Chapter 7
Managing the Phytotoxicity and Inconsistent Nematode Suppression in Soil Amended with Phytonematicides

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7.1 Introduction

The global withdrawal of environment-unfriendly synthetic nematicides from agrochemical markets resulted in the emergence of various alternatives for managing plant-parasitic nematodes (Chedekal 2013; Stirling 2014). However, the introduced alternatives had inherent drawbacks. For instance, most crude extracts from plants with acceptable efficacies on suppression of nematodes were highly phytotoxic and could therefore not be sanctioned for use in crop husbandry. The European and Mediterranean Plant Protection Organization (EPPO 2010) and other such legal entities in various countries have zero tolerance on products that induce phytotoxicity on crops which are being protected against pests. Invariably, some non-phytotoxic products have had inconsistent results on target pests, which raised credibility issues for their registration. Incidentally, most products to be used in agricultural pests such as plant-parasitic nematodes have to undergo registration after intensive efficacy and non-phytotoxic trials.

Plant-parasitic nematodes are among the most injurious soilborne pests in cropping systems, with yield losses ranging from 5 % to 15 % (Stirling 2014) and translating to billions of US dollars (Chitwood 2002; Khan et al. 2008). Following the withdrawal of highly effective nematicides, the use of nematode-resistant genotypes had been in the forefront as a management strategy of choice in reducing

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nematode densities to below injurious levels. However, in plant genotypes without nematode resistance such as watermelon (*Citrullus lanatus*), peppers (*Capsicum annuum*) and potatoes (*Solanum tuberosum*), the yield losses escalate to as high as 50 % and at times to complete crop failure due to infection by the root-knot (*Meloidogyne* species) nematodes (Pofu et al. 2012). Reliance on nematode resistance was not sustainable due to the existence of nematode races and sensitivity of nematode-resistant genotypes to environmental factors such as high soil temperature (Dropkin 1969), salinity (Mashela et al. 1992) and honeydew-inducing foliar insects (Pofu et al. 2012). Lack of nematode-resistant genotypes in certain economically important crops and incompatibility of intergeneric nematode-resistant rootstocks and scions also negated the widespread adoption of nematode resistance technology (Pofu et al. 2012). Notwithstanding the listed drawbacks and the degree of nematode resistance in a given cultivar, the extent of crop losses is also depended upon the aggressiveness of the target nematode. For example, *Meloidogyne* species, lesion nematode (*Pratylenchus* species), sting nematode (*Belonolaimus longicaudatus*) and burrowing nematode (*Radopholus similis*) are highly aggressive, and, therefore, each may induce excessive damage to the host plants. In contrast, the citrus nematode (*Tylenchulus semipenetrans*) is not aggressive, but could be highly damaging in soils with salinity problems (Mashela and Nthangeni 2002; Duncan 2009).

Management of plant-parasitic nematodes in cropping systems is indispensable if crop enterprises are to be profitable and thereby improve food security on a global scale. Due to various setbacks on nematode resistance, organic amendments and/or other biological agents were tested on a grand scale for the suppression of population nematode densities. Notably, higher plants, biocontrol agents and fungi have since provided a broad spectrum of active compounds for use in nematode management (Chitwood 2002; Okwute 2012; Chedekal 2013). Phytonematicides as an alternative management strategy in nematode suppression comprise a class of plant-based nematicides, which are available as aqueous plant extracts (Egunjobi and Afolami 1976; Rossner and Zebitz 1987; Chedekal 2013), methanol plant extracts (Usman 2013), ethanol plant extracts (Khan et al. 2008), oilcakes (Muller and Gooch 1982), essential oils (Meyer et al. 2008), fermented crude plant extracts (Kyan et al. 1999; Ncube 2008; Pelinganga and Mashela 2012; Pelinganga et al. 2013a), powders (Ahmad et al. 2013) or granules (Mashela et al. 2008, 2011, 2012).

Phytonematicides differ from conventional organic amendments, which may include crop residues, manures, compost, organic manures, agro-industrial wastes and sewage sludge (Castagnone-Sereno and Kermarrec 1991; D'Addabbo 1995; Thoden et al. 2011; Stirling 2014). Generally, phytonematicides were introduced to mitigate the drawbacks of conventional organic amendments in suppression of plant-parasitic nematodes (Mashela 2002), which include (1) inconsistent results in nematode suppression; (2) large quantities (10–500 t/ha) which were required to achieve nematode suppression; (3) unavailability of the materials; (4) high transport costs to haul the materials from the production site to that of use; (5) negative period, with the subsequent time-lag to allow for microbial decomposition in order to avoid negative periods; and/or (6) decrease in soil pH, which inherently

imbalances the availability of essential nutrient elements in the soil (Jafee et al. 1994; Belair and Tremblay 1995; McSorley and Gallaher 1995; Mashela 2002; Kimpinski et al. 2003; Thoden et al. 2011; Stirling 2014). Inputs for most phytonematicides are locally collected from indigenous plants (Muller and Gooch 1982; Akhtar and Malik 2000; Oka 2010; Mashela et al. 2011; Ahmad et al. 2013), which possess complex allelochemical compounds (Chitwood 2002; Okwute 2012). In purified formulation, most phytonematicides lose their nematode suppression capabilities (Wuyts et al. 2006; Oka 2010; Ntuli and Caboni 2012; Okwute 2012) and are accompanied by unacceptable high phytotoxicity levels on crops being protected against nematodes (Mian and Rodriguez-Kabana 1982a, b; Meyer et al. 2008). Generally, phytonematicides rely on allelochemicals as their active ingredients and are used in vivo for defence against invading pathogens (Rice 1984; Inderjit et al. 1999). Roots of certain allelochemical-producing plants exude copious quantities of allelochemicals to provide competitive edge against competitors during interference (Inderjit et al. 1999; Rice 1984). The objective of this overview was to provide the dosage model as an alternative strategy in managing plant-parasitic nematodes with specific reference to addressing efficacy, phytotoxicity and inconsistent result issues of phytonematicides.

7.2 Distinction Between Phytonematicides and Organic Amendments

In the original overview on organic amendments, Muller and Gooch (1982) noted that between 1971 and 1981, out of 33 organic amendment trials, those with at least 91 % success frequency on nematode suppression were in the form of powders and oilcakes from neem (*Azadirachta indica*), peanuts (*Arachis hypogaea*) and castor (*Ricinus communis*). Later, other reviews (Alam 1993; Ferraz and de Freitas 2004; Oka 2010) confirmed that neem extracts, particularly those from seed kernels, had high bioactivities on nematode populations. Mashela et al. (2011) introduced a classical model on the ground leaching technology (GLT) system, with the research focus being on powdered plant products from selected plant organs with the view of ameliorating the numerous drawbacks of conventional organic amendments in smallholder tomato (*Solanum lycopersicum*) farming systems in South Africa. In the GLT system, powdered materials were derived from unshelled dried castor bean, fever tea (*Lippia javanica*) leaves, wild cucumber (*Cucumis myriocarpus*) fruit and wild watermelon (*Cucumis africanus*) fruit. In all cases, the four products each consistently reduced population densities of *Meloidogyne* species and *T. semipenetrans*. Overall, the GLT system uses 0.2–0.7 powdered materials/ha for 4000 tomato plants when compared with 10–500 t organic amendments/ha required to effect consistent results in nematode suppression (Mashela 2002). In order to distinguish the powdered materials with their small quantities required in suppression of nematodes relative to large quantities required in conventional

organic amendments, the former were referred to as phytonematicides (Mashela et al. 2011). Phytonematicides at the concentration used are intended to consistently suppress population densities of the target nematodes, while stimulating growth of the protected crops instead of inhibiting plant growth and productivity (Pelinganga 2013). Certain phytonematicides can be highly effective in nematode suppression. Incidentally, the efficacy of powdered materials from *C. myriocarpus* fruit in nematode suppression was similar to those of aldicarb and fenamiphos nematicides (Mashela et al. 2008).

The major distinctions between phytonematicides and conventional organic amendments could be:

1. The empirically based small quantities applied to achieve consistent nematode suppression under diverse conditions as opposed to large quantities.
2. In GLT system there is gradual release of active ingredients from crude extracts into the rhizosphere which is achieved through irrigation water or rainfall as opposed to microbial degradation in conventional organic amendments.
3. Phytonematicide products mimic synthetic chemical nematicides since they could be commercially packaged in relatively small containers with label information which includes active ingredients, along with efficacy features.
4. Phytonematicides like most non-fumigant nematicides do not have negative periods and could therefore be applied as post-planting products.
5. These products are required to comply with relevant legislation in terms of avoiding health risks to end users, nontarget organisms and the environment.

The drawback of the GLT system was its high labour costs since products were manually applied, which rendered the system less appealing to large commercial tomato producers (Mashela et al. 2011). An alternative technology, referred to as botinemagation (Mashela et al. 2011), was developed for use in large-scale tomato farming systems, where crude extracts from fermented plant organs were used through drip irrigation systems. Using dried fruits of *C. myriocarpus* and *C. africanus* fruits, fermented crude extracts as liquid formulations consistently reduced population densities of *Meloidogyne* species in tomato production (Pelinganga et al. 2013a, b).

Not all plant organs contain allelochemicals with nematicidal properties. In South Africa, Van Wyk et al. (2002) listed 372 plant species on the basis of their toxicity to humans and animals, which were for the purpose of this discussion classified into six using their degree of toxicity (Table 7.1). Approximately 22.6, 18.3 and 6.7 % of the listed plants were described as being poisonous, very poisonous and deadly, respectively, to humans and livestock. The degree of toxicity to humans and animals does not confer a plant a better status to be a candidate for serving as source of phytonematicides. *Cucumis myriocarpus* and *R. communis*, from which two phytonematicides were developed for the GLT system (Mashela 2002; Mashela and Nthangeni 2002), were regarded as being poisonous and very poisonous, respectively (Van Wyk et al. 2002). However, the deadly oleander (*Nerium oleander*) and tamboti (*Spirostachys africana*) did not have phytonematicidal properties against *Meloidogyne* species (Mashela et al. 2011).

Table 7.1 Count and percent count of plants clustered according to the degree of toxicity to humans and livestock in South Africa

Classification	Count	%
Not really poisonous	14	3.8
Poisonous	84	22.6
Very poisonous	68	18.3
Deadly	25	6.7
Causes skin allergies or contact dermatitis	18	4.8
Poisonous to animals	163	13.8
Total	**372**	**100**

Statistics developed from Van Wyk et al. (2002)

In contrast, certain plants listed as 'not really poisonous', namely, fever tea (*Lippia javanica*) and *Brassica* species, produced potent phytonematicides (Mashela et al. 2010). Among the listed plant species, only 0.8 % plant species were tested against nematodes in South Africa, with only 0.55 % having some nematicidal properties. At a global level, among the 45 papers of biological control agents of nematodes discussed at the 2014 International Congress of Nematology in Cape Town, South Africa, 42, 36, 18 and 4 % were on botanicals, fungi, bacteria and enzymes, respectively. The highest percentage of phytonematicide papers clearly illustrated the potential importance and interest in this group of biological control agents in plant nematology.

7.3 Efficacy of Phytonematicides

The majority of in vitro trials have had in excess of 90 % suppression of nematode numbers from phytonematicides (Okwute 2012). However, due to their high phytotoxicities and restricted measures (EPPO 2010), a large number of botanicals with potent nematicidal properties do not make it beyond in vitro tests. Notwithstanding the high rejection of most products, detailed assessments on mode of action for certain phytonematicides had been undertaken.

7.4 Mode of Action of Phytonematicides

The distinguishing feature of synthetic pesticides is their single active ingredients, with clearly defined bioactivities. In synthetic insecticides, such single active ingredients had high incidents of insect resistance, particularly in insects with high reproductive capabilities (Nzanza and Mashela 2012). However, although certain nematode species have high reproductive capabilities, resistance to synthetic nematicides in plant-parasitic nematodes had not been observed (Van Gundy and McKenry 1975). In contrast to synthetic pesticides, phytonematicides have multiple active ingredients, with complementary modes of action. For instance, in

wild garlic (*Tulbaghia violacea*), the plant bulb contains sacrid volatile oils and sulpho-oxides—each being a derivative of allicin, which has insecticidal and nematicidal properties (Vijayalakshmi et al. 1996; Nzanza and Mashela 2012; Mashela et al. 2012). In insects the mode of action for the allicin derivatives had been identified as antifeedant, repellent and insecticidal (Vijayalakshmi et al. 1996; Dhanalakshmi 2006). Similarly, in insects, azadirachtin in neem had been shown to have antifeedant, repellent and anti-ovipositor properties, with capabilities for delaying or preventing moulting in insects. Apparently, using phytopesticides confers a broad spectrum of active ingredients, with multiple modes of action. In phytonematicides, observations on mode of action had been limited to chemotaxis, juvenile motility, egg hatch, juvenile mortality or juvenile paralysis, with limited information on behavioural responses of adult nematodes.

7.4.1 Chemotaxis

Chemotaxis is a phenomenon where nematodes direct their movement according to the gradient of selected chemical cues in the environments (Bargmann and Mori 1997). Positive chemotaxis occurs when movement is towards the increasing gradient of chemical cues. Conversely, movement towards the opposite direction of the increasing gradient is described as negative chemotaxis (Bargmann and Mori 1997). The nematode is literally exposed to both liquid- and airborne volatilised chemicals in the air-water interface of the soil, which could either be water-soluble and/or volatile chemoattractants or chemorepellents. According to Bargmann and Mori (1997), water-soluble chemoattractants are detected by chemoreceptors in nematodes at micromolar concentrations, while the volatile chemoattractants are detected at picomolar concentrations. Water-soluble and volatile chemoattractants are used for short- and long-distance chemotaxes, respectively (Prot 1980). In contrast, water-soluble and volatile chemorepellents are toxic and could cause either paralysis or death of the nematode. In phytonematicides, both chemoattractants and chemorepellents are important. Chemoattractant phytonematicides may disorientate the nematode from being guided by chemoattractant cues produced by potential host plants, thereby deferring penetration and attack of host by nematodes (Wuyts et al. 2006). In contrast, chemorepellents may induce various behavioural changes in the nematode, including paralysis and death (Bargmann and Mori 1997).

The body of a nematode is ‘wired’ with chemoreceptors, particularly on the frontal and cervical regions (Ferraz and Brown 2002), suggesting that chemoattractants and chemorepellents play important roles in behavioural activities of nematodes. Plants release numerous chemicals through exudation, leaching, volatilisation and microbial degradation for different reasons (Stirling 2014). Similarly, phytonematicides release potent chemicals either through leaching, volatilisation or microbial degradation (Mashela et al. 2011, 2012). Generally, increasing concentrations of phytochemicals could interfere with chemotaxis in

one of three ways: no effect (neutral chemotaxis), attract (positive chemotaxis) and repel (negative chemotaxis). Responses characterised by these three phases in the environment subscribe to density-dependent growth (DDG) curves (Salisbury and Ross 1992; Liu et al. 2003), which constitute an important part of this review.

Using purified phytochemical compounds, Wuyts et al. (2006) demonstrated that certain chemical compounds from *Philenoptera violacea* in the Fabaceae family had similar and/or different effects on chemotaxis—which is dependent much on the nematode species. In their work (Wuyts et al. 2006), among the tested chemical compounds produced through the shikimic acid pathway, 26 % repelled *R. similis*, 2.6 % attracted this nematode, while 45 % were neutral. In contrast, of the 37 % tested chemical compounds on *P. penetrans*, even those that were chemorepellent to *R. similis* had no effect on this nematode. In contrast, some chemorepellents to *R. similis* were also repellent to *M. incognita*. Although the approach used by Wuyts et al. (2006) did not provide information on physiological activities of the target chemicals in nematode bodies, it provided broad clues in terms of what we want to convey using DDG patterns later on in this overview. The three nematode species used depicted neutrality to the largest number of chemical compounds produced through the mevalonate pathway, followed by inhibition of motility and then repellence as depicted in chemotaxis (Wuyts et al. 2006). A remarkable feature in the work of Wuyts et al. (2006) was, therefore, the agreement of their observations with the concept of DDG patterns, particularly with the observed repeated neutral responses.

7.4.2 Motility

Juveniles from unhatched eggmasses which were previously exposed to crude extracts from leaves of Borelin remained motile, while those exposed to crude extracts of garlic bulb or neem seed kernels had impact on juvenile motility (Agbenin et al. 2005). According to DDG principles, different concentrations of phytonematicides might have no effect (neutral) on, stimulate and/or inhibit motility of nematodes (Salisbury and Ross 1992; Liu et al. 2003). Wuyts et al. (2006) observed that a chemical compound which was neutral in one nematode species could inhibit juvenile motility in another nematode species, vice versa. Similarly, those that were chemoattractants in chemotaxis for one nematode species might be neutral and/or inhibitive in juvenile motility for another nematode species. Oka et al. (2000) showed that essential oils from 12 of 27 plants immobilised more than 80 % *M. javanica* J2s after a 2-day exposure, with immobilisation being amenable to DDG patterns.

7.4.3 Egress

Egress in *M. incognita* was inversely proportional to concentrations of crude extracts from garlic and neem (Agbenin et al. 2005; Chedekal 2013). Although egress is a physical process, in most plant-parasitic nematodes, it is stimulated by external chemical cues from roots (Prot 1980). According to Wuyts et al. (2006), some phytochemical compounds were neutral towards egg hatch, while others were inhibitive. In contrast, one flavanone, which is a hesperetin chemical compound, was both stimulatory and inhibitive to egress in *R. similis* (Wuyts et al. 2006). Most active ingredients from phytonematicides have the capability to penetrate eggmasses, where J1s become exposed to aqueous solutions (Hirschmann 1985; Parmar 1987; Agbenin et al. 2005). Incidentally, the materials interfered with stylet development, rendering it incapable of piercing through the eggshell and, therefore, resulting in complete failure of egress (Hirschmann 1985; Parmar 1987).

Using in vitro trials, essential oils from 27 different plant species, at 1000 μL/L only 30 % of plants inhibited egress, while at 600 μL/L only 15 % of plants had oils with inhibitive properties (Oka et al. 2000). Ojo and Umar (2013) demonstrated that crude extracts from testa of cocoa bean (*Theobroma cacao*) plants had significantly higher effects on egress of *M. javanica* than oil palm fibre, with differences attributed to different chemical constituents. Cocoa bean testa contains alkaloids and flavonoids, with egress inhibition being directly proportional to the concentration of the listed chemical compounds (Ojo and Umar 2013). However, in the same study, Ojo and Umar (2013) observed that oil palm fibre, which was devoid of alkaloids and flavonoids, had negligent effects on egress. Okeniyi et al. (2013) demonstrated increasing concentrations (0, 10, 25, 50 and 100 %) of leaf crude extracts from the coastal golden leaf (*Bridelia micrantha*), euphorbia (*Mallotus oppositifolius*), abeere (*Hunteria umbellate*) and citron (*Citrus medica*)—each increased inhibition of egress in *M. incognita*. Removal of eggs from the chemical compounds resulted in reversal of the extract effects.

7.4.4 Mortality

In vitro exposure of *Meloidogyne* J2s to crude extracts from hen's nettle (*Fleurya interrupta*), panicled peristrophe (*Peristrophe bicalyculata*) and king of bitters (*Andrographis paniculata*) resulted in 100 % mortalities (Mukherjee and Sukul 1978). Similarly, high *Meloidogyne* J2 mortalities were observed in crude extracts from leaves of marigold (*Tagetes* species), Indian gooseberry (*Emblica officinalis*) and Christ's thorn (*Carissa carandas*) during in vitro exposure (Toida and Moriyama 1978; Haseeb et al. 1980). Also, in vitro exposure of *M. incognita* J2s to crude extracts or aqueous extracts from fresh leaves of various plants resulted in high mortalities (Agbenin et al. 2005; Chedekal 2013). Similarly, crude extracts of either cocoa bean testa or oil palm fibre resulted in high mortalities of *M. javanica*

juveniles (Ojo and Umar 2013). Juvenile mortalities were directly proportional to increasing concentrations of phytonematicides and exposure time (Agbenin et al. 2005). In some instances, 'mortalities' were reversible when J2s were removed from the chemicals (Wuyts et al. 2006).

7.4.5 Paralysis

Paralysis involves irreversible interference of nematicides with the nervous systems of J2s. Generally, affected J2s can still wiggle, but have complete loss of coordinated mobility. Phytonematicide-induced paralysis reports on plant-parasitic nematodes are uncommon. An exceptional case is that in Ntalli et al. (2011), where paralysis of *Meloidogyne* J2s was regularly observed when exposed to aliphatic ketones from rue (*Ruta chalepensis*).

7.5 Variation in Efficacy of Phytonematicides

Incidentally, biological entities respond to various abiotic and/or biotic factors through a myriad of complex processes and mechanisms. For instance, when various plant-parasitic nematodes infect plants at population densities below the tolerance limit, plant growth is invariably stimulated (Wallace 1973), while at high population densities, growth is reduced (Seinhorst 1967). Similarly, infection by different nematode species on various legumes either stimulated, had no effect on or inhibited nodulation and/or nitrogen fixation (Huang 1987). Vesicular-arbuscular mycorrhizal (VAM) fungi on various host plants also resulted in positive, neutral or negative growth responses (Smith 1987). Different fertilisers and/or salinity levels can also induce such growth responses in plants. In soil allelochemical residue (SAR) trials, it was shown that while SAR effects from one phytonematicide stimulated growth of the successor crop, SAR effects consistently reduced population densities of *Meloidogyne* species (Mashela and Dube 2014), with reduced population densities subscribing to similar inconsistent growth patterns (Zasada and Ferris 2003). Mashela (2014) showed that SAR effects had inhibitive effects on nodulation by *Bradyrhizobium japonicum* in cowpea (*Vigna unguiculata*). Others (Mashela and Dube 2014) argued that for phytonematicides to be successful, their inhibition concentration range to nematodes should overlap the stimulation range to the crop being protected against nematodes.

Sites of action in organisms by allelochemicals are not yet established. However, cucurbitacins from fruits of wild *Cucumis* species were shown to have the potential to inhibit cell division in cancer at high concentrations, while the materials were highly cytotoxic to healthy cells (Lee et al. 2010). In contrast, when used at low concentrations, cytotoxicity was avoided, but division of healthy cells was stimulated, thereby rendering the materials cancerous (Lee et al. 2010). These

observations in cancer trials provided clues on the site of action of cucurbitacins—the cellular level.

Reports which demonstrated that conventional organic amendments increased population densities of nematodes in Europe (Belair and Tremblay 1995; Kimpinski et al. 2003), had no effect on nematode numbers in Florida, USA (Jafee et al. 1994; McSorley and Gallaher 1995) and reduced nematode numbers (Stirling 2014) raised credibility issues on organic amendments due to the 'perceived' inconsistent results (McSorley 2011). The efficacy of phytonematicides is dependent upon the concentration of allelochemicals in the organ used for processing the intended products. Generally, the accumulation of secondary metabolites in organs varies from season to season (Mudau et al. 2008), with high inconsistent results in nematode suppression and high phytotoxicities during certain seasons. However, the variability that leads to inconsistent results should not be confounded with DDG patterns in allelochemical-containing products. Although the variability of concentrations of allelochemicals in a particular organ could be associated with DDG patterns in certain cases, DDG principles are primarily related to responses of living entities in response to increasing concentrations of allelochemicals ex vitro. In organs such as fruits or bulbs where the accumulation of secondary metabolites appears to level off with maturity, variability in efficacy of phytonematicides on nematode suppression had mostly been due to different concentrations in the processed product (Meyer et al. 2008). Generally, sources that result in the final product being of high variability are undesirable, particularly when commercial products are envisaged. On the basis of the three phases (stimulation, neutral and inhibition) being characterised by different concentration ranges, one could argue that the various materials of plant origin did not have 'inconsistent' results on nematode suppression, but what was being observed in a particular time was a reflection of differences in concentrations with respect to the allelochemicals involved.

7.5.1 *Density-Dependent Response Patterns in Phytonematicides*

At low concentrations, crude extracts of neem leaf were shown to stimulate growth of maize (*Zea mays*) and tomato seedlings, while at high concentrations, the opposite occurred (Egunjobi and Afolami 1976; Rossner and Zebitz 1987). Similarly, Inderjit et al. (1999) noted that at low concentrations root leachate from golden crownbeard (*Verbesina encelioides*) consistently stimulated plant growth of various plant species. Also, at low concentrations, nemarioc-B phytonematicide stimulated growth of tomato seedlings, where the product was viewed as having a 'fertiliser effect' (Mashela 2002). However, detailed analysis of essential nutrient elements in leaves did not support the 'fertiliser effect' view since the product had negligible effect on accumulation of essential nutrient elements. In subsequent

studies (Mafeo et al. 2011a, b; Pelinganga et al. 2012, 2013a, b), it was shown that various plant variables (y-axis) when subjected to lines of the best fit on increasing concentrations of nemarioc-A (x-axis) invariably resulted in quadratic relationships, which is a strong indicator for the existence of DDG patterns (Salisbury and Ross 1992; Liu et al. 2003). A myriad of complex models regarding DDG patterns exist in biological entities, including plant-parasitic nematodes (Ferris and Wilson 1987; Duncan and McSorley 1987). The DDG tenets are closely related to the original conceptual framework of the carrying capacity (Nicholson 1933), which had since been used in a wide range of disciplines. DDG patterns have three distinct growth responses: stimulated, saturated (neutral) and inhibited growth (Salisbury and Ross 1992; Liu et al. 2003), with biological indices which had been used to unravel diverse biological responses to increasing pressures from their environments. DDG principles have the ultimate aim of improving decision-making systems in sustainable management of natural resources. Generally, plants, nematodes and microbes respond to increasing concentrations of allelochemicals through DDG patterns (Rice 1984; Ferris and Wilson 1987; Zasada and Ferris 2003; Liu et al. 2003), with attempts to investigate the mechanisms involved still being at conceptual stages, except that the site of action is at the cellular level (Lee et al. 2010).

Biological entities respond to increasing concentrations of allelochemicals in phytonematicides through DDG patterns, which comprise three phases, namely, stimulation, neutral and inhibition phases (Salisbury and Ross 1992; Liu et al. 2003; Pelinganga et al. 2012, 2013a, b). DDG patterns are an advanced modification of the 1933 Nicholson's carrying capacity model, which had been adapted and used in various disciplines. Liu et al. (2003) quantified concentrations of allelochemicals which lead to three stages that characterise DDG patterns for various organisms using the curve-fitting allelochemical response dosage (CARD) computer-based model. The CARD model quantifies the three phases through seven biological indices: (1) threshold stimulation (D_m) = the allelochemical concentration that initiates the stimulation phase, (2) saturation point (R_h) = the concentration that terminates stimulation or starts the neutral phase, (3) 0 % inhibition (D_0) = the concentration that terminates the neutral phase, (4) 50 % inhibition (D_{50}) = the concentration at half the distance of the inhibition phase, (5) 100 % inhibition (D_{100}) = the concentration at the end of the inhibition phase), (6) the sensitivity index (k) = provides the degree of sensitivity of an organism to the test product and (7) the coefficient of determination (R^2) = provides the degree of the strength of the CARD model. Generally, stimulated ($D_m - R_h$) and inhibited ($D_0 - D_{100}$) growth concentrations are ideal representatives for phytonematicides and herbicides, respectively. The CARD model had since been empirically adapted to generate phytonematicide concentrations which stimulate plant growth while reducing population densities of nematodes using fruits as organs of preference in order to avoid confounding variability of allelochemical concentrations in the source and the actual concentration of allelochemicals in the processed product (Mafeo and Mashela 2010; Pelinganga and Mashela 2012). Using the three phases of the CARD model, we are currently in a position to argue that observations that

nematode populations were not consistently suppressed by application of conventional organic amendments, which were dubbed 'inconsistent' since the materials sometimes stimulated (Belair and Tremblay 1995; Kimpinski et al. 2003), had no effect on (Jafee et al. 1994; McSorley and Gallaher 1995; Thoden et al. 2011) or inhibited population densities of nematodes (Mashela et al. 2011), were biologically incorrect. Incidentally, it should also be noted that not all plant organs or species have allelochemicals which have potent nematicidal properties (Mashela et al. 2011). In most plants with nematicidal allelochemicals, due to their autoallelopathy, the chemical compounds in vivo are compartmentalised in organs not always preferred for use in conventional organic amendments. For instance, in *C. myriocarpus* fruit, cucurbitacin A is compartmentalised in seeds (Jeffrey 1978), which are hardly used in conventional organic amendments for fear of spreading the 'weed' through seed dispersal. Similarly, in neem the active ingredient, azadirachtin, is primarily concentrated in seed kernels (Parmar 1996).

Another important feature of DDG patterns in the CARD model is that the variable (y-axis) and the concentration of allelochemicals (x-axis) invariably have quadratic relationships (Salisbury and Ross 1992; Liu et al. 2003; Pelinganga et al. 2013a, b; Pelinganga and Mashela 2012). On this basis, should there be a positive linear instead of quadratic relationship between dependent and independent variables, results could be suggesting that the concentrations of the phytonematicide used were within the stimulation range—as observed in various trials. Incidentally, no effective response of dependent variables over increasing independent variable levels could suggest that phytonematicide concentrations were either within the neutral range (R_h–D_0) or below D_m. In contrast, negative linear relationship invariably suggested that the concentration of allelochemicals tested was within the inhibition range (D_0–D_{100}). In biology, literature is replete with responses to abiotic and/or biotic factors that can be described as relationships that have positive (D_m–R_h), neutral (R_h–D0) or negative linear responses (D_0–D_{100}) (Salisbury and Ross 1992). Interestingly, such responses had not attracted attention as those in conventional organic amendments, where the responses were broadly viewed as evidence that phytonematicides were unsuitable for use in management of plant-parasitic nematodes since they were unpredictable.

7.5.2 *Fluctuations in Concentrations of Allelochemicals In Vivo*

Allelochemicals in plants are produced for 'unknown' physiological roles through various pathways, with the major ones being the (a) shikimic acid pathway, (b) malonic acid pathway and (c) mevalonic acid pathway (Lai 2008). Concentrations of any allelochemical within the pathways are in continuous state of fluctuation as depicted by a large number of precursors and reversible chemical reactions which are linked to the end of glycolysis just prior to the Krebs cycle of respiration

(Lai 2008). Primarily, the formation of secondary metabolites helps to remove excess end products along the respiration pathway, particularly the acetyl co-A at the end of glycolysis (Campbell 1990). Responses to a phytonematicide in the plant being protected against nematodes are primarily a reflection of the phytonematicide concentration level at the time that particular organ was harvested for the development of the phytonematicide in question. For instance, phytonematicides derived from leaves such as those of fermented crude extracts from *L. camara* plants have the tendency of being highly inconsistent in nematode suppression due to seasonal variation of active ingredients in leaves. Also, the drying conditions of the organ after harvest might have deleterious effects on concentrations of the allelochemicals (Makkar 1991). For instance, shade-, sun- and oven-dried plant materials from the same plant organ may eventually contain different chemical concentrations due to differential chemical losses through volatilisation. Also, exposure of the harvested materials to rainfall as is common in maturation of conventional organic amendments may result in leaching out of allelochemicals since they are primarily nonstructural.

Timing of nematode sampling with reference to the initial application time of the phytonematicide could also be an important factor to consider in the perceived 'inconsistent' effect of organic materials in nematode management. In GLT systems, nematode sampling for *Meloidogyne* species was empirically established at 56 days after inoculation of plants (Mashela et al. 2011). When Maila and Mashela (2013) increased the sampling time from 56 to 150 days in citrus seedlings treated with nemarioc-AG and nemafric-BG, the highest population densities of *T. semipenetrans* were in phytonematicide-treated plants than in untreated controls. The unexpected observation was explained on the basis of cyclic growth patterns of population nematode densities, which subscribe to DDG patterns due to inherent competition for infection sites (Fig. 7.1). Generally, soon after the application of a phytonematicide, the product reduced population nematode densities, while those in untreated control increased, resulting in a situation where growth in the two populations was unsynchronised in a way that when the treated reached the trough the control was reaching the peak (Maila and Mashela 2013). By 150 days, nematode numbers under the untreated control were approaching the trough, while those from the treated seedlings were approaching the peak after reaching the trough within approximately 56-day application interval. Pofu and Mashela (2014) quantified the cyclic growth of population nematode densities of *Meloidogyne* species in four hemp (*Cannabis sativa*) cultivars and concluded that from inoculation to the peak of the nematode densities approximately 56 days were required, which was in agreement with the 56-day application interval for phytonematicides in GLT systems.

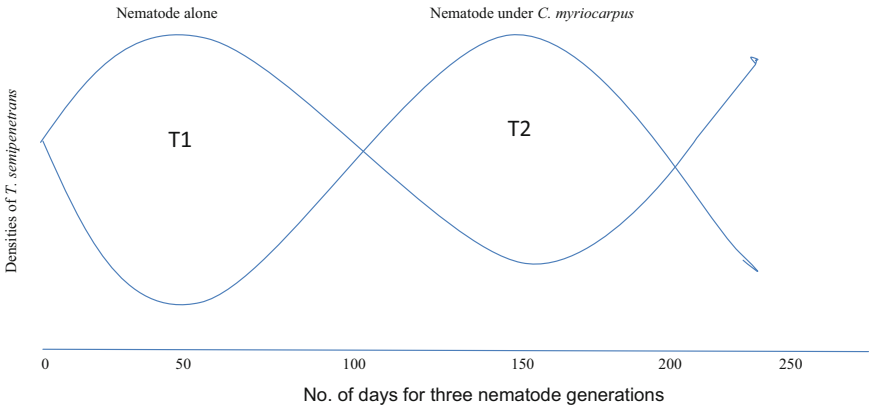

Fig. 7.1 Relative cyclic population densities of *Tylenchulus semipenetrans* on rough lemon under untreated control and nemarioc-AG-treated pots at 150 days after inoculation with 25,000 nematodes

7.5.3 Confounding Survival Adaptations with Phytonematicidal Effects

Nematodes have evolved unique survival strategies, which rendered them the status of 'undefeatable enemies' after attempts to annihilate them failed. The survival strategies had been classified into (1) intrinsic adaptations in the life cycle of the nematode and (2) extrinsic rapid responses to environmental stresses (McSorley 2003). Intrinsic adaptations occur at three levels: (a) diapause in the egg (J1), (b) developmental dormancy prior to egress in various nematodes and (c) sex reversal, mainly in *Meloidogyne* species (Triantaphyllou 1973; Papadopoulou and Triantaphyllou 1982). Extrinsic rapid responses to environmental stresses (cryptobiosis = anabiosis) involve modifications in nematode cuticles which eventually decrease their permeability to water and related gases during J2, J3 and J4 stages, depending on the nematode species (Bird and Bird 1991; McSorley 2003). Cryptobiotic responses to drought, low temperature, osmotic stress, low oxygen and high concentrations of toxic chemical compounds had been referred to as anhydrobiosis, cryobiosis, osmobiosis, anoxybiosis and chemiobiosis, respectively (McSorley 2003). Both intrinsic and extrinsic adaptations might in many respects be confounded to nemastatic responses observed in non-fumigant synthetic nematicides (Van Gundy and McKenry 1975). For example, when eggs used in hatching in vitro trials are allowed gradual permeation of chemicals to J1s, juveniles may enter the diapause stage, with the resultant failure of egress. Similarly, when cryptobiosis coincides with the application of any nematicide, the product might be rendered unfit for the intended purpose. Notwithstanding, conditions should be improved and specified during in vitro trials to establish efficacy of phytonematicides on nematodes in order to avoid confounding survival strategies

induced by gradual adverse effects on various stages of nematodes with the effects of phytonematicides.

7.6 Magnitude of Phytotoxicity in Phytonematicides

Allelochemicals as active ingredients in phytonematicides are naturally phytotoxic to other plant species during interference (Wuyts et al. 2006; Okwute 2012; Ntuli and Caboni 2012). In banana (*Musa acuminata*) trial, application of 200–400 g powdered neem seed kernels per mat at 6-month application interval induced phytotoxicity—characterised by complete wilting prior to fruiting (Musabyimana et al. 2000). Additionally, in survivor plants, the inflorescence failed to emerge (Musabyimana et al. 2000), resulting in a condition called choking, where the inflorescence could not emerge through the whorl of the pseudostem. Wild garlic (*Tulbaghia violacea*) bulbs contain sacrid volatile oils and sulpho-oxides—both being derivatives of allicin (Vijayalakshmi et al. 1996). Crude extracts of garlic bulb at 50 % concentration reduced population densities of plant-parasitic nematodes, but was highly phytotoxic to tomato seedlings (Sukul et al. 1974; Egunjobi and Afolami 1976). However, at 20 % concentration, there were no noticeable effects on tomato plant growth, while the product suppressed population densities of *M. incognita* (Agbenin et al. 2005). Oil from clove (*Eugenia caryophyllata*), when drenched using 0.1, 0.2 and 0.3 % concentrations at 0, 2, 5 and 7 days prior to transplanting cucumber (*Cucumis sativus*), muskmelon (*C. melo*), pepper and tomato seedlings, all concentrations were highly phytotoxic to all crops while reducing nematode populations (Meyer et al. 2008). Sensitivities of seedlings to clove oil from *E. caryophyllata* varied with plant species, with tomato seedlings being the most sensitive among all the test plants (Meyer et al. 2008). Generally, at transplanting, seedlings from various crops were all affected by oil at 0.2 and 0.3 % concentrations. The product contains eugenol as an active ingredient, which is naturally herbicidal at low concentrations (Walter et al. 2001; Tworkoski 2002; Waliwitiya et al. 2005; Bainard et al. 2006; Boyd and Brennan 2006; Boyd et al. 2006). Incide ntally, oilcakes from different plant species have high levels of phytotoxicity to various crops at various concentrations (Haseeb et al. 1980; Mian and Rodriguez-Kabana 1982a, b; Muller and Gooch 1982; Parmar 1996). Ahmad et al. (2013) demonstrated that ground leaves of adulsa (*Justicia adhatoda*) at 3 % (w/w) concentration were highly phytotoxic to tomato seedlings. Similar phytotoxic effects were observed from high concentrations of *L. camara* root extracts on various plant species (Shaukat et al. 2003).

Two phytonematicides from fruits of indigenous *Cucumis* species in South Africa are available in granular formulation, nemarioc-AG and nemafric-BG (Mashela et al. 2011), and liquid formulation, nemarioc-AL and nemafric-BL (Pelinganga et al. 2013a). Nemarioc-AG phytonematicide was shown to be highly phytotoxic to eight monocotyledonous and ten dicotyledonous crops, with most crops failing to emerge when 5 g crude extracts were applied as pre-emergent

drenches (Mafeo and Mashela 2009a, b, 2010). Similarly, both nemafric-BL and nemarioc-AL were highly phytotoxic to tomato seedlings when applied at above 10 % concentration after transplanting (Pelinganga and Mashela 2012; Pelinganga et al. 2013a, b). Nemafric-BL has cucurbitacin B ($C_{32}H_{48}O_8$), while nemarioc-AL contains two active ingredients, namely, cucumin ($C_{27}H_{40}O_9$) and leptodermin ($C_{27}H_{38}O_8$) (Rimington 1938; Jeffrey 1978). Except in rare cases such as pyrethrins that account for 80 % global uses of botanical pesticides, in purified form most active ingredients of phytonematicides, including azadirachtin-containing products, are not effective on nematode suppression, while they are highly phytotoxic to crops (Wuyts et al. 2006; Okwute 2012). Subsequently, most active ingredients in phytonematicides are applied as crude extracts.

7.7 Management of Phytotoxicity in Phytonematicides

Due to phytotoxicity and its zero tolerance in m ost legislative frameworks on products used in agriculture, literature is replete with efficacy trials which do not go beyond in vitro status. Using the concept of DDG patterns, there are basically three concentration ranges, namely, stimulation, neutral and inhibition concentration ranges (Fig. 7.2). Using the latter, we developed the concept of mean concentration stimulation range in an attempt to answer the farmers' question 'How much concentration of nemarioc-AL or nemafric-BL to apply?' which was followed by 'What is the application interval for the recommended concentration?'. The two questions were empirically answered, with avoidance of phytotoxicity and the efficacy of the products on nematode suppression in mind.

7.7.1 Establishing the Mean Stimulation Concentration Range

The potential uses of the CARD model rely on the availability of empirically generated data (Mafeo et al. 2011a, b, c; Pelinganga et al. 2012, 2013a, b). As an illustration, an experiment was conducted on tomato plants inoculated with 5000 eggs and second-stage juveniles (J2s) of *M. incognita*/plant and subjected to 0, 2, 4, 8, 16, 32 and 64 % concentrations of nemafric-BL (Fig. 7.3). At 56 days after initiating the treatments, plant variables were subjected to analysis of variance, with significant ($P \leq 0.05$) treatment means (Table 7.2) being further subjected to the CARD model to generate the quadratic relationships.

From the CARD-generated biological indices (Table 7.3), the actual values of R_h for the variables measured were computed (Table 7.4). The mean actual D_m and actual R_h values were used to establish the concentration stimulation range (CSR), which is representative of the stimulated growth in the test plant (Table 7.5).

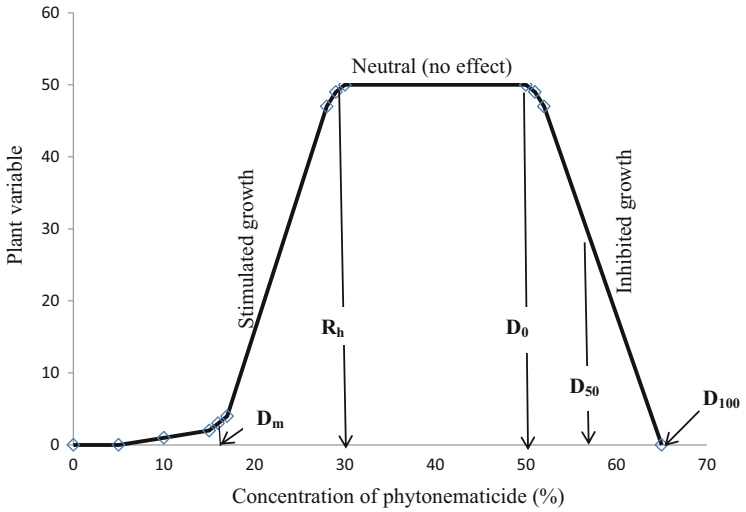

Fig. 7.2 Three distinct growth responses in density-dependent growth patterns

Fig. 7.3 Tomato seedlings for generating mean stimulation concentration range

Half the distance of the integrated CSR is referred to as the mean concentration stimulation range (MCSR). By definition, MCSR is the concentration of a phytonematicide which stimulates plant growth, while suppressing population densities of the target nematode (Pelinganga et al. 2013a; Mashela et al. 2014) and is quantified as:

Table 7.2 Means of plant growth in tomato seedlings in responses to increasing concentration of nemarioc-AL phytonematicide

	Dry shoot (g)	Dry root (g)	Plant height (cm)	Stem diameter (mm)
0	12.030	3.474	97.390	7.556
2	12.180	3.159	100.330	6.916
4	12.180	3.891	97.900	7.218
8	12.630	3.331	95.640	7.509
16	12.160	3.264	102.880	7.007
32	9.140	2.338	94.030	6.725
64	8.620	1.650	89.330	6.543
ns	ns	ns	ns	ns

ns not significant at $P \leq 0.05$

Table 7.3 Curve-fitting allelochemical response dosage-generated biological indices on tomato seedlings over six concentrations of nemarioc-AL phytonematicide

Biological index	Dry shoot mass	Dry root mass	Plant height	Stem diameter	Mean
Threshold stimulation (D_m)	2.533	2.195	2.734	1.534	2.224
Saturation point (R_m)	0.713	0.321	1.98	0.078	0.773
0 % inhibition (D_0)	11.482	9.209	19.942	5.420	10.961
50 % inhibition (D_{50})	164.897	59.003	2899.739	1603.200	957.165
100 % inhibition (D_{100})	703.5	170.3	2902.473	1604.735	1110.842
k	4	1	2	4	–
Sensitivity ranking: $\sum k = 11$					
$P \leq$	0.01	0.01	0.01	0.05	

Table 7.4 Demonstration of how MCSR is computed from threshold stimulation and saturation point biological indices

	Concentration of nemarioc phytonematicide				
Biological index	DSM^x	DRM	PHT	SDR	Mean
Threshold stimulation (D_m)	2.533	2.195	2.734	1.534	2.249
Saturation point (R_m)	0.713	0.321	1.980	1.612	0.773
Actual R_h value	3.246	2.516	4.714	1.612	3.022
Mean concentration stimulation range (MCSR)					2.63 %

MCSR $= (D_m + \text{adjusted} \quad R_h)/2 = (D_m + D_m + R_h)/2 = (2D_m + R_h)/2 = D_m + (R_h/2) = 2.244 + (0.773)/2 = 2.244 + 0.3865 = 2.6305 = 2.63$ % concentration would be non-phytotoxic to tomato plants

$$\text{MCSR} = (D_m + \text{adjusted } R_h)/2 \; = (D_m + D_m + R_h)/2 = (2D_m + R_h)/2$$
$$= D_m + (R_h/2)$$

Using actual mean D_m and R_h, values in the MCSR formula provided the values of 2.63 and 2.99 % for nemafric-BL and nemarioc-AL, respectively, in tomato plants (Pelinganga 2013). The MCSR value, which is empirically based on a series of phytonematicide concentrations, should be interpreted alongside the overall k-value of the plant to the test phytonematicide. The usefulness of a given product for use as a phytonematicide is entirely dependent on the overall sensitivity ($\sum$k) of the plant being protected to the product used (Liu et al. 2003). The k-values, which are plant and product specific, are generated using the CARD model and are defined as the number of *In* $(D+1)$ transformations that serves as a biological indicator of the degree of sensitivity of an organism to increasing concentrations of an allelochemical (Liu et al. 2003). The lower is the mean k-value, the higher is the sensitivity of the plant to the test allelochemicals and vice versa (Liu et al. 2003; Mafeo and Mashela 2010; Pelinganga and Mashela 2012). In CARD model, as the mean sensitivity ($\sum$k/n) values increase, coefficients of determination (R^2) also increase to a peak, where $k = $ i, followed by decreases from $i+1$ transformations until the model stops running (Liu et al. 2003). The three DDG patterns and the selected biological indices for nemarioc-AL phytonematicide on tomato plants were illustrated for various potential purposes (Fig. 7.4).

In both nemafric-BL and nemarioc-AL phytonematicides, MCSR values were established as being equivalent to 3 % concentration (Pelinganga et al. 2013b). In other words, for every 3 L stock solution of nemafric-BL or nemarioc-AL phytonematicides, 100 L chlorine-free water is used for application through drip irrigation. After empirically determining the amount to be applied per irrigation, the next step is to determine the application interval, which allows the computation of the application frequency—a factor required in the computation of dosage (D) = MCSR × application frequency.

7.7.2 Determining Phytonematicide Application Interval

The application interval (T) in days for the derived MCSR cannot be established using the CARD model since the latter is exclusively used when the x-axis represents increasing concentration of allelochemicals (Liu et al. 2003). The concept 'weeks-of-30-day-month' for the x-axis was developed for *Meloidogyne* species, where the x-axis was equivalent to 0, 1, 2, 3 and 4 'weeks-of-30-day-month' (Pelinganga and Mashela 2012; Pelinganga et al. 2013b). The unit 'weeks-of-30-day-month' was developed to enhance the capability of a phytonematicide to break the life cycle of *Meloidogyne* species since their life cycles under optimum conditions in tropical and subtropical areas is approximately 30 days. In *T. semipenetrans* with the life cycle of approximately 42 days, the unit would be

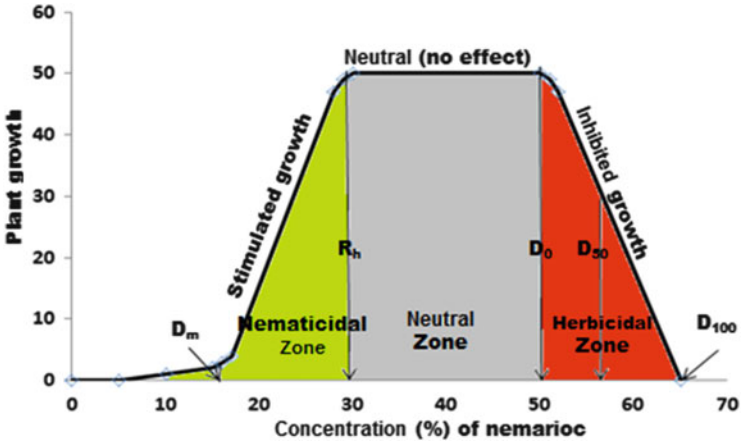

Fig. 7.4 Application of concentration model

'weeks-of-42-day-month'. Since empirical information is required to establish the application interval, experiments are usually established with each tomato seedling inoculated with 5000 eggs and J2s of *M. incognita* under greenhouse conditions (Pelinganga and Mashela 2012). Nematode population densities were managed using the empirically established MCSR value of 3 % for nemafric-BL at 0-day (untreated control), 7.5-day (1 week × 30 days/4 weeks), 15-day (2 weeks × 30 days/4 weeks), 22.5-day (3 weeks × 30 days/4 weeks) and 30-day (4 weeks × 30 days/4 weeks) application interval. At 56 days after the treatment, plant variables (y-axis) are then subjected to ANOVA, with significantly ($P \leq 0.05$) different treatment means being subjected to lines of the best fit to generate the quadratic relationships ($Y = b_2x^2 + b_1x + a$), where the optimum application interval was determined using $x = -b_2/2b_1$ in weeks (Table 7.5). In nemafric-BL 3 % and nemarioc-AL 3 %, the application intervals were 18 and 16 days, respectively (Pelinganga et al. 2012, 2013a). Doubling the concentration from 3 % to 6 % concentration had negligent effect on application interval of nemarioc-AL phytonematicide, but increased that of nemafric-BL from 18 days (2.40 weeks × 30 days/4 weeks) to 20 days (Pelinganga et al. 2013a).

In the use of nematicides applied into the soil, the concept of dosage is important and should be distinguished from dose and concentration (Van Gundy and McKenry 1975). Dose is an amount of chemical taken up by the target pest to effect detrimental behavioural changes, which may include disruption of juvenile development in eggs, egress, disoriented motility and/or mortality in nematodes (Van Gundy and McKenry 1975). In contrast, dosage (D) is the product of concentration (C) and the application frequency (T_{ca}), which could be summarised as:

$$D(\%) = C\ (\%) \times\ T_{ca}$$

The T_{ca} is the proportion of the crop cycle (days) to the application interval (days),

Table 7.5 Optimum application interval of nemarioc-AL phytonematicide at 3 % concentration on tomato seedlings

Variable	Quadratic relation	R^2	x
Dry root mass (g)	$Y = -0.3838x^2 + 1.5878x + 6.901$	0.92	2.07
Dry shoot mass (g)	$Y = -1.3405x^2 + 5.0903x + 44.374$	0.64	2.82
Dry fruit mass (g)	$Y = -0.8833x^2 + 4.9781x + 17.914$	0.65	1.90
Plant height (cm)	$Y = -0.7202x^2 + 3.6208x + 57.275$	0.88	2.51
Stem diameter (mm)	$Y = -0.3427x^2 + 1.1775x + 12.945$	0.65	1.72
			2.20

with the factor being unit-less. For instance, at 56 days under greenhouse or microplot conditions, T_{ca} values for nemafric-BL 3 % and nemafric-AL 3 % were 3.11 and 3.50, respectively. The model is primarily for seasonal crops, but can also be adapted for perennial crops since nematode population dynamics for various crops, particularly in citriculture, are well established (Duncan 2009).

7.8 Soil Allelochemical Residual Effects

The soil allelochemical residue (SAR) effects investigate post-application effects of phytonematicides on various successor crops and nematode population densities. Increasing the concentration and shortening the application intervals inherently increase the dosage in the soil and, therefore, might defeat the purpose of establishing the MCSR and T_{ca} values which are intended to ameliorate phytotoxicity. Doubling the concentration of phytonematicides may have negligent effects on the application interval, but serious consequences on dosage (Pelinganga et al. 2013a) and, thereby, SAR effects. The SAR effects of phytonematicides from *Cucumis* species were shown to have inhibitive effects of nodulation in *B. japonicum* (Mashela and Dube 2014) while having stimulation effects on growth of sweet-stem sorghum (Mashela 2014). In both cases, SAR effects reduced population nematode densities of *Meloidogyne* species. Additional work is still being under way to understand the chemistry of the SAR effects from phytonematicides.

7.9 Conclusion

Higher plants provide a broad spectrum of active ingredients for use in the management of plant-parasitic nematodes, with their principal drawback being phytotoxicity since their active ingredients comprise allelochemicals. The development of a phytonematicide where phytotoxicity is to be avoided consists of a series of steps. Firstly, there is need to establish whether the plant organ intended for use as a

phytonematicide has the potential to reduce population nematode densities under in vitro and/or ex vitro conditions. Secondly, a series of concentrations with known bioactivity effects on the target nematode under greenhouse conditions are used to establish the MCSR value, which is a non-phytotoxic concentration to the crop which is to be protected against nematodes. Thirdly, the MCSR is used to establish the application interval (days), which should be based on the unit that would allow the product to interrupt the life cycle of the target nematode. Using the proposed procedures, commercial phytonematicides could be a reality in the management of plant-parasitic nematodes.

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