>Garl</td>
<td>Allicin &lt; 1 ppm</td>
<td>8.0 L</td>
<td>0.25 mL</td>
</tr>
<tr>
<td>Cont</td>
<td>Water</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**IN VITRO EXPERIMENTS**

Second-stage juveniles (J2s) were obtained from an Italian population of *M. incognita* reared on tomato *Solanum lycopersicum* (L.) Karst. ex Fawr. cv. Naxos in the greenhouse of the University of Naples Federico II. The Meloidogyne species used in our experiments have been morphological and molecular identified previously (D’ERRICO et al., 2014). Egg masses from infested tomato roots were collected and hatched by tap water to obtain J2s to use in the experiments. Freshly hatched J2s (24 hours old) were used. The irreversibility or reversibility of J2s mobility was determined by rinsing and shifting immobile nematodes to distilled water (GIACOMETTI et al., 2010; D’ERRICO et al., 2017a; D’ERRICO et al., 2017b). Forty J2s were added to individual wells, each containing the solution under assess-
means were compared using Student-Newman-Keuls multiple comparison test. The level of significance was set at \( P < 0.05 \) in all the analyses.

**RESULTS**

**IN VITRO EXPERIMENTS**

The effect of each solution on J2's mobility is shown in Fig. I. Although the J2s mobility has been daily observed for 28 days, in Fig. I are reported only the most representative days. After 24 h, Fost demonstrated high nematicotoxic activity with 90% of nematode immobility, Aba 25% of nematode immobility and three concentrations of tannin extract (T30, T40, T50) immobilized 5% of J2s. Whereas no nematicidal activities in the other tannin extract (T75, T12 and T15) immobilized 5% of J2s.

Table 2 – Codes identifying pot treatments and effects of different concentrations of aqueous solutions of tannins and treated and untreated controls on the root-knot nematode *Meloidogyne incognita*.

<table>
<thead>
<tr>
<th>Treatment codes</th>
<th>Dose at transplant (kg/ha(^a))</th>
<th>Dose g/L (^b) (\text{H}_2\text{O})</th>
<th>Application time</th>
<th>Plant heights (cm)(^b)</th>
<th>Nematodes 10 cm(^2) soil(^c)</th>
<th>RGI(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T30</td>
<td>6 kg</td>
<td>0.30 g</td>
<td></td>
<td>45.69 a</td>
<td>71.38 b</td>
<td>607 a</td>
</tr>
<tr>
<td>T40</td>
<td>8 kg</td>
<td>0.40 g</td>
<td></td>
<td>47.81 a</td>
<td>71.56 b</td>
<td>595 a</td>
</tr>
<tr>
<td>T50</td>
<td>10 kg</td>
<td>0.50 g</td>
<td>at transplant</td>
<td>45.56 a</td>
<td>70.63 b</td>
<td>597 a</td>
</tr>
<tr>
<td>T75</td>
<td>15 kg</td>
<td>0.75 g</td>
<td></td>
<td>46.13 a</td>
<td>71.88 b</td>
<td>596 a</td>
</tr>
<tr>
<td>T10</td>
<td>20 kg</td>
<td>1.00 g</td>
<td></td>
<td>45.94 a</td>
<td>71.94 b</td>
<td>586 a</td>
</tr>
<tr>
<td>T12</td>
<td>25 kg</td>
<td>1.25 g</td>
<td></td>
<td>47.63 a</td>
<td>71.13 b</td>
<td>590 a</td>
</tr>
<tr>
<td>T15</td>
<td>30 kg</td>
<td>1.50 g</td>
<td></td>
<td>47.19 a</td>
<td>71.81 b</td>
<td>585 a</td>
</tr>
<tr>
<td>T30a</td>
<td>6 kg</td>
<td>0.30 g</td>
<td></td>
<td>46.31 a</td>
<td>75.56 ab</td>
<td>458 c</td>
</tr>
<tr>
<td>T40a</td>
<td>8 kg</td>
<td>0.40 g</td>
<td></td>
<td>46.25 a</td>
<td>76.75 b</td>
<td>454 c</td>
</tr>
<tr>
<td>T50a</td>
<td>10 kg</td>
<td>0.50 g</td>
<td></td>
<td>47.44 a</td>
<td>75.44 a</td>
<td>457 c</td>
</tr>
<tr>
<td>T75a</td>
<td>15 kg</td>
<td>0.75 g</td>
<td></td>
<td>48.38 a</td>
<td>77.56 ab</td>
<td>457 c</td>
</tr>
<tr>
<td>T10a</td>
<td>20 kg</td>
<td>1.00 g</td>
<td></td>
<td>48.44 a</td>
<td>78.38 ab</td>
<td>451 c</td>
</tr>
<tr>
<td>T12a</td>
<td>25 kg</td>
<td>1.25 g</td>
<td></td>
<td>48.19 a</td>
<td>79.38 ab</td>
<td>450 c</td>
</tr>
<tr>
<td>T15a</td>
<td>30 kg</td>
<td>1.50 g</td>
<td></td>
<td>47.88 a</td>
<td>80.06 ab</td>
<td>448 c</td>
</tr>
<tr>
<td>T10b</td>
<td>20 kg</td>
<td>1.00 g</td>
<td>at transplant</td>
<td>45.06 a</td>
<td>78.06 ab</td>
<td>511 b</td>
</tr>
<tr>
<td>T15b</td>
<td>30 kg</td>
<td>1.50 g</td>
<td>+ 2 weeks later (10 kg/ha(^a))</td>
<td>45.19 a</td>
<td>78.69 ab</td>
<td>500 b</td>
</tr>
<tr>
<td>Fost</td>
<td>10.0 L</td>
<td>0.50 mL</td>
<td>at transplant</td>
<td>46.50 a</td>
<td>89.75 a</td>
<td>371 d</td>
</tr>
<tr>
<td>Aba</td>
<td>5.0 L</td>
<td>0.25 mL</td>
<td>+ 2 weeks later (5 kg/ha(^a))</td>
<td>47.25 a</td>
<td>80.31 ab</td>
<td>381 d</td>
</tr>
<tr>
<td>Garl</td>
<td>5.0 L</td>
<td>0.25 mL</td>
<td>at transplant</td>
<td>46.25 a</td>
<td>80.00 ab</td>
<td>390 d</td>
</tr>
</tbody>
</table>

\(^a\) Plant heights were determined at 30 DAT and 60 DAT.

\(^b\) Final nematode population density of *Meloidogyne incognita* (Kofoid & White) Chitwood in 10 cm\(^2\) soil was extracted by cotton-wool filter method. Nematode root galling index (RGI) was obtained using a 0-10 scale where 0 = no galls and 10 = 100% of roots galled.

Data are arithmetic means of four replications and means separated with the Student-Newman-Keuls test (\( P < 0.05 \)).

In the subsequent experiment (Fig. II), the immobile J2s exposed to all the other concentrations of tannin extract were ≤ to 20%. The immobilization of J2s exposed to tannin extract increased over time and about 50% in T30, T75, T10, T12 and T15 solutions of J2s were immobilized within 10 days, except for T50 (45%). The best performance at 10 days was shown by T40 (70%). Overall, during 10 days of experiment, all the solutions tested were statistically different compared to the untreated control (water). However, the nematode mobility in tannin extract at different concentrations was still lower than all the other solutions. During the first two weeks, viability of juveniles was suppressed in Garl solution and tannic extract at the highest concentration (T15). All concentrations with tannins from 0.30 to 1.2 g L\(^{-1}\) showed a nematode immobility in the range of 60-70%. A clear response of J2s to tannin extract was established from 15 to 20 days and a significant paralysis of J2s (up to 80%) was evident after 20 days of exposure. At 25 days, all the J2s exposed to T30, T40, T50, T10 and T12 were immobilized. Results showed that the viability of *M. incognita* J2s was significantly reduced (\( P < 0.05 \)) over time by all concentrations of tannin extract throughout the experiment but slowly and in different ways. All the concentrations of tannin extract (0.30, 0.40, 0.50, 0.75, 1.00, 1.25, 1.50 g L\(^{-1}\)) had significant effects on J2 mobility compared to the water control. However, differences between some tannin extract concentrations were not found.

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In the subsequent experiment (Fig. II), the immobile
Fig. I – Percentage of immobile *Meloidogyne incognita* ([Kofoid & White] Chitwood) second-stage juveniles (J2s) after exposure to seven concentrations of tannin extract from 0.30 to 1.50 g L⁻¹ (T30, T40, T50, T75, T10, T25 and T15) and treated (Fost, Aba and Garl) and untreated (Cont, water) controls over time (days). Values are means of four replicates ± SD.

Fig. II – Cumulative percentage of mobile *Meloidogyne incognita* ([Kofoid & White] Chitwood) second-stage juveniles (J2s) incubated in distilled water after exposure to seven concentrations of tannin extract from 0.30 to 1.50 g L⁻¹ (T30, T40, T50, T75, T10, T25 and T15) and treated (Fost, Aba and Garl) and untreated (Cont, water) controls over time (days). Data are averages of four replicates.
nematodes recovered their motility in water. This recovery was observed until J2s death. Generally, the mobility of J2s was increased with exposure period to water. After exposure to different concentrations of tannin extract, mobile J2s were in the range of 21% for T15 to about 50% for T30 and T10 after ten days rinse in water and these latter two concentrations (T30 and T10) were the highest peaks of recovery reached. Overall, the recovery in water of J2s previously immobolized by different concentrations of tannin extract (T30, T40, T50, T75, T10 and T15) was delayed in comparison with Aba and Garl and there were still alive J2s during an observation period of 25 days but it was not statistically significant. J2s immobilized by Garl also recovered their motility in water until death at 15 days. Only 4% of J2s, pre-exposed to Fost, recovered their motility just for two days; also, J2s pre-exposed to Aba recovered their motility but this recovery was shorter (until 10 days) than J2s pre-exposed to tannin extract and garlic extract. However, all J2s tested died gradually in time. The low percentage of J2s died in the control was considered physiological.

**IN VIVO EXPERIMENTS**

All 20 treatments did not significantly increased plant heights compared to the untreated control at 30 DAT (Table 2). Whereas, plant heights measured at 60 DAT were statistically different among treatments. The treated plants belonging to the first group (T30, T40, T50, T75, T10, T25 and T15) were not statistically different within them and in comparison to the untreated control (water). Also the treated plants belonging to the second group (T30a, T40a, T50a, T75a, T10a, T25a and T15a) were not statistically different within them but only when compared to the untreated control. The same results were shown by plants belonging to the third group (T10b and T15b). So there were no statistically significant differences between the second and the third group when compared to Aba and Garl treated plants. The highest plants were recorded in pots treated with Fost.

Treatments with tannin extract applied only at transplant did not significantly reduced RGI in comparison to untreated control. The second group of tannin treated plants reduced RGI in comparison to untreated control, but RGI resulted significantly higher than that recorded in Fost and Aba treated plants. The plants treated with Garl had the same RGI recorded for the second group of plants treated with tannin extract. The third group of tannin treated plants (T10b and T15b) had the same RGI recorded for the first group of tannin treated plants and for untreated control. When tannin extract was applied only at transplant (first group) did not reduced the soil nematode population compared to the control. Applications of tannin extract at transplant and two weeks later (T10b and T15b) reduced the soil nematode population compared to the untreated control but less than the tannin extract treatments applied to the second group of plants and treated controls. However, the values of soil nematode population recorded in the second group of tannin treated plants were statistically higher than those in treated controls (Fost, Aba and Garl). No treatment showed any phytotoxic effects at the concentrations tested.

**CONCLUSIONS**

Phytochemicals can play an important role in the sustainable management of plant parasitic nematodes in organic and conventional systems (D’ADDABBO et al., 2014). The results of the present *in vitro* study revealed that the tannin extracts from *C. sativa* had effects on *M. incognita* J2s mobility. The immobility of nematodes increased with increasing concentration and longer exposure times. Likely, these factors were the main responsible for nematode control. The recovery of J2s mobility upon transfer to distilled water indicated that tannin extract caused, in the majority of cases, a reversible paralysis on *M. incognita*. However, although J2s may recover their mobility under laboratory conditions, likely they are too weak to locate host plant roots in the field (HADDOCK et al., 2004).

In the pot experiments the significant differences among the three application times demonstrated the positive effect of repeated tannin applications. Based on our results, the best treatment repetition regimen, irrespective of concentration, was the applications of tannin at transplant, 14 days after transplantation and then every 7 days, because we reached the highest nematicidal effect without phytotoxicity on tomato plants. Tannins applied once at transplant did not produce statistically different effects in comparison to the untreated control. However, repeated applications of tannins reduced disease parameters less than treated controls with exception of RGI in comparison to garlic extract. No effect of tannin extract on stimulation of tomato plant height was observed. Likely, the small differences detected on plant heights among treatments were due to the activity of nematodes that has contributed to malfunctioning of root system.

The tannins content in roots could be involved in passive plant defence working as chemical barriers in the roots for the nematode invasion (TAYLOR & MURANT, 1966). It has been recognized that tannins may be chemical signals that *Meloidogyne* species utilize to recognize plant hosts and locate areas for root penetration (OHIRI & PANNU, 2010). Application of tannins to soil, in pre-planting and at planting, could move up the feeding oriented-behavior of J2s and disorient them spreading “fake” chemical signals aimed to a reduction in the root searching efficiency of J2s that would either prevent or delay their attack; also post-planting applications, via drench or via drip irrigation, could have similar effects. According to other authors, we suppose that the observed nematostatic effect of tannins combined with their characteristics of attractants might represent a possible strategy to control RKN (HEWLETT et al., 1997; MAISTRELLO et al., 2010). Previous studies on the effects of tannins on plant parasitic nematodes reported a grave phytotoxicity to the host plant, instead we demonstrated that tannins at the tested concentrations are safe for tomato crop. However, the beneficial effect of natural phytochemicals is a promising area of nematode management (CHITWOOD, 2002) and tannins have great potential in environmentally friendly integrated pest management programs (ABID et al., 1997). In conclusion, the use of SaviotaN® appears promising for the control of *M. incognita* in sustainable agriculture. The best performance may be obtained with short-term crops, low nematode population densities and to supplement other control strategies such as conventional treatments. However, further information is needed to investigate the effect of tannins in field experiments using different nematode-crop combinations and soil types.

**REFERENCES**


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