Nematode Control by the Use of Antagonistic Plants

Dinka GRUBIŠIĆ [1](✉)
Gordana UROIĆ [1]
Arijana IVOŠEVIĆ [2]
Martina GRDIŠA [2]

Summary

Plant parasitic nematodes (PPN) cause significant economic damage to a wide range of crops. Although soil treatment with synthetic nematicides has given some protection and increased yields, many of them are being banned from the world market, because like other pesticides used in conventional agriculture, they might cause adverse damage to the agricultural ecosystem. Also, trends toward organic farming and sustainable agriculture continue to rise. Thus, the uses of alternative nematode management practices, which are safe and economically acceptable, are increasingly used. Among them, the use of antagonistic plants is a very attractive alternative. Some of antagonistic plants give the benefit of a green manure. Also, some nematocidal substances have been isolated from them and have drawn the attention of the pesticide industry. The use and the effect of the most investigated antagonistic plants like marigold (*Tagetes* spp.), neem (*Azadirachta indica* A. Juss.), species from the genus *Brassica*, *Crotolaria*, *Mucuna*, etc. are presented in this review. It is necessary to continue the discovery of the new effective nematode antagonists and to develop techniques for their more efficient utilization. Furthermore, it is important to explore other benefits of antagonistic plants that would stimulate their cultivation by the farmers.

Key words

antagonistic plants, brassicas, marigold, neem, nematode control, plant parasitic nematodes

[1] University of Zagreb, Faculty of Agriculture, Department of Agricultural Zoology, Svetošimunska cesta 25, 10000 Zagreb, Croatia
✉ e-mail: djelinic@agr.hr
[2] University of Zagreb, Faculty of Agriculture, Department of Seed Science and Technology, Svetošimunska cesta 25, 10000 Zagreb, Croatia

Received: June 18, 2018 · Accepted: July 23, 2018

Introduction

Plant parasitic nematodes (PPN) cause significant economic damage to a wide variety of crops worldwide. After crops are infected with nematodes, crop yield and quality are reduced, either directly from root or aerial plant parts deformation caused by nematode feeding, or indirectly from infection by other pathogens that results from nematode damage (Wang et al., 2007). PPN are usually controlled by using nematicides, resistant varieties and crop rotation. Additional methods used for managing nematodes in agriculture include soil solarization, and the use of organic amendments, trap crops, microbial biocontrol agents and plants that are antagonistic to PPN. Like the most of pesticides, nematicides could be dangerous to the human health, environment, wildlife, beneficial organisms, and also may induce selection of resistant PPN populations. There is an increasing interest in environmentally friendly tactics for nematode control, particularly as fumigants as the other chemical nematicides are becoming more limited (Schneider et al., 2003). Because of the high costs of nematicides, their use, especially in developing countries, is limited and therefore the producers must rely on non-chemical methods for pest control. Some cultural practices and the use of antagonistic plants for control of nematodes are a promising alternative (Ferraz and Grassi de Freitas, 2004).

Plants considered to be antagonistic are those that negatively affect the population of nematodes, like trap plants, unfavorable hosts and those containing nematicide/nematostatic compounds in their tissues, which can be released into the external environment or act only within the plant (Ferraz and Valle, 1997 cited by Moreira et al., 2015). These plants not only serve to control the infestation of PPN, but can also be used as green cover, organic matter, or for improving the general quality of the soil (Moreira et al., 2015). Planting of some legumes as green manure results in reduction of nematode population density and fixation of the nitrogen from the atmosphere into soil, improving soil fertility (Ferraz and Grassi de Freitas, 2004). Some cover crops, when incorporated, bring organic material to the soil and augment the activity of antagonistic fungi (Ferraz and Grassi de Freitas, 2004). There is also an interesting effect of some antagonistic plants on soil organisms. Bacterial isolates from rhizosphere (rhizobacteria) of some plant species (e. g. *Mucuna deeringiana* /Bort./ Merr., *Ricinus communis* L., *Canavalia ensiformis* /L./ DC and *Secale cereale* L.) demonstrate an antagonism towards PPN and differ from the rhizobacteria isolated from soybean (Kloepper et al., 1991 cited by Ferraz and Grasside Freitas, 2004). These rhizobacteria isolated from antagonistic plants significantly reduced the incidence of *Meloidogyne incognita* and *Heterodera glycines* in soybean plants when compared to the bacteria isolated from soybean roots. The results indicate that rhizospheres of antagonistic plants may be useful sources of biological control agents for PPN (Kloepper et al., 1992 cited by Ferraz and Grassi de Freitas, 2004).

There are many nematocidal substances that can be isolated from antagonistic plants. These botanical extracts have some advantages over synthetic pesticides; 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 with a wide spectrum of use while retaining a selective action within each pest class. Furthermore, they are derived from renewable resources (Quarles, 1992). Some of the main species of antagonistic plants and their use in plant protection are presented in more detail.

Tagetes spp.

The genus *Tagetes* (marigold), family *Asteraceae*, contains 56 species, of which only six annuals and three perennials are currently cultivated (Ferraz and Grassi de Freitas, 2004). The most commonly cultivated as ornamentals throughout the world are *Tagetes erecta* L., *Tagetes patula* L., *Tagetes lunulata* Ort and *Tagetes tenuifolia* Cav. (Soule, 1996). Marigold may reduce population of PPN by multiple mechanisms of action, by acting as a non–host or a poor host, producing allelopathic compounds that are toxic or inhibit PPN development by creating an environment that favors nematode antagonistic flora or fauna (Wang et al., 2001) or by behaving as a trap crop (Pudasain et al., 2008). Marigold roots release the chemical compound alpha-terthienyl. Alpha-terthienyl has nematocidal, insecticidal, antiviral and cytotoxic properties and it is one of the most toxic naturally occurring compound found to date (Gommers and Bakker, 1988). However, in the field, it is not clear whether marigolds inhibit development of nematodes because of the alpha-terthienyl itself or because they are non-host for certain nematodes. Contrary to the belief that nematodes are damaged and killed outside the roots, some authors claim that the effect appears inside the roots, which was correlated with the strongly nematocidal tiophenes, present in plants and rarely elsewhere in the nature (Suatmadji, 1969). Measurements of the size and activity of the microbial population in soils cropped with marigolds in the field and in the greenhouse, showed that they were not depleted and that it is unlikely that the nematode control by this plant is due to the release into the soil of biocidal agent (Topp et al., 1998 cited by Ferraz and Grassi de Freitas, 2004).

Most of the results indicate that *Tagetes* spp. are very efficient in nematode control, especially for *Pratylenchus* and *Meloidogyne* species. However, different species and varieties of *Tagetes* may present distinct reaction to the same nematode and different nematode populations and may also present different behavior when challenging the same *Tagetes* varieties (Ferraz and Grassi de Freitas, 2004). Despite some variation in marigold effectiveness among cultivars, protector crop, nematode species, and temperature ranges, several studies have shown that in some instances using marigold resulted in more effective nematode control than some fumigants and chemical nematicides (Hooks et al., 2010). Evenhuis et al. (2004) hypothesized that marigold controlled nematode populations to greater depths than the soil fumigant and that marigold debris remained in the soil after the rotation continue to exert its toxic effect on *Pratylenchus penetrans* over time. For that purpose, marigold can also be incorporated as a green manure (Siddiqui and Alm, 1987b cited by Hooks et al., 2010) and applied as a plant extract similar as nematicides (Mateeva and Ivanova, 2000 cited by Hooks et al., 2010). Crop rotation with marigolds has been found to have similar effects as growing a non – host crop in reducing PPN (Hooks et al., 2010).

The most frequently used species of the genus are *T. patula, T. erecta* and *T. minuta.*, however, *T. patula* usually provides better nematode control then the other species. In general, they are used in crop rotation but in many situations, intercropping is very effective (Ferraz and Grassi de Freitas, 2004). *T. patula* suppressed *P. penetrans* and *Pratylenchus pratensis* (Oostenbrink, 1960 cited by Hooks et al., 2010; Conijn et al., 1996) and the four *Meloidogyne* species; *Meloidogyne arenaria*, *Meloidogyne incognita*, *Meloidogyne javanica*, and *Meloidogyne hapla* (Suatmadji, 1969). *T. erecta* also suppressed *M. arenaria*, *M. incognita*, and *M. javanica*, but not *M.*

hapla (Suatmadji, 1969). *Meloidogyne* spp. juveniles are not able to fully develop in the roots of *T. erecta* (Ploeg and Maris, 1999). Several studies found that *T. erecta* acted as a trap crop by arresting the development of *Meloidogyne* spp. juveniles [(Daulton and Curtis, 1963; Rangaswamy et al., 1993; Ploeg and Maris, 1999b) cited by Hooks et al., 2010]. Abid and Maqbool (1990) reported that the number of root galls due to *M. javanica* in tomato plants grown side by side with *T. erecta* was significantly lower when compared to tomato grown alone. Also, root length, shoot weight and number and weight of fruits were higher in plants grown with *Tagetes*. Intercropping *T. erecta* and eggplant in *M. javanica* infested soils resulted in better growth of eggplant and reduction of the final nematode population up to 40% (Dhangar et al., 1995 cited by Ferraz and Grassi de Freitas, 2004). Soil populations of *M. incognita*, *M. javanica* and *Meloidogyne acrita* were also reduced and yield of eggplant increased when *T. patula* was planted within or between the rows of eggplant (Varma et al., 1978).

In the investigation conducted by Suatmadji (1969), when exposed to *M. hapla*, *T. patula* developed few or very small giant cells and galls, the nematode often died, and giant cells often degenerated. *M. hapla* survived for four weeks in the roots, but the development beyond the second-stage juvenile (J2) in *T. patula* and *T. minuta* was hardly noticeable, and only few J2 became adults in *T. erecta*. Belcher and Hussey (1977 cited by Ferraz and Grassi de Freitas 2004) found that *T. patula* acted as a trap crop to *M. incognita* as it allowed nematode penetration, but giant cell formation was not initiated and juveniles did not develop beyond the early second stage. A hypersensitive necrotic reaction was observed where the juvenile had attempted to feed. Similar observations were made by Rangaswamy et al. (1993) and Motsinger et al. (1977) who also noticed that some varieties of *T. patula* act as trap crops for *Meloidogyne* spp. Trap crop mechanisms operate mainly against endoparasitic nematodes, which may partially explain more consistent activity of marigold against endoparasitic than ectoparasitic nematodes (Hooks et al., 2010).

Planting marigold offers an alternative to methyl bromide fumigation if integrated with other cultural, chemical and biological tactics (Noling and Becker, 1994). Soil solarization can be efficiently integrated with marigold. Nematodes weakened by sublethal heat may be controlled better by marigold and the mortality caused by using both cultural methods could be increased more than by applying either method alone (Hooks et al., 2010). Planting of marigold also has some limitations. Ploeg and Maris (1999) found that impact of soil temperature on the ability of *Tagetes* to suppress nematode differs among *Tagetes* cultivars. Pudasini et al. (2008) proposed that *P. penetrans* population declined at a slower rate during the last three weeks of the marigold growth because its nematocidal effect reduces as it senesces. For that reason, the decision on planting date of various marigold cultivars could be of great significance. Future research should focus on searching for marigold with a longer vegetative stage which may also reduce the loss of nematocidal effect of aged marigold (Pudasini et al., 2008). In this way problem of marigold self-seeding and thus becoming a weed in the next cash crop could be diminished. Also, marigold may compete for water and nutrients with cash crop when used as intercrop, resulting in yield reductions (Miller and Aherens, 1969 cited by Hooks et al., 2010). These authors suggested interplanting marigolds when crop plants achieve dominance and are no longer sensitive to plant competition. Natarajan et al. (2006) suggested applying extracts from *T. erecta* to prevent farmers from compromising yield loss due to competition. Because marigold is mostly marketed as an ornamental plant, seeds are costly compared to cover crop seeds, so despite its positive effect in PPN suppression, the integration of marigolds into nematode pest management programs is practically nonexistent in commercial farm operations. However, if more marigold seeds were to be commercialized for cover cropping, seed costs would be probably more affordable (Hooks et al., 2010).

Some unknown marigold species may be a good host for ring (*Criconemoides mutabile*), stubby – root (*Paratrichodorus teres*), sheath (*Hemicycliophora similis*), reniform (*Rotylenchulus robustus*) and pin (*Paratylenchus sp.*) nematodes (Lehman, 1979). Wang et al. (2003a cited by Hook et al., 2010) reported that *T. erecta* was a good host for reniform nematode. Mentioned data should be considered while planning the use of marigold in PPN control.

Azadirahta indica A. Juss

Neem (*Azadirachta indica* A. Juss.) is known as a 'wonder tree' due to its many uses in medicine, agriculture, industry, as a shade tree, firewood, etc. (Ferraz and Grassi de Freitas, 2004). It has been found that neem materials can affect more than 400 insect pests [(National Research Council, 1992; Siddiqui and Alam, 1993; Kumar et al., 1996; Martinez, 2002; Mourão et al., 2004) cited by Silva et al., 2008], mites, nematodes, fungi, bacteria and even few viruses (Ferraz and Grassi de Freitas, 2004) and snails (Mostafa and Abdel-Megeed, 1996 cited by Khalil, 2013). Its vegetative parts reduce populations of several nematode species that attack soybean [(Vyas, 1993; Gupta et al., 1993; Khurma and Singh, 1997; Akhtar and Akhtar, 2000) cited by Silva et al., 2008] and several other plants, i.e., tomato (Rossner and Zebitz, 1986 cited by Silva et al., 2008), okra (Rao et al., 1997 cited by Silva et al., 2008), cowpea (Mojumder and Mittal, 2003 cited by Silva et al., 2008), pigeon pea (Nageswari and Mishra, 2001, 2005 cited by Silva et al., 2008), and rice (Prasad et al., 2005 cited by Silva et al., 2008). Bioactive products from neem tree are effective in controlling approximately 16 species of PPN (Akhatar and Alam, 1993). The nematocidal effect of neem is supposed to be related to the naturally occurring chemicals, e.g. azadirachtin, nimbin, salannin, nimbidin, kaempferol, thionemone, quercetin and others (Ferraz and Grassi de Freitas, 2004). Active neem constituents can be absorbed through plant roots and systemically moved upward through the plant's xylem tissues [(Gill and Lewis, 1971; Larew, 1988; Osman and Port, 1990; Nisbet et al., 1993) cited by Khalil, 2013], and therefore they could be used to manage PPN through soil application, especially against plants' root feeders.

Neem has been tested in many ways to control PPN. These include mulching with fresh or dried leaves, usage of leaf extracts as soil amendment, application of root exudates, seed coating with neem extract or oil, seed or kernel powder used for soil treatment or seed coat, root dipping in neem leaf extracts (Ferraz and Grassi de Freitas, 2004), as saw dust (Akhtar, 1998), application as a drench into the soil (Trifonova and Atanasov, 2011).

The results of Trifonova and Atanasov (2011) showed that application of the 0.5 % neem oil and NeemAzal® (0.3%) caused significant inhibitory effect on the multiplication of *Globodera rostochiensis* and decreased reproduction rate to 66.6%. The best reduction of 77-78% in population density was obtained by the application of neem oil in combination with plant extracts of

Nicotiana tabacum L. (0.5%) and *Veratrum album* L. (1.0%) treatments. For the best effect in the management of the control of potato cyst nematodes it is necessary to apply plant extracts in the hatching time of the juveniles. Bare-root dip treatment with neem oil was effective in reducing population density of *M. incognita* on tomato (Akhtar and Mahmood, 1993). According to Akhtar and Alam (1991), Khurma and Singh (1997) and Upadhyay et al. (2003) the seed and leaf extracts of neem caused 100% juvenile mortality of the root-knot nematodes and some free-living nematodes on potato. In investigation of Moreira et al. (2015), neem (but also *Crotolaria*, *Mucuna*, *Sorghum* and *Guandu* bean) was incorporated as fresh plant in soil with tomato plants infested by *M. javanica* and *Meloidogyne enterolobii*. The most promising results were observed in the neem and sorghum plants. Atungwu et al. (2009) evaluated the efficacy of neem leaf powder and organic fertilizer for management of *M. incognita* in soybean under the laboratory conditions. Neem leaf powder applied alone gave the highest reduction in gall formation (92.59 %) and nematode multiplication (82.07 %), followed by the combination of neem leaf powder and organic based fertilizer, and organic based fertilizer applied alone. Increases in grain yield ranged from 103.08 to 108.46 % in response to organic soil amendment. Various products prepared from neem such as leaf powder, sawdust and oilseed cake were tested for their activities against PPN (*Hoplolaimus indicus*, *Helicotylenchus indicus*, *Rotylenchulus reniformis* and *M. incognita* juveniles), a predatory nematode (*Dorylaimus elongatus*) and free-living nematodes in the chickpea (*Cicer arietinum* L.) field. Soil-amendments with these materials resulted in a significant decrease of PPN relative to control plots. In contrast, populations of predatory and free-living nematodes increased. Oilseed cake was the most effective, though all the neem products and urea markedly suppressed PPN. All the treatments resulted in increased fresh and dry weights, the height and the number of pods on chickpea plants (Akhtar, 1998). Extract of neem seed was used to control the root knot nematode *M. javanica* of sweet gourd and was found to be lethal to juvenile stage compared to the extracts of bark and neem leaf (Yasmin et al., 2003). Pot experiment with all the extracts in both side drench and root-dipping methods appeared to give significant suppression in root galling L_2 and L_3 populations of the nematode. Sivakumar and Gunasekaran (2011) investigated management of root knot nematodes in tomato, chilly and eggplant with neem oil formulations. Their results clearly indicated that the neem oil formulation NO 60EC (C) as seed dressing and seedling bare root dip had significantly reduced the root galling by *M. incognita,* by recording the lowest root knot index of 2.0, lowest soil population of nematodes and the highest eggplant fruit yield. The formulation NO 60EC (C) was found to be the best against *M. incognita* in all the three crops tested under field condition. However, the treatments were on par with Carbofuran 3G at 1.0 kg a.i./ ha as soil application.

Comparing the cost factor and environmental hazards the neem oil formulation is the most suitable and ideal method of nematode management. Kumar and Khanna (2006) conducted investigation under glass-house conditions in order to test the effects of five neem-based nematicide products in the form of drenches on the growth of tomato and population density of *M. incognita,* which is the root-knot nematode. All the tested formulations suppressed nematode multiplication and root galling severity significantly and improved plant growth. At low concentrations the most effective formulations against the root-knot nematode were neem seed kernel extracts and Econeem. At the highest concentration of 1.0% all the neem formulations were equally effective. Neem seed kernel extracts drench produced the best plant growth, closely followed by Econeem. Javed et al. (2008) investigated the effects of neem formulations applied as soil drenching on the development of second stage juveniles (J2) of *M. javanica* on roots of susceptible tomato cv. Tiny-Tim controlled environment. Seven days after the transplant, three neem formations (neem cake, Aza 5 mg and 10 mg) were drenched at 10 ml per pot. At 10 mg Aza was found the most effective in protecting the roots against nematode infection. Suneem (azadirachtin 80% a.i.) was used to coat tomato seeds for protection against *M. incognita* that resulted in reduced number of *M. incognita* juveniles and root galls (Akhtar and Mahmood, 1997). Khan et al. (2012) recorded that incorporation of dry neem leaves in the soil integrated in treatment with *Paecilomyces lilacinus* not only completely eliminated the suppressive effect of the nematodes but also induced an additional increase of 5-6% in the yield of eggplant.

There are many neem based pesticidal formulations on the market (Margosan – O, Nimbecidine, Neemgold, Neemazal, Neemax, Fortune Aza, Neemix, Achook, Neemrich, Neemark, Econeem, Rakshak, Repelin, Welgrow, Azatin, Turplex, Align, Bioneem, Benefit, etc.) which are mainly used as insecticides, but some of these products have also shown good nematocidal properties (Ferraz and Grassi de Freitas, 2004). Azadirachtin is considered the only relatively safe pesticide, which would not cause environmental risk, and would not cause an ecological problem in the microbial community in soil (Kizilkaya et al., 2012). The potential for mobility of formulated azadirachtin in soil is very low and the accumulation in the environment is not expected (Sadre, 1983). Azadirachtin has been found to degrade rapidly due to environmental factors such as UV radiation in sunlight, heat, air moisture, acidity and enzymes present in foliar surfaces (Khalil, 2013). It was reported that the formulated azadirachtin breaks down rapidly in 100 hours in water or light, and does not cause long-term effects (Sadre, 1983). Azadirachtin has also been reported to be non-mutagenic and it appears to have no apparent mammalian toxicity (Khalil, 2013). A study clarified that pure azadirachtin is not toxic to humans (Beard, 1989 cited by Khalil, 2013), while a more recent study estimates that at least 15 mg of azadirachtin per kg of body weight could be taken safely by humans each day, which is well within the range for use as a pesticide (Boeke et al., 2004 cited by Khalil, 2013). Neem products are safe for spiders, adults of numerous beneficial insects and eggs of many predators (Coccinellids) and due to their relative selectivity it can be recommended for use in programs of integrated pest management (Schmutterer, 1997).

Brassica spp.

All *Brassica* spp. have shown to release bio-toxic compounds or metabolic byproducts that exhibit broad activity against bacteria, fungi, insects (Bohinc et al., 2013; Laznik et al., 2014), nematodes and weeds. Brassicas produce glucosinolates which are b-D-thioglucosides, distinguished from one another by differences in their organic side chains (R groups). Glucosinolates, classified as aliphatic, aromatic or indole forms, occur in all plant parts and degrade via enzymatic hydrolysis. As a result of tissue damage, the relatively non-reactive glucosinolates react with myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1), which is stored separately in the cell, to yield nitriles, epithionitriles, thiocyanates and isothiocyanates

(ITCs) (Brown and Morra, 1997; Fahey et al., 2001). ITCs are highly toxic compounds of varying volatility. They are general biocides whose activity results from irreversible interactions with proteins (Brown and Morra, 1997). Chen et al. (2007) also state that pest suppression is believed to be the result of glucosinolate degradation into biologically active sulfur containing compounds - thiocyanates. Brassicas cover crops are often mowed and incorporated to maximize their natural fumigant potential. This is because the fumigant chemicals are produced only when individual plant cells are ruptured (Chen et al., 2007). The biotoxic activity of brassicas and mustard cover crops is low compared to the activity of commercial fumigants. It varies depending on species, planting date, growth stage, climate and tillage systems. To maximize pest suppression, incorporation should occur during vulnerable life stages of the pest (Chen et al., 2007).

Nematocidal brassicas can accumulate the majority of glucosinolates either in the root system (catch effect) or in the stems and leaves (biofumigant effect). The first process is the most suitable to control cyst nematodes. Brassicas catch crops attract the juvenile stages of endoparasitic nematodes working as a trap, since these, after root penetration, are poisoned by hydrolysis products and are not successful in completing their developmental cycle in 10–12 weeks, during the intercropping time. Consequently, the nematode population in soil progressively decreases. At full flowering the plants are chopped and immediately incorporated at around 20 cm depth. A light irrigation sprinkled after incorporation in soil, aims at promoting the glucosinolate hydrolysis and the subsequent isothiocyanate release (Lazzeri et al., 2004). The nematocidal effect of a catch crop is produced during the whole cultivation time, while its incorporation as a green manure shows an overall amending effect, increasing the organic matter amount and improving soil fertility. The biofumigant effect during incorporation is only secondary (Curto, 2008).

Jagodič et al. (2017) investigated the chemotactic response of infective juveniles (IJs) of the entomopathogenic nematodes (EPNs) *Steinernema feltiae, Steinernema kraussei, Heterorhabditis bacteriophora, and Steinernema carpocapsae,* to the synthetic volatiles (e. g. dimethyl sulfide, dimethyl disulfide, allyl isothiocyanate, etc.) typically emitted by damaged *Brassica nigra* roots. The temperature was the main factor influencing the movement of different EPNs species towards volatile compounds tested. All the tested compounds efficiently repelled *S. kraussei* suggesting that sulfur compounds and glucosinolate breakdown products could play an important role in EPNs navigation.

In Wyoming, oilseed radish (*Raphanus sativus* L.) and yellow mustard (*Sinapsis alba* L.) reduced the sugar beet cyst nematode populations by 19-75%, with greater suppression related to greater amount of cover crop biomass. Curto (2008) demonstrated that in Italy the only method suitable to reveal heavy nematode infestations with more than 300–400 eggs - J2, is excluding the sugar beet cultivation and sowing biocidal brassicas intercrops for soil recovery. In Maryland (USA), rapeseed, forage radish and a mustard blend did not significantly reduce incidence of soybean cyst nematode. In a series of studies (Chen et al., 2007) rapeseed, arugula and mustard were studied as alternatives to fumigation in the control of *Meloidogyne chitwoodi* in potatoes. The brassicas cover crops were usually planted in late summer (August) or early fall and incorporated in spring before planting mustard. Results were promising, with nematodes reduced up to 80%, but because of the very low damage threshold, green manures alone cannot be recommended for adequate control of *M. chitwoodi* in potatoes. The current recommended alternative to fumigation is the use of rapeseed or mustard cover crop plus the application of MOCAP. This regimen costs about the same as fumigation.

The viability of potato cyst nematode (PCN) populations (*Globodera pallida*) was evaluated in three field experiments using *Brassica juncea* (L.) Czern., *Raphanus sativus* L, and *Eruca sativa* Mill. amendments (Ngala et al., 2015). Following cover crop incorporation, field plots were planted with susceptible potatoes to evaluate the biofumigation effects on PCN reproduction. In experiment where these species were summer cultivated and autumn incorporated, PCN population in post-potato harvest was reduced ($P = 0.03$) in *B. juncea* treated plots, while *R. sativus* prevented further multiplication; in experiment where overwintered brassicas cover crops were spring incorporated, there was no significant effect on PCN reproduction. In experiment with either *B. juncea* or *R. sativus* untreated or treated with metconazole, PCN populations were reduced. Rahman and Somers (2005 cited by Penfold and Collins, 2012) investigated the capacity of brassica plants and their associated seed meals to reduce soil nematode populations in a badly infested vineyard. In a 3-year-old vineyard, they found that the 'Nemfix' cultivar of *B. juncea* L., grown in the mid-row and then side-thrown under the vine, provided a 13-fold reduction in root-knot nematode populations in the vine row, 36 weeks after treatment. Rahman et al. (2011 cited by Penfold and Collins, 2012) conducted a pot experiment over three continuous years, and found that in the third year, 'Nemfix' green manure applied to vines inoculated with *M. javanica* increased yield by 69–101%, compared to the unamended control. Vanstone and Lantzke (2006 cited by Penfold and Collins, 2012) suggest that green manure cover crops need to be covered with soil to be effective. The above-ground biomass must therefore be mown and thrown onto the vine row and covered with soil. It was recommended that such treatments should be applied in consecutive years to have a long-lasting impact on nematode populations. It was also stated that biofumigation would not be as effective in older vines, where female nematodes and their egg masses could be safely buried within root tissues (Penfold and Collins, 2012). Brassica cover crops are a good tool and an excellent rotation crops, but pest management results are inconsistent from year to year and in different geographic regions. Different species and varieties contain different amounts of bioactive chemicals. More research is needed to further clarify the variables affecting the release and toxicity of the chemical compounds involved (Chen et al., 2007).

Other antagonistic plant species

The genus *Mucuna* (velvet bean) comprises more than 100 species. The genus is well known for its nematocidal effects, although itself it is not immune to a number of nematode species and the mode of action on nematodes is not clear yet (Ferraz and Grassi de Freitas, 2004). In the experiment of Asmus and Ferraz (1988) 65% reduction of *M. javanica* juveniles was determined in field where *Mucuna atterima* (Piper & Tracy) Holland was cultivated for 100 days and incorporated into soil, while 200% increase in the nematode population occurred after tomato was cultivated instead of *M. atterima*.

Some species of the genus *Crotalaria* are recommended to be included in crop rotation to control nematodes. Villar and

Zavaleta-Mejía (1990) found that soil incorporation of *Crotalaria longirostrata* Hook. & Arn residues (due to toxic compounds in the plant tissues) was more effective in control of *M. incognita* and *M. arenaria* than the intercropping with tomato under laboratory conditions, while the root exudates of *C. longirostrata* were found to be nematocidal to *Meloidogyne* spp. juveniles. *Crotalaria* spp. are also highly resistant to *Pratylenchus brachyurus*, *Pratylenchus zeae* and *Rotylenchulus reniformis* (Silva et al., 1989b, 1989c cited by Ferraz and Grassi de Freitas, 2004).

Some grass species (family *Poaceae*) have shown antagonistic effect against PPN. They fit in rotation for annual crops and can be interplanted as cover crops in perennials (Ferraz and Grassi de Freitas, 2004). Rodriguez-Kabana et al. (1988a, b, 1989a, 1992 cited by Ferraz and Grassi de Freitas, 2004) reported that sesame (*Sesamum indicum* L., fam. *Pedaliaceae*), in rotation with peanut or soybean provided efficient control of *M. arenaria*, *M. incognita* and *Heterodera glycines*. Extracts of leaf, shoot and root; dried root powder and essential oils of basils (*Ocimum* spp, fam. *Lamiaceae*), mainly *Ocimum sanctum* L., *Ocimum basilicum* L. and *Ocimum americanum* L. have shown strong nematocidal properties (Ferraz and Grassi de Freitas, 2004). There are also many other plants with nematocidal properties like *Ruta graveolens* L. (family *Rutaceae*), *Datura metel* L. and *Datura stramonium* L. (family *Solanaceae*), *Chenopodium* L. spp. (family *Chenopodiaceae*), *Calotropis procera* (Aiton) W. T. Aiton (family *Asclepiadacae*), *Artemisia* L. spp. (family *Asteraceae*) and many other which were applied in the control of many important PPN (Ferraz and Grassi de Freitas, 2004).

Natural nematicides may also be found under the sea. Twelve seaweed species were screened against *M. javanica* (Atta-Ur et al., 1997). Exposure to crude extracts of *Jolina laminarioides*, *Cystoseira trinodis* and *Zoanthid* species caused over 50% mortality of *M. javanica*. Zoanthamine, a white crystalline compound isolated from *Zoanthid* species, was effective against the juveniles (Atta-Ur et al., 1997).

Conclusion

Although benefits of growing antagonistic plants exist, including soil improvement and lessening of pests and diseases, in some cases no yield increase and income improvements are recorded, while there is some increase in the production cost. In order to make the management of PPN by antagonistic plants more acceptable, discovery of new effective nematode antagonists and the development of new, stable and low cost nematocidal or nematostatic formulations is essential. Also, the investigations and development of techniques for improved utilization and application of antagonistic plants or their active components is of great importance. Thus, techniques of application like bare root dipping in plant extracts, seed dressing or association of antagonistic plants with nematophagous fungi were explored in the last decade. Since the most antagonistic plant species are not cash crops it is also desirable to find additional applications for the selected antagonist as compensation for the expenses to grow them and to make this method more interesting to farmers. Although the use of antagonist plants, as a sound agronomic conservationist practice is not the first farmer's thought, it is also important to increase the number and in-depth evaluation studies of potential and known antagonists, on to what extent their use is safe in regard to natural enemies of nematodes and to non – target organisms.

—

References

Abid M., Maqbool M. A. (1990). Effects of inter – cropping of *Tagetes erecta* on root – knot disease and growth of tomato. International Nematology Network Newsletter 7: 41-42

Akhtar M., Alam M. M. (1991). Integrated control of plant-parasitic nematodes on potato with organic amendments, nematicide and mixed cropping with mustard. Nematol. Mediterr. 19: 169-171

Akhtar M., Alam M. M. (1993). Utilisation of waste materials in nematode control: A review. Bioresour Technol 45: 1-7

Akhtar M., Mahmood I. (1993). Control of plant-parasitic nematodes with nimin and some plant oils by bare-root dip treatment. Nematol. Mediterr 21: 89-92

Akhtar M., Mahmood I. (1997). Control of root-knot nematode *Meloidogyne incognita* in tomato plants by seed coating with suneem and neem oil. J Pestic Sci 22: 37-38

Akhtar M. (1998). Biological control of plant-parasitic nematodes by neem products in agricultural soil. Appl Soil Ecol 7 (3): 219-223

Asmus R. M. F., Ferraz S. (1988). Antagonismo de algumas especies vegetais, principalmente leguminosas, *Meloidogyne javanica*. Fitopatol. Bras. 13: 20-24

Atta-Ur R., Khan A. M., Shabir M., Abid M., Chaudhary M. I., Nasreen A., Maqbool M. A., Shameel M., Sualeh R. (1997). Nematicidal activity of marine organisms. Pakistan J Nematol 15: 95-100

Atungwu J. J., Ademola A. A., Aiyelaagbe I. O. O. (2009). Evaluation of organic materials for inhibition of nematode reproduction in soybean. Afr Crop Sci J 17 (4): 167-173

Bohinc T., Košir I. J., Trdan S. (2013). Glucosinolates as arsenal for defending Brassicas against cabbage flea beetle (*Phyllotreta* spp.) attack. Zemdirbyste-Agriculture 100 (2): 199-204

Brown P. D., Morra M. J. (1997). Control of soil-borne plant pests using glucosinolate-containing plants. Adv Agron 61: 167-231

Chen G., Clark A., Kremen A., Lawley Y., Price A., Stocking L., Weil R. (2007). Brassicas and mustards. In: Managing Cover Crops Profitably, 3rd Edition (Clark A., ed), Sustainable Agriculture Research and Education (SARE) program, Beltsville, MD, USA, pp. 81-90

Conijn C. G. M., Molendijk L. P. G., Schepman M., Koster A. T., Schenk A. M. E., Kroonen Backbier B., Gommers F. J., Brinkman H. (1996). *Tagetes* and root lesion nematodes. Gewasbescherming 27: 106-110

Curto G. (2008). Sustainable methods for management of cyst nematodes. In: Integrated Management and Biocontrol of Vegetable and Grain Crops Nematodes. Integrated Management of Plant Pests and Diseases, vol 2. (Ciancio A., Mukerji K. G., eds), Springer, Dordrecht, pp. 221-237

Evenhuis A., Korthalas G. W., Molendijk L. P. G. (2004). *Tagetes patula* as an effective catch crop for long-term control of *Pratylenchus penetrans*. Nematology 6: 877-881

Fahey J. W., Zalcmann A. T., Talalay P. (2001). The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry 56: 5-51

Ferraz S., Grassi de Freitas L. (2004). Use of antagonistic plants and natural products. In: Nematology-Advances and perspectives; volume II, Nematode Management and Utilisation (Chen Z. X., Chen S. Y., Dickson D. W., eds), CABI Publishing, Wallingford, UK, pp. 931-977

Gommers F. J., Bakker J. (1988). Physiological diseases induced by plant responses or products. In: Diseases of nematodes (Poinar G.O. Jr., Jansson H-B., eds.) Vol. I., CRC Press Inc., Boca Raton, FL, USA, pp. 3-22

Hooks C. R. R., Wang K-H, Ploeg A., McSorley R. (2010). Using marigold (*Tagetes* spp.) as a cover crop to protect crops from plant-parasitic nematodes. Applied Soil Ecology 46: 307-320

Jagodič A., Ipavec N., Trdan S., Laznik Ž. (2017). Attraction behaviours: are synthetic volatiles, typically emitted by insect-damaged *Brassica nigra* roots, navigation signals for entomopathogenic nematodes (*Steinernema* and *Heterorhabditis*)? BioControl 62: 515-524

Javed N., Anwar S. A., Fyaz S., Khan M. M., Ashfaq M. (2008). Effects of neem formulations applied as soil drenching on the development of root-knot nematode *Meloidogyne javanica* on roots of tomato. Pak J Bot 40 (2): 905-910

Khalil M. S. (2013). Abamectin and Azadirachtin as Eco-friendly Promising Biorational Tools in Integrated Nematodes Management Programs. J Plant Pathol Microbiol 4: 174 doi:10.4172/2157-7471.1000174

Khan M. R., Mohiddin F. A., Ejaz M. N., Khan M. M. (2012). Management of root-knot disease in eggplant through the application of biocontrol fungi and dry neem leaves. Turk. J. Biol. 36: 161-169

Khurma U. R., Singh A. (1997). Nematicidal potential of seed extracts: in vitro effects on juvenile mortality and egg hatch of *Meloidogyne incognita* and *M. javanica*. Nematol. Mediterr. 25: 49-57

Kizilkaya R., Akca I., Askin T., Yimaz R., Olekhov V. (2012). Effect of soil contamination with azadirachtin on dehydrogenase and catalase activity of soil. Eurasian J. Soil Sci. 2: 98-103

Kumar S., Khanna A. S. (2006). Effect of neem-based products on the root-knot nematode, *Meloidogyne incognita*, and growth of tomato. Nematol. Mediterr. 34: 141-146

Laznik Ž., Trdan S., Vučajnk F., Bohinc T., Vidrih M. (2014). Cruficerous plants - use as bio-fumigants in potato against wireworms. Acta Agric Scand B Soil Plant Sci 64 (7): 606-614

Lazzeri L., Curto G., Leoni O., Dallavalle E. (2004). Effects of glucosinolates and their enzymatic hydrolysis products via myrosinase on the root-knot nematode *Meloidogyne incognita* (Kofoid *et* White) Chitw. J Agric Food Chem 52: 6703-6707

Lehman P. S. (1979). Factors influencing nematode control with marigolds. Nematology Circular No. 50. Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, FL.

Moreira F. J. C., Barbosa da Silva M. C., Araujo Rodrigues A., Neves Tavares M. K. (2015). Alternative control of root-knot nematodes (*Meloidogyne javanica* and *M. enterolobii*) using antagonists. International Journal of Agronomy and Agricultural Research 7 (2): 121-129

Motsinger R. E., Moody E. E., Gay C. M. (1977). Reaction of certain french marigold (*Tagetes patula*) cultivars to three *Meloidogyne* spp. J Nematol 9: 278

Natarajan N., Cork A., Boomathi N., Pandi R., Velavan S., Dhakshnamoorthy G. (2006). Cold aqueous extracts of African marigold, *Tagetes erecta* for control tomato root knot nematode, *Meloidogyne incognita*. Crop Prot 25: 1210-1213

Ngala B. M., Haydock P. P., Woods S., Back M. A. (2015). Biofumigation with *Brassica juncea*, *Raphanus sativus* and *Eruca sativa* for the management of field populations of the potato cyst nematode *Globodera pallida*. Pest Manag Sci (5): 759-769

Noling J. W., Becker J. O. (1994). The challenge of research and extension to define and implement alternatives to methyl bromide. J Nematol 26: 573-586

Penfold C., Collins C (2012). Cover crops and plant-parasitic nematodes. Wine Australia Factsheet June 2012

Ploeg A. T., Maris P. C. (1999). Effect of temperature on supression of *Meloidogyne incognita* by *Tagetes* cultivars. J Nematol 31: 709-714

Pudasain M. P., Viaene N., Moens M. (2008). Hatching of the root – lesion nematode, *Pratylenchus penetrans*, under the influence of temperature and host. Nematology 10: 47-54

Quarles W. (1992). Botanical pesticides from *Chenopodium*. IPM Practicioner 14 (2): 1-11

Rangaswamy S. D., Reddy P. P., Joshi S. (1993). Histopathological and histochemical investigation on antagonistic trap crops (marigold and mustard) and susceptible tomato infested with *Meloidogyne incognita*. Current Nematology 4: 203-206

Sadre N. L., Deshpande V. Y., Mendulkar K. N., Nandal D. H. (1983). Male antifertility activity of *Azadirachta indica* in different species. Proceedings of the 2nd International Neem Conference, Rauischholzhausen, Germany, pp. 473-482

Schmutterer H. (1997). Side effects of neem (*Azadirachta indica*) products on insect pathogens and natural enemies of spider, mites and insects. J. Appl. Entomol. 121: 121-128

Schneider S. M., Rosskopf E. N., Leesch J. G., Chellemi D. O., Bull C. T., Mazzola M. (2003). Alternatives to methyl bromide – preplant and post-harvest. Pest Manag Sci 59: 814-826

Silva J. C. T., Oliveira R. D. L., Jham G. N., Aguiar, N. D. C. (2008). Effect of neem seed extracts on the development of the Soybean Cysts Nematode. Trop Plant Pathol 33 (3): 171-179

Sivakumar M., Gunasekaran K. (2011). Management of root knot nematodes in tomato, chilli and brinjal by neem oil formulations. J Biopest 4 (2): 198-200

Soule J. A. (1996). Novel annual and perinneal *Tagetes*. In: Progress in new Crops (Janick J., ed), ASHS Press, American Society for Horticultural Science, Alexandria, USA, pp. 546-551

Suatmadji R.W. (1969). Studies on the effect of *Tagetes* species on plant parasitic nematodes. H. Veenman en Zonen, Wageningen, The Netherlands, pp. 132 pp. Available at: http://edepot.wur.nl/192253/ [Accessed 03.05.2018].

Trifonova Z., Atanasov A. (2011). Control of potato cyst nematode *Globodera rostochiensis* with some plant extracts and neem products. Bulg J Agric Sci 17 (5): 623-627

Upadhyay K. D., Dwivedi K., Uttam S. K. (2003). Effect of some plant extracts on the mortality and hatching of *Meloidogyne incognita* and *Heterodera cajani* in festing pigeonpea. Nematol. Mediterr. 31: 28-32

Varma M. K., Sharma H. C., Pathak V. N. (1978). Efficacy of *Tagetes patula* and *Sesamum orientale* against root knot of eggplant Plant Dis Rep 62: 274-275

Villar E. M. J; Zavaleta Mejía E. (1990). Effect of *Crotolaria longirostrata* Hook y Arnott on root galling nematodes (*Meloidogyne* spp.). Rev Mex Fitopatol 8: 166-172

Wang K-H., Sipes B. S., Schmitt D. P. (2001). Suppression of *Rotylenchulus reniformis* by *Crotalaria juncea*, *Brassica napus*, and *Tagetes erecta*. Nematropica 31 (2): 235-249

Wang K-H, Hooks C. R., Ploeg A. (2007). Protecting crops from nematode pests: Using marigold as an alternative to chemical nematicides. University of Hawaii, Honolulu (Plant Disease; PD-35).

Yasmin L., Rashid M. H., Nazim Uddin M., Hossain M. S., Hossain M. E., Ahmed M. U. (2003). Use of Neem Extract in Controlling Root-knot Nematode (*Meloidogyne javanica*) of Sweet-gourd. Plant Pathol J 2: 161-168

acs83_40