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Organic and Inorganic Nitrogen Amendments to Soil as Nematode Suppressants

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Abstract: Inorganic fertilizers containing ammoniacal nitrogen or formulations releasing this form of N in the soil are most effective for suppressing nematode populations. Anhydrous ammonia has been shown to reduce soil populations of *Tylenchorhynchus claytoni, Helicotylenchus dihystera,* and *Heterodera glycines.* The rates required to obtain significant suppression of nematode populations are generally in excess of 150 kg N/ha. Urea also suppresses several nematode species, including *Meloidogyne* spp., when applied at rates above 300 kg N/ha. Additional available carbon must be provided with urea to permit soil microorganisms to metabolize excess N and avoid phytotoxic effects. There is a direct relation between the amount of "protein" N in organic amendments and their effectiveness as nematode population suppressants. Most nematicidal amendments are oil cakes, or animal excrements containing 2–7% (w:w) N; these materials are effective at rates of 4–10 t/ha. Organic soil amendments containing mucopolysaccharides (e.g., mycelial wastes, chitinous matter) are also effective nematode suppressants.

Key words: amendments, biological control, fertilizers, microbial ecology, nonchemical control, pest management, waste management.

Man has added organic and inorganic amendments to soil for centuries to improve soil fertility and increase crop yield. The nematicidal effect of some of these amendments has been recognized for some time, and reviews on the subject have been published (46,61). In developed countries the availability of effective nematicides has superseded the use of fertilizers and organic amendments for control of nematodes. Consequently, most of the research on the use of organic amendments for suppressing nematodes has been conducted in developing countries, principally in India (46). Linford et al. (29) were the first to study the nematicidal effects of organic amendments, incorporating chopped pineapple (*Ananas comosus*) leaves into soil for control of *Meloidogyne* spp. in cowpea (*Vigna unguiculata*). They noted that the population of free-living nematodes increased while that of *Meloidogyne* spp. decreased, and suggested that the increased organic matter supported microbial and animal species antagonistic to nematodes. Since then, many different types of organic amendments have been applied to a variety of crops to reduce populations of plant parasitic nematodes. Table 1 presents a list of selected nitrogenous amendments and the nematodes they have been found to be effective against.

As the potential of amendments to control parasitic nematodes became recognized, researchers began more complex studies. Miller et al. (40,41) and Kirmani et al. (27) varied the C:N ratio of organic amendments and found that when more nitrogen was available, nematode control was enhanced. Other researchers examined different forms of nitrogen to determine their relative effectiveness against nematodes. Eno et al. (15) demonstrated the effectiveness of anhydrous ammonia in field soil infested with species of *Hoplolaimus, Criconemoides, Trichodorus,* and *Belonolaimus.* More recently, Rodríguez-Kábana et al. (53,55) reexamined the nematicidal properties of anhydrous ammonia. In greenhouse studies, ammonia reduced soil populations of *Tylenchorhynchus claytoni* and *Helicotylenchus dihystera* when applied at rates of 62 mg N/kg soil or higher; root populations of *H. dihystera* or of *Hoplolaimus galeatus* were reduced only with rates of 125 mg N/kg soil. In three field experiments with soybean (*Glycine max*), planting time applications of anhydrous ammonia at rates of 0–224 kg N/ha were relatively ineffective in reducing late-season juvenile population densities of *Meloidogyne arenaria* (Neal) Chitwood, although significant yield increases were obtained in one experiment in response to the treatments. In another field experiment, ammonia at 56 and 112 kg/ha reduced population den-

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TABLE 1. Selected amendments to soil studied for their activities against plant-parasitic nematodes.

Amendment	Nematode	Test plant	Reference
Oil-cakes			
Castor bean	*Heterodera schachtii, Meloidogyne javanica, Meloidogyne* spp.	Tomato, sugarbeet	28
	Tylenchulus semipenetrans	Citrus	30,33
Karanj	*Meloidogyne* spp.	Tomato, okra	58
Castor, linseed, mahua, margosa, sesame, peanut	*M. javanica*	Tomato, okra	58,61
Castor, mahua, margosa, mustard, sesame, peanut	*M. incognita*	Tomato	19,26
Mustard, etc.	*Meloidogyne* spp.	Tomato	20
Fish	*M. hapla*	Sugarbeet	69
Castor, neem, peanut	*Helicotylenchus erythrinae, H. indicus, M. incognita, Rotylenchulus reniformis, Tylenchorhynchus brassicae*	Tomato, eggplant	25
Neem	*Helicotylenchus, Pratylenchus*	Maize, mung bean, wheat	50
Cotton seed, linseed, neem, mustard, peanut, sesame	*Hirschmanniella oryzae*	Rice	34
Castor, mahua, neem, peanut	*Aphelenchus avenae, Ditylenchus cyperi, M. incognita, T. brassicae*	Spinach	3
Karanj, neem	*M. incognita*	Okra	13
Cotton seed, mustard, sesame	*M. incognita*	(In vitro)	59
Mustard, peanut	*M. incognita, Meloidogyne* spp.	Pepper	64
Manures and composts			
Farm yard manure, compost	*H. rostochiensis*	Potato	65
Farm yard manure	*Heterodera* spp.	Turf	21
Steer manure, chicken manure, liquid fish	*T. semipenetrans*	Citrus	33
Composted timothy hay	*P. penetrans*	Strawberry	42
	Meloidogyne spp., *T. semipenetrans*	Citrus	30
Crotalaria, Kentucky blue grass, marigold	*M. hapla*	Sugarbeet	69
Pressmud, farm yard manure	*Meloidogyne* spp.	Okra	4
Chicken manure	*M. incognita*	Several	8,12
Chopped margosa, leaves, melia, sesbania, crotalaria	*M. javanica*	Tomato, okra	60
Miscellaneous			
Chitin	*M. incognita*	Tomato	31
	T. semipenetrans	Sweet orange	32
	P. penetrans	Cucumber	39
Alfalfa, lespedeza, oat hays	*M. incognita*	Tomato	24

sities of juveniles of *Heterodera glycines* in soil samples collected 14 days after planting. These field experiments also demonstrated that planting time applications of ethylene dibromide (4.7–18.6 liters/ha) together with anhydrous ammonia (56 or 112 kg N/ha) resulted in a soybean yield increase and accompanying control of *M. arenaria* and *H. glycines* superior to that obtained when each chemical was applied singly. Similar results were also obtained with combinations of ammonia and 1,3-dichloropropene in other soybean field experiments for control of *M. arenaria* and *M. incognita* (55). Rodríguez-Kábana et al. (53) were in agreement with Vassalo (66) in attributing the nematicidal properties of anhydrous ammonia principally to its plasmolysing effect in the immediate vicinity of its application point in the soil; however, their data on the effectiveness of ammonia against *H. glycines* also suggested other mechanisms were operating. They believed it possible that ammonia could exert a selective influence for microbial antagonists of *H. glycines,* particularly fungi (43,44,48,49). It was reasoned that since NH_4^+-N is the preferred source of N for many soil fungi (10), some fungal parasites of *H. glycines* could have increased in numbers following applications of NH_3 to soil. Proliferation of such fungal parasites in turn could have resulted in the observed reductions in *H. glycines* juvenile populations.

Walker (68) studied organic—peptone, soybean meal, skim milk, urea—and inorganic—KNO_3, $(NH_4)_2SO_4$, $(NH_4)_2CO_3$, NH_4OH—nitrogen sources and found that ammoniacal and organic nitrogen sources were more detrimental to nematodes than nitrate. Other common fertilizers also have been studied, and findings generally indicate that those containing ammoniacal nitrogen are more damaging to nematodes than those with nitrate nitrogen (6–8, 23,56).

Since ammoniacal nitrogen is detrimental to nematodes, urea has been studied as a nematicide. The compound is readily converted to ammonia by urease present in the soil, a necessary conversion if urea is to be effective both as a fertilizer and as a nematicide. Mojtahedi and Lownsbery (45) performed in vitro experiments in which urea was added to a mixture of nematodes with and without the addition of urease or urease-producing bacteria; nematodes were killed only in the presence of urease. Urea is a good nematicide when applied at levels in excess of 300 kg N/kg soil (22,52). Such high rates of urea result in significant accumulations of nitrate and ammoniacal N in soil and phytotoxicity (22). The phytotoxic effects of urea are due to the narrow C:N ratio of urea; there is insufficient available carbon in soil treated with nematicidal rates of urea alone to permit microbial utilization of all the available nitrogen. These detrimental accumulations of ammonia and nitrate can be overcome by supplementing urea amendments with additional available carbon. Studies at Auburn University have shown that combinations of blackstrap molasses plus urea (52) or of hemicellulosic paper waste plus urea (22) are not phytotoxic and are as nematicidal as additions of urea alone. In addition, these combination treatments stimulate microbial activity resulting in marked increases not only in urease activity but also in other soil enzymatic activities associated with microbial metabolism. In all cases, soil receiving the combination treatments had much greater enzymatic activity than soil treated with urea alone. The observed increase in numbers of microbivorous nematodes relative to soil treated with urea only reflected the increased microbial activity in soil treated with the combination treatments. This was interpreted as resulting from the short life cycle of these nematodes and the availability of large bacterial populations in soil that received the combination treatments.

The superiority of ammonia-releasing nitrogen sources over nitrates for suppressing nematodes has been demonstrated in experiments with *Rotylenchulus reniformis* on olives (6), *Criconemoides xenoplax* on plum (45), and *Tylenchulus semipenetrans* on citrus (5,7).

Studies of the nematicidal properties of inorganic N fertilizers indicate that to be effective these materials must be applied at levels far in excess of those required for crop fertilization. This perhaps explains why in some studies (57,67) inorganic N fertilizers, even ammonia-releasing materials, when applied at fertilizer rates have been ineffective against plant parasitic nematodes. There is also evidence that different rates are required according to the nematode species present. Eguiguren et al. (14) observed no significant decreases in *Tylenchorhynchus* spp. but reported a suppression of species of *Criconemoides* and *Trichodorus* in response to the same rate of nitrogen fertilizer. Miller (38) found urea inhibited *Pratylenchus penetrans* and *Hoplolaimus* sp. but was ineffective against *Tylenchorhynchus* sp.

The high rates of N required for consistent nematicidal activity from ammoniacal fertilizers can be expected to result in significant phytotoxicity through the accumulation of metabolic byproducts in the soil. As was demonstrated for urea, however, this can be obviated by formulating the fertilizers with carriers containing sufficient available carbon to ensure adequate stimulation of microbial metabolic activity. It should be possible through careful choice of appropriate organic carriers to enhance the nematicidal properties of inorganic N fertilizers, avoid phytotoxic effects, and provide excellent plant nutrition.

Additions of nitrogenous organic manures to soil have often been reported to reduce population densities of plant parasitic nematodes. Most efficacious of these amendments are those with low C:N ratios that release ammonia in soil. Effective materials of this type are oil meals and cakes, composts and animal ordures, and green manures. Table 1 presents a list of the types of materials utilized for nematode control, the target nematodes, and the crop species on which they have been tried.

There is a direct relation between the amount of Kjeldahl-determined N in organic amendments and their effectiveness as nematode population suppressors (36). Most nematicidal amendments contain 2–7% (w:w) N; these materials are effective when incorporated at rates of 4–10 t/ha. Soil incorporation of these materials stimulates the soil microflora leading to the release of ammonia through the activity of proteolytic and deaminating enzymes. These amendments typically result in the enhancement of soil urease activity and the accumulation of ammoniacal and nitrate nitrogen (35,37,51). As with urea, these amendments can be phytotoxic if sufficient carbon is not available to support metabolism of the added nitrogen. This phenomenon was illustrated in a recent study with chicken litter and oil cakes of peanut and cotton for control of *M. arenaria* (35). All three materials were effective in suppressing the nematode; however, the oil cakes (C:N ratios of 7) were phytotoxic, whereas the chicken litter (C:N = 10) was not.

Whereas the nematicidal effects of ammoniacal inorganic fertilizers and high-N organic amendments can be attributed for the most part to microbial activities connected with the N cycle in soil, very little is known about the roles of individual microbial species in suppressing nematodes—specifically, which microorganisms are able to decompose or transform the amendments in soil leading to the destruction of nematodes. Also, what, if any, toxins active against nematodes are released or synthesized through microbial activity during transformation of the nitrogenous amendments? Is there any relation between the proteolytic activity of the microflora and the ability of these microorganisms to destroy or parasitize nematodes? Are the enzymes needed to decompose the proteins of the amendments the same as those involved in the destruction of the nematode eggs, juveniles, or adults?

Some organic amendments are known to contain substances toxic to nematodes (1,2,4,5,28,61,62,63); thus, when added to the soil in sufficient quantity, they suppress nematode activity directly. Most interesting among nitrogenous amendments for control of nematodes are those containing chitin or similar mucopolysaccharides. Chitin is widely distributed in nature, being the second most common polysaccharide after cellulose (47). Crustacean chitin and chitinous materials of microbial origin (e.g., cell walls of some fungi) are readily available as waste products of the food and pharmaceutical industries. Chitin is a component of the middle layer of the tylenchoid egg shell (9). When chitin is added to soil it is depolymerized through chitinase activity in the soil (47,51). The end result of this process is the release of *N*-acetylglucosamine and presumably deamination of the sugar with consequent accumulation of ammoniacal N and nitrates in the soil (51). There is a particular microflora associated with the decomposition of chitin in soil (18,37,54). Chitin amendments are effective for control of *M. incognita* in tomato (31), reduction of the incidence of *Tylenchulus semipenetrans* on orange (32), and reduction of *Pratylenchus penetrans* and *Tylenchorhynchus dubius* on cucumber (39). Studies at Auburn University have shown that chitin amendments are also effective in the control of *H. glycines* (54) and *M. arenaria* (18,37). More important, with some exceptions an association has been established between the chitinolytic ability of fungi and their capacity to destroy nematode eggs (16–18,54). Several fungal species isolated from soil treated with chitin are able to decompose the polymer and are known parasites of eggs of *Meloidogyne* spp. or *H. glycines*, or have been isolated from diseased eggs of these nematodes (18,54). As with other nitrogenous amendments, chitin amendments can be phytotoxic because of the relatively narrow (6.4) C:N ratio of the polymer; however, this problem can be eliminated with additional available carbon (11). There is also evidence that some of the phytotoxic effects caused by chitin amendments may be related to changes in soil pH (unpubl.).

Results from the chitin studies have demonstrated that it is possible to choose the composition of an amendment to be added to soil so as to stimulate the development of a microflora parasitic or destructive to nematodes. Conceivably, organic amendments could be developed to select for microorganisms capable of decomposing the proteins or other materials that make up nematode cuticles or other structures. Stimulation of a selected microflora could then be coupled with the addition to soil of compounds capable of rendering nematodes or their eggs susceptible to attack by the microorganisms.

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