Industrial Crops and Products 42 (2013) 447–453

Contents lists available at SciVerse ScienceDirect

Industrial Crops and Products

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

Nematicidal activities of *Cannabis sativa* L. and *Zanthoxylum alatum* Roxb. against *Meloidogyne incognita*

Tariq Mukhtar[a,*], Muhammad Zameer Kayani[b], Muhammad Arshad Hussain[c]

[a] *Department of Plant Pathology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan*
[b] *Green Belt Project, Department of Agriculture, Rawalpindi, Pakistan*
[c] *Plant Pathology Section, Regional Agricultural Research Institute, Bahawalpur, Pakistan*

ARTICLE INFO

Article history:
Received 28 March 2012
Received in revised form 12 June 2012
Accepted 17 June 2012

Keywords:
Root-knot nematodes
Hemp
Toothache tree
Ovicidal
Larvicidal
Infectivity

ABSTRACT

Because of being costly and pernicious to the environment and human health, the use of nematicides has become prohibitive in many countries and the management of plant parasitic nematodes using antagonistic plants can be a very attractive alternative. In the present studies the effectiveness of aqueous extracts of *Cannabis sativa* and *Zanthoxylum alatum* was assessed on hatching, mortality and infectivity of *Meloidogyne incognita* at different concentrations viz. S, S:1, S:5, S:10, S:25, S:50 and S:100. Both the plants had significant effects on juvenile mortality and hatching inhibition in a dose-dependent manner. Mortality and hatching inhibition caused by *C. sativa* were significantly higher than that of *Z. alatum*. Time duration also affected mortality and hatching inhibition significantly. Significant inhibition in invasion of *M. incognita* juveniles on cucumber cv. Royal Sluis was observed by different treatments with extracts. *M. incognita* juveniles exposed to 'S' extracts of *C. sativa* and *Z. alatum* for 24 and 48 h caused no infection. Exposure for 12 and 6 h caused more than 95 and 90% reductions in infectivity of *M. incognita* juveniles respectively. Similarly, soil drench and root dip treatments also caused significant reductions in infection. Reduction in infectivity was found to be significantly higher with extracts of *C. sativa* as compared to *Z. alatum* and decreased in a dose-responsive manner. The results of the studies showed that the extracts of test plants, commonly found locally, possess high potentials for the control of root-knot nematodes and could be the possible replacement for synthetic nematicides.

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1. Introduction

Root-knot nematodes (*Meloidogyne* spp.) are considered among the top five major plant pathogens and the first among 10 most important genera of plant parasitic nematodes in the world. The disease caused by this nematode is often the only, or one of the few, nematode diseases of crops known to farmers owing to its spectacular symptoms. They are worldwide in distribution, attacking a wide range of agricultural crops (Sasser and Freckman, 1987). In addition, these parasites interact with other disease causing organisms to produce disease complexes, break down resistance against other pathogens and reduce plant tolerance to environmental stress (Taylor, 1979).

Nematicides are man made chemicals which are released into the environment for the control of nematodes. Pesticides' residues, however, have been linked to cancer, liver diseases and hypertension and are a potential health hazard for farmers who handle them as well as for consumers of pesticide-treated plant products. The World Health Organization estimates that there are nearly 375,000 cases of poisoning each year in developing countries. The estimated number of fatalities from agrochemical exposure is at least 20,000 every year, the majority being in developing countries (WHO, 1990; Rosenstock et al., 1991; Pimental et al., 1992; Kishi et al., 1995). The nematicides are also reported to cause environmental pollution. Methyl bromide causes ozone depletion and has been banned. Chemicals are not only harmful to livestock and plants but also to beneficial fauna and flora of the soil. Thus, due to threats caused by the chemicals and removal of key nematicides from the market, alternatives involving the use of nematicidal and nematostatic plants are now increasingly being advocated for the control of plant parasitic nematodes. According to Quarles (1992) botanicals present some advantages over synthetic pesticides, such as: they can provide novel compounds that pests are not yet able to inactivate; they are less concentrated and thus potentially less toxic than pure compounds; they biodegrade rapidly, and may possess multiple modes of action making possible a wide spectrum of use while retaining a selective action within each pest class, and they are derived from renewable resources. Other advantages include cheapness and ready availability over the conventional nematicides. Their environmental safety in an environmentally

* Corresponding author. Tel.: +92 51 9290239; fax: +92 51 9290771.
E-mail address: drtmukhtar@uaar.edu.pk (T. Mukhtar).

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http://dx.doi.org/10.1016/j.indcrop.2012.06.027

conscious world also holds promise for their acceptability and use by resource-poor farmers.

Extracts of various plants have been found effective for the control of root-knot nematodes (Ahmad et al., 2004; Abo-Elyousr et al., 2010; Hussain et al., 2011a). Anthelmintic properties of *Cannabis sativa* L. and *Zanthoxylum alatum* Roxb. have also been reported. *C. sativa*, belonging to the family Cannabaceae, is a self-grown plant widely found in the Pothowar region of the country. Cannabis, also known as marijuana, has been applied in different ways and formulations to repel pests and as a pesticide. Its use as a companion crop averted many plant pathogens, weeds and insect pests. Use of dried flowers and leaves of cannabis has been very effective in killing or repelling plant pathogenic nematodes, mites, insect pests, weeds, etc. (Kayani et al., 2012). There are reports that extracts of cannabis significantly reduced populations of phytopathogenic nematodes, bacteria, fungi, protozoans, insects, mites and weeds. Similarly, pure cannabinoids, contained in cannabis have also killed or prevented insects, fungal and bacterial pathogens (McPartland, 1997).

Toothache tree (*Zanthoxylum alatum* Roxb.), a member of the family Rutaceae, grows widely in the hilly tracks of Pothowar. It is widely used in the native medical system for the relief of flatulence and works as a vermifuge and stomachic. The stick of the plant is effective in toothache. The seeds and fruits of the plant are effectual in fever, indigestion and roundworm expulsion (Mehta et al., 1981; Kalia et al., 1999). The objectives of the present studies were to assess the activities of aqueous extracts of *C. sativa* and *Z. alatum* on hatching, mortality and infectivity of *M. incognita* with a goal of identifying novel leads for nematicides or for use as soil amendments.

2. Materials and methods

2.1. Collection of plant material

The leaves of hemp (*C. sativa*) were collected from Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi and those of toothache tree (*Zanthoxylum alatum*) from Kotli Sattian, District Rawalpindi. The leaves were washed under tap water to remove dust particles.

2.2. Preparation of extracts

Twenty-five grams each of *C. sativa* and *Z. alatum* leaves was ground with 75 mL of distilled water in a warring blender, left for 12 h and filtered twice through Whatman filter paper No. 1. The extracts were then filtered through 0.2 μm Millipore filter. The extracts thus obtained were arbitrarily designated as standard 'S' and used as stock solutions. The subsequent concentrations viz. S:1, S:5, S:10, S:25, S:50 and S:100 were prepared by adding requisite amount of distilled water.

2.3. Multiplication of M. incognita

The nematode, *M. incognita*, used in the studies was obtained from a single egg mass and multiplied on a highly susceptible tomato cultivar (Money maker). Three-week-old tomato seedlings were transferred in earthen pots containing 4 kg of formalin sterilized sandy clay loam soil (sand, 63%; silt, 17%; clay, 19%; pH, 7.5; organic matter, 1.13%) and inoculated with about 5000 freshly hatched second stage juveniles (J2s) of *M. incognita* maintained on tomato cv. Money maker one week after transplantation. The inoculated plants were kept on the bench of the glass house of the department at 25 ± 2 °C and watered as needed.

2.4. Collection of juveniles

For collection of juveniles, eggs were extracted using sodium hypochlorite (Hussey and Barker, 1973). The eggs were then processed on extraction trays for emergence of second stage juveniles (Whitehead and Hemming, 1965). The freshly hatched juveniles were collected in a beaker, standardized and concentrated.

2.5. Collection of egg masses

M. incognita infected tomato plants were uprooted and gently washed under running tap water. Uniform-sized egg masses were collected using forceps. The eggs per egg mass were counted (Mukhtar and Pervaz, 2003).

2.6. Mortality bioassay

To see the effects of leaf extracts on the mortality of *M. incognita* J2s, 1 mL of extract from each concentration of both the plants was poured individually into 3.5-cm-dia. plastic plates and 50 μL of juvenile suspension containing about 80 freshly hatched juveniles of *M. incognita* was added. The plates containing distilled water served as control. There were five replications for each treatment. The plates were incubated at 25 ± 1 °C and mortality was recorded after 6, 12, 24, 48 and 72 h. Nematodes were considered alive if they moved or appeared as a winding shape (El-Rokiek and El-Nagdi, 2011) and were considered dead if they did not move when probed with a fine needle (Abbasi et al., 2008).

2.7. Hatching bioassay

For hatchability bioassay, 1 mL of leaf extract from each concentration was poured individually into 3.5-cm-dia. plastic plates. A single uniform-sized egg mass containing about 250 eggs was placed in each plate. The plates containing distilled water served as control. There were five replications for each treatment. The plates were incubated at 25 ± 1 °C. Hatching was recorded after 2, 4 and 6 days. After each count the egg masses were washed with 1 mL of sterilized distilled water in their respective plates and transferred to fresh extracts of the same concentration.

2.8. Infectivity bioassay

2.8.1. Experiment 1: infectivity bioassay using extract treated juveniles

In this experiment the infectivity bioassay was done using extract treated juveniles and there was no contact of the plants with the extracts. Freshly hatched second stage juveniles were treated with different concentrations of the extracts of both the plants for 6, 12, 24 and 48 h and washed with distilled water three times. Cucumber seeds (cv. Royal Sluis) were sown in 200 mL plastic cups filled with autoclaved soil (sand, 63%; silt, 17%; clay, 19%; pH, 7.5; organic matter, 1.13%). Seven days after emergence, the cucumber seedlings were inoculated with 300 juveniles treated with different concentrations of both the antagonistic plants by making holes around the stems of plants. Each treatment was replicated five times. The cucumber seedlings were placed in a growth chamber at 25 °C in a completely randomized design with 16 h photoperiod for one week. The seedlings were watered daily. After one week, the plants were carefully uprooted; their roots were washed free of soil and stained with acid fuchsin (Byrd et al., 1983). The nematodes were counted in the whole root system of each plant using a stereomicroscope.

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