Industrial Crops & Products 108 (2017) 195–200

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

Artemisia annua compounds have potential to manage root-knot and potato cyst nematodes

Trifone D'Addabbo[a,*], Maria Pia Argentieri[b], Vincenzo Radicci[a], Francesco Grassi[c], Pinarosa Avato[b]

[a] Institute for Sustainable Plant Protection, National Council of Research, Via Giovanni Amendola 122, Bari, 70126, Italy
[b] Department of Pharmacy – Drug Sciences, University of Bari Aldo Moro, Via Edoardo Orabona 4, 70125 Bari, Italy
[c] Council for Research and Experimentation in Agriculture – CAR, Via Gino Rizzo, 73047 Monteroni, Lecce, Italy

ARTICLE INFO

Keywords:
Artemisia annua
Root-knot nematodes
Globodera rostochiensis
Sustainable control

ABSTRACT

Previous *in vitro* investigation of the nematotoxic effect of *Artemisia annua* and its main phytochemicals have indicated that this plant can be a potential source of nematicidal products. Following this previous study, the objective of this work was to assess the nematicidal activity of soil treatments with meal and water extract from dry biomass of *Artemisia annua* on the root-knot nematodes *Meloidogyne incognita* and *M. hapla* and on the cyst nematode *Globodera rostochiensis* in experiments on potted tomato and potato, respectively. Meal was applied at 5, 10, 20 or 40 g kg^{-1} soil rates, whereas soil treatments with 25, 50, 100 and 250 g L^{-1} water solutions of the extract were applied either in a single application or splitted into four treatments. Gall formation and multiplication of both *M. incognita* and *M. hapla* on tomato roots were significantly reduced by all soil treatments with *A. annua* meals. Both single and splitted application of *A. annua* water extract effectively reduced *M. incognita* population on tomato roots and in the soil. In the experiment on potato, almost all rates of *A. annua* meal and extract significantly reduced soil population density of *G. rostochiensis*, without any statistical difference among the two applications. The nematicidal activity of *A. annua* products on root-knot nematodes resulted not statistically different or even higher than that of the nematicide fenamiphos. A significant increase of growth was always recorded on tomato and potato plants as a side effect of treatments with meal or water extract. Data from these experiments seem to indicate that products from *A. annua* can be potential candidates for the formulation of new nematicides suitable for a sustainable nematode management, though field trials are still needed for an effective demonstration of a commercial exploitation.

1. Introduction

Phytoparasitic nematodes are generally included among the difficult controlled pests (Perry and Moens, 2011). It has been estimated that plant parasitic nematodes cause about $157 billion global agricultural losses per year, most of which due to root-knot nematodes, *Meloidogyne* species, unanimously considered the most harmful nematodes because of their worldwide occurrence and heavy yield losses caused to a large range of crops (Abad et al., 2008; Wesemael et al., 2011). An economic significance can be assumed also by the potato cyst nematode, *Globodera rostochiensis* Wollenweber, due to the serious problems caused in the major potato (*Solanum tuberosum* L.) growing areas of the world (Turner and Evans, 1998).

Synthetic nematicides have been the primary method for nematode control throughout the past century, but the de-registration of the most hazardous chemical formulations has emphasized the need to investigate products less harmful to the environment such as plant-derived nematicidal formulations (D'Addabbo et al., 2014).

Industrial formulations of extracts and biomasses from plants belonging to a wide range of botanical families are increasingly evaluated for a sustainable phytoparasitic nematode management, due to their content of bioactive compounds with biocidal properties (Chitwood, 2002; Ntalli and Caboni, 2012).

Soil amendments with fresh or dry biomasses from biocidal plants and derived granular or pelleted formulations have been widely investigated for suppressiveness to phytoparasitic nematodes (Akhtar and Malik, 2000; Hynes and Boyetchko, 2006). Soil treatments with green or dry biomasses or seed meal of *Brassica* crops releasing nematotoxic volatiles represents a successful application of this technique (Avato et al., 2013). Soil amendments with saponin-rich dry top and root material from *Medicago sativa* L. were also reported to reduce soil population density of the root-knot nematode *M. incognita* Kofoid et White

* Corresponding author.
E-mail address: trifone.daddabbo@ipsp.cnr.it (T. D'Addabbo).

http://dx.doi.org/10.1016/j.indcrop.2017.06.025
Received 16 March 2017; Received in revised form 6 June 2017; Accepted 9 June 2017

(Chitw.) on tomato (*Lycopersicon esculentum* Mill.) and of cyst nematodes *G. rostochiensis* and *Heterodera carotae* Jones on potato and carrot (*Daucus carota* L.), respectively, either in pot experiments and in field trials (D'Addabbo et al., 2009, 2011).

The nematicidal activity of raw and formulated water extracts from a wide range of plants has been contrastingly documented. Treatments of soil infested by *M. incognita* with a cold water extract from plant biomass of African marigold (*Tagetes erecta* L.) effectively reduced root gall indices of tomato (Natarajan et al., 2006), as well as water extracts from different mint (*Mentha* spp.) species exhibited significant nematicidal activity against the same nematode species (Caboni et al., 2013). Waters extract from quillay (*Quillaja saponaria* Molina) bark have been industrially exploited since many years for the production of nematicide formulations effectively applied against a broad range of phytoparasitic nematodes (Giannakou, 2011). Adversely, a slight or poor nematicidal activity has been documented for the water extracts from other plants such as *Inula viscosa* and *Myrtus communis* (Oka et al., 2006, 2012). A positive influence on plant growth and crop yield has been also stated as a side effect of soil treatments with some biomass or water extracts from nematicidal plants (D'Addabbo et al., 2005, 2011; Natarajan et al., 2006), though phytotoxic effects of extracts from plants with a nematicidal potential are also documented (Zhang et al., 2014).

Sweet wormwood, *Artemisia annua* L. (Asteraceae), is an old Chinese herb traditionally used in medicine and industrially exploited for the antimalaric properties of artemisinin, its active metabolite (Li et al., 2000). In addition to conventional uses, *A. annua* seems to have a great potential of application for the production of new nematicidal products, due to the presence of a high number of bioactive phytochemicals, mainly sesquiterpene lactones and phenolic chlorogenic acids (Bhakuni et al., 2001; Tan et al., 1998; Carbonara et al., 2012; D'Addabbo et al., 2013), as well as to the production of a large plant biomass and to adaptability to poor soils of marginal areas not or scarcely suitable to agricultural crops.

Species from genus *Artemisia* have been acknowledged for a nematicidal activity on root-knot nematodes (Al-Banna et al., 2003; Dias et al., 2000; Korayem et al., 1993). Nematicidal properties of *A. annua* have been documented by *in vitro* studies on the toxicity of a water extract from the plant aerial parts and its main components, i.e. caffeic acid, chlorogenic acid, artemisinin on different nematode species (D'Addabbo et al., 2013; Pandey 1990; Shakil et al., 2004). Adversely, no data are available on the nematicidal activity of soil treatments with plant biomass or water extracts from *A. annua* and their influence on plant growth.

Therefore, the main objective of this study focused on the assessment of nematicidal effect of soil treatments with meal or water extract from dry biomass of *A. annua* on root-knot nematodes *M. incognita* Kofoid et White (Chitw.) and *M. hapla* Chitwood and on the cyst nematode *G. rostochiensis* reared on potted tomato and potato, respectively, as well as of their side effects on plant growth.

2. Materials and methods

2.1. Plant material

Aerial parts from plants of *A. annua* (cv. Cronos) cultivated under experimental field conditions at Monteroni (Lecce, Apulia, Italy) were harvested at the flowering stage, air dried at temperatures below 40 °C and then moderately fine pulverized for all the subsequent experiments.

2.2. Nematode inocula

The populations of *M. incognita* and *M. hapla* were recovered from infested roots of greenhouse tomato at Leverano (Lecce, Apulia, Italy) and parsley in field at Monopoli (Bari, Apulia, Italy), respectively. Both populations were then reared on tomato *cv.* Roma in glasshouse at 25 ± 2 °C and 20 ± 2 °C, respectively. Plants were uprooted after a 60-day maintenance and roots were finely chopped. Six 10-g samples were taken from root biomass infested by each *Meloidogyne* species and processed with a 1% sodium hypoclorite water solution (Hussey and Barker, 1973), in order to quantify eggs and juveniles density in preparation of the utilization of infested root biomass as inoculum. The inoculum for the experiment on potato was represented by cysts of *G. rostochiensis* recovered from a naturally infested soil at Polignano (Bari, Apulia, Italy) and analyzed for their average content of eggs and juveniles (J2) by counting under a light stereoscope.

2.3. Extract preparation and chemical analysis

Boiling distilled water was added to the plant material (25, 50, 100 or 200 g drug to 1 L water) and mixtures left standing for 24 h, manually stirred from time to time and then filtered through a conventional filter paper (Whatman grade 1) and freeze-stored until used.

HPLC/DAD/ELSD and ESI–MS/MS chemical analyses of the same extract were previously carried out (Carbonara et al., 2012), whereas in this study the extract was furtherly characterized for its composition in primary metabolites such as essential and non-essential amino acids and sugars by using ^{1}H NMR technique. Measurements were recorded at 25 °C on a 500 MHz Agilent-500 spectrometer, operating at a proton NMR frequency of 499.79 MHz. The sample was run in D_2O and acquired with the following parameters: 128 scans requiring 10 min and 26 s acquisition time; 0.10 Hz/point; pulse, (PW) = 30° (9 μs); RD, = 2 s. A presaturation sequence was used to suppress the residual H_2O signal.

2.4. Experiments in potting mixes

Steam sterilized sandy soil was added with chopped tomato roots infested by *M. incognita* or *M. hapla*, or with cysts of *G. rostochiensis* up to reach an initial population density of 20 eggs and *J2* mL^{-1} soil. In the first two experiments, soil infested with *M. incognita* or *M. hapla* was mixed with the *A. annua* meal at 5, 10, 20 or 40 g kg^{-1} soil rates and then poured into 1.2 L clay pots. In the following two experiments, soil infested with *M. incognita* or *G. rostochiensis* was amended with the same meal rates used in the previous experiments and then poured into 1.2 L clay pots, or was irrigated with 250 mL of 25, 50, 100 and 200 g L^{-1} water extracts after transferring in 1.2 L clay pots. Meal treatments were always applied three weeks before tomato transplanting or potato sowing, whereas the water extract was applied as soil drench either as a single treatment one week before tomato transplanting or potato sowing or as 4 weekly treatments with 1/4 of the full dosage starting from tomato transplant or potato sowing. Non-treated soil, either infested and non-infested, and soil treated with a granular formulation of nematicide fenamiphos (5% a.i.), broadcast on soil surface at the rate of 30 g m^{-2} c.p. one week before tomato transplanting or potato sowing were used as controls. Five pots were provided as replicates of each treatment in all the four experiments. Pots were arranged in a randomized block design on the benches of a greenhouse maintained at a constant 25 ± 2 °C (*M. incognita*) or 20 ± 2 °C (*M. hapla* and *G. rostochiensis*) temperature. In the experiments on *M. incognita* and *M. hapla*, a one-month-old seedling of tomato cv. San Marzano was transplanted in each pot, whereas a tuber of potato cv. Spunta was sown in pots with soil infested by *G. rostochiensis*. In all experiments, plants were regularly irrigated during the two-month permanence in greenhouse, but did not receive any fertilizer, as to evidenciate the growth effect of experimental treatments.

At the end of each experiment, plants were uprooted and fresh weight of aerial parts and roots of each plant was recorded. Gall formation on tomato roots infested by *M. incognita* or *M. hapla* was evaluated according to a 0–5 scale (0 = no galls, 1 = 1–2 galls, 2 = 3–10 galls, 3 = 11–30 galls, 4 = 31–100 galls and 5 > 100 galls) (Taylor and Sasser, 1978). Final population density of *M. incognita* was