INDIGENOUS PLANT NEMATICIDES AND AGRO–ECOLOGICAL SYSTEMS

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ABSTRACT

In tropical countries of the world, farmers are faced with many plant protection issues and phytosanitary risks. Such issues include but are not limited to food insecurity, lower income in traditional low-input agroecosystems, adverse effects of pesticide use on man and the environment in intensive systems and export restrictions due to strict regulations on quarantine pests and limits on pesticide residue in farm produce. In order to make more food available to growing populations in these countries, pesticidal extracts and other preparations of plant origin can be used for management of pests and diseases. Also, vegetational diversity in agroecosystems can be utilized to reduce pests and diseases by the following mechanisms: (1.) disruption of life cycle of pathogenic pests, (2) allelopathy effects, (3) stimulation of specific below-ground antagonists of pests or induction of general soil suppressiveness and (4) physiological resistance of cultivated crops due to improved crop nutrition.

I. INTRODUCTION

Farmers, particularly in the tropics, are faced with dramatic plant protection issues/phytosanitary risks resulting in:

- Food insecurity and reduced income in traditional low–input agrosystems e.g in subsistence systems in Sub-Saharan Africa.
- Adverse effects of pesticide use on human health and on the environment in and around intensive systems.
- Export restrictions due to strict regulations imposed by exporting countries concerning quarantine pests and minimum limits on pesticide residues.

To provide more and better food to populations in both the south and northern hemispheres in a sustainable manner, there is a need for a shift from agrochemistry to agroecology. Agroecology is based on the optimization of biological interactions and regulations in agroecosystems, and its application to crop protection (Deguine, 2008).

Agroecosystem diversification at different scales is one of the two pillars of the agroecological approach, alongside soil quality enhancement (Nicholls and Altieri, 2004; Ferron and Deguine, 2005; Deguine *et al*., 2008). In addition to agronomic benefits (Malezieux *et al.*, 2009), introducing vegetation diversity in agrosystems may lead to different pest and diseases regulation processes.

Even though increased vegetation diversity and the general biodiversity it induces at different trophic levels lead to more efficient natural control of pests and diseases in agroecosystems in perhaps the majority of cases (Andow, 1991), vegetational diversification *per se* is no guarantee of a reduction in the impact of pests and diseases (Helenius, 1998). In addition, diversified systems are generally more difficult to manage than simplified ones (Malezieux *et al*., 2009). Wood and Lenne (2001) suggested that some sustainable natural systems consist of simple vegetation with a single dominant species, e.g. wild relatives of rice, sorghum and wheat in simple, extensive, often annual stands. Hence, there is a need for caution when recommending vegetation diversification to improve pest and disease control.

A better understanding of the mechanisms involved is critical to explain how, where and when exceptions to this principle are likely to occur. In addition, tools are needed to evaluate, develop and monitor agroecosystems based on enhanced ecological processes of pest and disease control by optimized, rather than maximized, vegetational diversification or on “mimics” of such mechanisms if need be.

The level of active ingredients in plants with pesticidal principles is largely dependent on the agro-ecological systems where they grow. Thus in studies that border on pest control with botanicals, a quantification and characterization of the pesticidal principles in pesticidal plants is imperative. This would ensure that such studies could be reproducible in any part of the world. Managing the agro-ecosystems with vegetation diversity exploits the principles of the following mechanisms: (1) disruption of life cycle of pests, (2) allelopathic effects of plants, (3) stimulation of specific below–ground antagonists of pests or induction of general soil suppressiveness and (4) physiological resistance due to improved crop nutrition.

II. MANAGEMENT OF PESTS AND DISEASES BY TAKING ADVANTAGE OF PLANT RESOURCES IN AGRO-ECOLOGICAL SYSTEMS

2.1 Disruption of Life Cycle of Pests

2.1.1 Hosts and non-host effects on pests and diseases

Crop rotation with non-host plants is the first general agronomic rule to avoid soil-borne diseases. Non-host effects at the field level over time disrupt the life cycle of soil-borne pests and diseases via below-ground processes. The effect targeted is a reduction in inoculum or in carry-over population due to the absence of the host plant.

2.2 Below-Ground Bottom-up Allelopathic Effects

2.2.1 Trap crop / suicidal germination inducers

These are effects that directly affect the feeding / infection attachment ability of the pest or disease on the host plant. Various plants are known to produce and release antibiotic components via two major processes: (1) root exudation and (2) release of components during plant decomposition after incorporation in the soil.

A good example of this mechanism is *Solanum sisymbriifolium* , which was introduced in the Netherlands as a trap crop for potato cyst nematodes (*Globodera* spp.), stimulated hatching (although slightly less than the susceptible potato crop) but was completely resistant, i.e no progeny cysts were formed (Scholte, 2000; Scholte and Vos, 2000; Timmermans *et al.*, 2005).

Brassicaceous green manures can also act as trap crops for nematodes (Thorup-Kristensen *et al*., 2003). The best documented case of their use for this purpose is that of the control of sugar beet nematodes (*Heterodera schactii*) in Europe (Muller, 1999; Schlathoelter, 2004). In lieu of chemical control, cover cropping with resistant plants allows the sustainable production of sugar beet in field infested with sugar beet cyst nematodes.

Some *Brassica* crops commonly used for biofumigation to control root-knot nematodes have also been shown to be suitable hosts during their growing stage, thus leading to an increase in the pathogens prior to the biofumigation process (Bernard and Montgomery- Dee, 1993; Mac Sorley and Frederick, 1995; Mc Leod *et al*., 2001; Stirling and Stirling, 2003). This underscores limitation to the use of plants with allelopathic effects in the control of plant parasitic nematodes.

Siam weed, *Chromolaena odorata*, is also reported to exhibit allelopathy.

2.3 Stimulation of Soil Pest-Pathogen Antagonists

2.3.1 Activation of general microflora and macrofauna

Introducing a selected plant may turn out to be a better option for building up beneficial populations than directly inoculating soil with beneficial microorganisms. For instance, Miethling *et al*. (2000) and Schloter *et al*. (2006) observed in the greenhouse that sowing *Medicago sativa and Secale cereale)* had a stronger impact on rhizospheric microbial communities than soil inoculation with *Sinorhizobium meliloti* or the origin of the soil.

Blanchart *et al*. (2006) reported higher densities of facultative phytophagous, bacterial-feeding and predatory nematodes, and lower densities of obligatory phytophagous (*Cricoemella, Scutellonema and Meloidogyne)* nematodes, resulting from intercropping maize with *Mucuna pruriens.*

2.3.2 Activation of specific pathogen–antagonist micro-organisms

The rhizosphere of some nematicidal plants like *Plantago major* and *Thymus officinalis* not only releases nematicidal compounds but also harbours nematode-antagonistic bacteria (Insunza *et al*., 2002).

These bacterial isolates produce hydrolytic enzymes, some of which are related to soil suppressiveness such as chitinase, which is reported to destroy the chitinous layer of nematodes, and chitinolytic bacteria, which are reported to be effective biological agents for the control of nematodes (Spiegel *et al.*, 1991; Tian *et al*., 2000) and also proteases.

2.4 Crop Physiological Resistance via Improved Nutrition

2.4.1 "Tolerance/compensation"-like resistance

In addition to their nematicidal activities, *Crotalaria* species (particularly *Crotalaria juncea*, a productive legume) also increase the yield of the follower crop due to improved soil nitrogen status (Wang *et al.*, 2003). Varied crop rotations contribute to better and more balanced soil fertility to support crop growth because each crop species has different nutritional requirements for optimum growth and development, and each draws on individual nutrients in the soil at different rates. This balance has been suggested to have a positive effect on crop resistance to pests and diseases (Krupinsky *et al.*, 2002).

An indirect positive effect of mulching and of the use of cover crops is thus better crop nutrition from minerals derived from the decomposition of organic matter, provided biofumigants with antibiotic effects on beneficial micro-organisms are not released. Mulching also limits evaporation and contributes to better water nutrition of crops (Scopel *et al*., 2004), making them better able to withstand attacks by pests or pathogens.

III. REPORTS OF SUCCESSFUL MANAGEMENT OF PLANT-PARASITIC NEMATODES WITH BOTANICALS IN DIFFERENT AGRO-ECOLOGICAL SYSTEMS

Plant products for use against pests and diseases have been examined under laboratory, glasshouse and field conditions. In most trials, botanicals were applied, alone or in combinations as sprays. Treatment of seeds with neem oil (NO) before planting and incorporation of de-oiled neem cake (DONC) into the soil is a common practice. In other cases, pesticidal plants have been incorporated in arable crop fields to release active ingredients or for allelopathy effects. Preparation of plants with pesticidal activity is cost effective; they are easy to prepare with minimum knowledge, and the process is competitive in terms of returns to the producer.

Neem (*Azadirachta indica*) is used extensively to control more than 200 pests and more than 25 plant diseases and nematodes because of different modes of action of the limonoids, terpenoids flavonoids, and other alkaloids present with azadirachtin being an important component (Gahukar, 1995; Akhtar, 2000). The content of toxic constituents differs; with more detected in roots of derris, *Derris elliptica* (Xie *et al*., 2004), and neem seed (Kaur *et al*., 2005). Ready-to-mix preparations are commercially available (Shanker and Parmar, 1999). Also producers can prepare the crude extract in water from neem leaves or seeds, leaves of several other plants, and emulsion of neem oil. Plant products act as sterilants, oviposition deterrents, feeding disruptions, and contact toxins.

Studies carried out by Singh (1965a, b) and Singh and Sitaramaiah (1971a, b) had shown that use of oil cakes as manure at the rate of 2,500 kg/ha provided a highly effective control of root knot nematodes, improved soil fertility and resulted in many-fold increase in yield of tomato and okra. The authors had also reported that application of wood, sawdust, at the same rate as above, followed by application of 120 kg N/ha through urea also controlled root knot disease. The effect of the treatment lasted in the subsequent crop also. The reduced incidence of root knot infection with such treatments was attributed to:

(i) Stimulation of root growth.
(ii) Stimulation of predaceous fungi, and
(iii) Production of toxic metabolites during the decomposition of the organic matter.

The mechanisms of nematode suppression by organic amendment remain complex but the basic activity is biofumigation by volatiles released during the decomposition process. Oil cakes enhance the activity of predaceous fungi that feed on nematodes (Singh *et al.,* 2002). The population of *Catenaria anguillulae* was increased several fold when the soil was amended with oilcakes of mustard, linseed, margosa or sesamum. Soil amendment with rapeseed meal is reported to reduce number of galls on tomato roots caused by *Meloidogyne arenaira*. Quantity of edible oil cakes effective against root knot nematodes can be reduced by combining with other biofertilizers.

The toxicity of ethanol and water extracts of leaves and bark of stem of neem (*Azadirachta indica*), leaves and roots of gliricidia (*Gliricidia sepium*), peels of cassava (*Manihot esculentus*) leaves and roots of siam weed (*Chromolaena odorata*) on eggs and second-stage juveniles of

Meloidogyne incognita was investigated *in vitro*. All concentrations of plant extracts tested inhibited hatching of eggs and killed second-stage juveniles. Ethanol extract of neem leaves at 40,000 mg/kg was the most effective in inhibiting egg-hatch (79.2% inhibition) and killing second-stage juveniles. Also ethanol (control) was more effective than water (control) in inhibiting egg hatch (22.9% inhibition) and killing second–stage juveniles of *M. incognita* (Fatoki and Fawole, 1999).

Adekunle and Fawole (2003a) investigated the effects of carbofuran and water extract of leaves of neem (*Azadirachta indica*) as compared to water extract of leaves and roots of siam weed (*Chromolaena odorata)* on the development and generation time of *Meloidogyne incognita* Race 2 infecting tomato under screenhouse conditions. Adult females (AF) *M. incognita* were first seen in control plants on day 30 after inoculation, and in plants treated with water extract of neem leaves at 20,000 mg / kg and 40,000 mg / kg, water extract of siam weed leaves at 20,000 mg/kg and 40,000 mg / kg on day 32; while they were first seen in plants treated with carbofuran at 1.5 kg a.i/hectare and 2.5 kg a.i. / hectare on day 36 after inoculation. Generation time of *M. incognita* in tomato plants treated with water extract of neem leaves at 40,000mg / kg, water extract of siam weed leaves at 40,000 mg / kg and carbofuran was 48 days for each treatment as comparted to 44 days in control plants at a temperature range of 28 to 34^0 C.

Field studies were conducted in 1998 and 1999 to investigate the effects of air-dried milled neem leaves, siam weed leaves and roots each at 30 kg / ha and 50 kg / ha, and carbofuran at 1.5 kg a.i / ha and 2.5 kg a.i / ha on *Meloidogyne incognita* infecting cowpea cv. IT 86D-715. Carbofunran-treated plants had highest grain yield (1.7 t / ha) and the least root galling (0.6) at harvest while carbofuran–treated soil had the least nematode population after harvest and population of *Pratylenchus* spp. was reduced by 83% *Helicotylenchus* spp. by 86.5% *Xiphinema* spp. by 89.1% and *Meloidogyne incognita* by 94.8%. This was followed by the grain yield in neem leaf-treated plants (1.35 t/ha) with a root galling of 1.4, while nematode population in neem leaf-treated soil was reduced as follows: *Pratylenchus* spp., 67.6%, *Helicotylenchus* spp., 64.1%, *Xiphinema* spp., 64.8% and *M. incognita* 83.4%. Grain yield in siam weed–treated plants (0.8 t/ha) with a root galling of 1.7, was higher than that of control plants and population of *Pratylenchus* spp. was reduced by 49.9% *Helicotylenchus* spp. by 59.1%, *Xiphinema* spp. by 63.2% and *Meloidogyne incognita* by 74.9% (Adekunle and Fawole, 2003b)

Neem leaves, Siam weed leaves and Siam weed roots which were found to show nematicidal activity against the root-knot nematode, *M. incognita* in laboratory, screenhouse and field trials were subjected to chemical analyses to ascertain the active ingredients in them. The analyses revealed that neem leaves contain tannins and amines including methylamine; siam weed leaves contain alkaloids, flavonoids and amides including benzamide and ketones including benzylethanone; while siam weed roots contain alkaloids, saponins, flavonoids, amides including benzamide and ketones including benzylethanone an o-hydroxylbenzanone. (Fatoki and Fawole, 2000).

Abbasi *et al.* (2005) confirmed the efficacy of soil amendment with neem cake in control of root knot of tomato caused by *Meloidogyne hapla.* Pahman and Somers (2005) used green manure with *Brassica juncea* (Indian mustard cv. Nemfix) and its seed meal in vineyards and obtained good control of *M. javanica* when the meal was applied in the rows not between rows. Broccoli plant residues are highly effective amendments against root knot nematodes. The role of mustard can be explained by the release of isothiocyanates during decomposition of glucosinolates. The cost of oil-cakes has increased manifold in India and the treatment becomes as costly as the use of nematicides. Oil cakes of mustard, sesamum, linseed are cattle feed.

Trials were conducted under laboratory conditions to investigate the nematicidal action of methanol extracts of *Sylibum marianum*, *Plantago lanceolata* and *Cassia fistula* against eggs and second stage juvenile (J_2) of *Meloidogyne incognita*. Extract of *P. lanceolata* was more active against *M. incognita* than other extracts as it inhibited egg-hatch by 75% and killed juveniles of the nematode within five days. Further studies revealed that butanol fractions of *P. lanceolata* were more active than other fractions in preventing egg-hatch and killing juveniles of the nematode. Of the group of compounds isolated from butanol fractions of *P. lanceolata*, the one collected in 5-15% methanol in chloroform exhibited a stronger nematicidal action against *M. incognita*. The results of this study may be useful in developing new nematicides of plant origin (Adekunle *et al*., 2007a).

Adekunle *et al.* (2007b) reported that essential oils are natural volatile substances found in a variety of plants. They investigated the toxicity of z-β-ocimene and dihydrotagetone (isolated from the oil of *Tagetes minuta*) to eggs and juveniles of *Meloidogyne incognita in vitro*. *Tagetes minuta* oil at 4%, 3%, 2% and 1% was strongly toxic to eggs and juveniles of *M. incognita*. Further studies revealed that dihydrotagetone and z-β-ocimene isolated from the oil showed strong nematicidal activity against *M. incognita*, with dihydrotagetone showing a higher level of toxicity than z-β-ocimene. The results of this study suggest that dihydrotagetone and z-β-ocimene isolated from *T. minuta* oil are potential sources of botanicals for control of the root-knot nematode, *M. incognita.*

The efficacy of sawdust in controlling root knot.reported in early 1970s by Singh and Sitaramaiah was later confirmed by Stirling (1989), Vawdrey and Stirling (1997), Stirling and Nikulin (1998). They obtained good control of root knot of tomato and ginger in field trials. Sawdust-amended soil was almost free of galls and had the lowest populations of root knot nematodes. However, chemical treatment gave better control. Powdered pine bark has also been used for control of *Meloidogyne arenaria* on soybean. Level of control increased with increasing amount of the amendment. The treatment was also effective against the soybean cyst nematode (*Heterodera glycines*). Gall and cyst formation was completely eliminated where the materials was used at 5% rate. Fungi populations were increased by powered pine bark. *Penicillium chrysogenum and Paecilomyces variotii* were the predominant fungal species. Nico *et al.*, (2004) have reported effective control of *M. incognita and M. javanica* by amendment of potting mixes with composted agro-industrial wastes such as dry cork, dry grapes residue after extraction of juice, dry rice husk etc.

Leucaena leucocephala is a small tropical tree used for a variety of purposes in agriculture, land management and homeopathic medicine. Quercetin, a flavonoid, was isolated and characterized from extracts of leaves of *L. leucocephala* and its effects on egg hatching and juvenile mortality of *Meloidogyne incognita* were investigated at 0.8, 0.4 and 0.2% in *vitro.* The compound was highly toxic to eggs and juveniles of the nematode at the three rates tested (Adekunle and Aderogba, 2008).

Adekunle (2009) reported that field trials were conducted for two consecutive years at the Teaching and Research Farm of the Obafemi Awolowo University in the tropical rainforest zone of Nigeria, to investigate the effects of *Meloidogyne incognita*, *Practylenchus* spp., *Partatylenchus* spp. and *Hoplolaimus* spp. on three okra (*Abelmoschus esculentus*) cultivars planted in 4-m alleys between 3-year-old leguminous trees, *Leucaena leucocephala* and *Gliricidia sepium.* A nematode-infested field without *L. leucocephala* and *G. sepium* was used as the control filed. The leguminous trees were pruned at 3-weekly intervals to prevent shading of okra and the prunings were mulched in the alley field. At the termination of the study, okra cultivars in the non-alley field had higher root-knot nematode galling indices than those in the alley field both the 2005 and 2006 trials. Fruit yields of okra cultivars were higher in the alley than the non-alley field. In the alley and non-alley fields, okra cv. 47-4 recorded the highest fruit yield in both years of the trial. Soil population densities of four genera of plant-parasitic nematodes increased in both the alley and non-alley fields. However, there was a much greater increase in the non-alley field, suggesting that *L. leucocephala* and *G. sepium* planted as alley crops have the potential to suppress nematode populations.

Leaf extracts of *Inula viscose*, which have been found effective against many fungal foliar pathogens, have strong nematicidal activity, particularly against the root knot nematodes. Addition of leaf powder to sand (0.1% w/w) greatly reduces the number of second stage juveniles of *M. javanicva.* Aqueous extract of the powder are less effective than the organic solvent extracts (Oka *et al*., 2001). Extraction of dry leaves with a mixture of acetone and n-hexane or n-hexane alone yields an oily paste. After evaporation of the solvent, the emulsifiable product can be diluted in water. A concentration of 0.01% (paste w/w) killed juveniles of *M. javanica* and reduced galling on roots (Oka *et. al.,* 2006)

Adekunle (2011) reported that field experiments were conducted in 2008 and 2009 in the tropical rainforest zone of Nigeria to investigate the effects of amendment of soil with seedlings of African marigold (*Tagetes erecta*) and sunn hemp (*Crotalaria juncea*) incorporated singly in plots on *Meloidogyne incognita* and yield of cowpea and soybean. The experimental field, which was naturally free of plant-parasitic nematodes, was inoculated with chopped roots of *M. incognita* race 2-infected *Celosia argentea* roots and planted to tomato to increase *M. incognita* population at the site.

Eight week-old marigold seedlings were incorporated in cowpea or soybean field and eight week-old sunn hemp seedlings were also incorporated in cowpea or soybean field. At the ends of the experiments, *M. incognita* population densities were significantly higher in control plots than those of the plots amended with marigold or sunn hemp with correspondingly higher grain yield in the amended plots in both cowpea and soybean fields in both years. A significantly higher population of the nematode and consequently, lower yield was associated with cultivar Ife Brown than cultivar Ife Bimpe of cowpea for each treatment whereas in soybean cultivars, the pattern was not definite. Also twelve seedlings of marigold or sunn hemp per plot incorporated into the soil produced significantly higher grain yield in cultivar Ife Brown of cowpea and cultivar TGX 1440 of soybean compared to six seedlings per plot. The results of this study suggest that incorporating marigold or sunn hemp in *M. incognita*-infected cowpea or soybean field has potentials to suppress *M. incognita* population and reduce nematode damage on yield of the associated leguminous crops.

(IV) FURTHER RESEARCH

There are areas in which further research on use of botanicals can be initiated. These include, but are not limited to the following:

1. There are many plant species in different agro-ecologies and different zones of the world that have potentials to manage plant-parasitic nematodes, which have not been tested (explored)

2. Water extracts are easily washed off plants due to heavy rains, and residual times in soil or on plants is <6 days; frequent application of botanicals are needed and as a result costs may increase (Lakshmisubramanian *et al*., 1998; Markandeya *et al.*, 2001). Research in needed to extend residuals by adding stickers, but it must also be determined if extended residuals would present ecological problems.

3. Plant products have poor contact toxicity (Gahukar, 1998) so they must be ingested by pests to be effective. Research should be oriented on how to improve effectiveness of plant-derived products.

4. Although EC-based formulations can be stored for up to a year (Kumar and Parmar, 2000), extracts of leaves, seeds, or cake prepared in water have to be applied within a few hours of

preparation because they are sensitive to high temperature and break down due to UV light (Gahukar, 1998). Stabilizers and antioxidants are necessary.

5. Many plant species have been exploited in plant protection and many others remain to be examined, for example, *Tagetes erecta* L., *Mentha* spp. *Parthenium hysterophorus* L., *Thuja occidentalis* L., *Allium cepa L*., and *Tridax procumbens* L. probably because: (1) raw materials are not readily available since some species are legally protected by regulation against exploitation, (2) proper methods of collection, storage, and quality verification of raw materials are not followed, (3) the quality of local preparations might be suspect, and (4) isolation/extraction synthesis and formulation of bioactive constituents is a long, expensive process (Jaglan *et al.* 1997). At the local level, extension education is needed to demonstrate the correct methods of collection and storage of plant materials, and decortication and preparation of crude extracts.

6. Patenting of plant products, particularly traditional preparations, generally does not occur in developing and under-developed countries and only a few products, that is, neem cake, neem oil, and pongam oil have been patented. Awareness campaigns on the current rules and regulations should prove useful to encourage development of natural pesticides.

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