

## Efficacy of Neem Seed Derivatives against Nematodes Affecting Banana

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Soil applications of powdered neem seed or neem cake at 100 g/plant at planting and, subsequently, at 3-month intervals, reduced the populations of *Pratylenchus goodeyi* Sher & Allen and *Meloidogyne* spp. on par with Furadan 5G (carbofuran) applied at 40 g/plant at planting and then at 6-month intervals to banana plants grown in 100-l containers with controlled levels of banana nematode infestations. Eight months after planting, banana plants treated with powdered neem cake, seed or kernel or with neem oil had 4 to 95 times fewer parasitic nematodes than the untreated control. However, only neem cake powder or neem seed powder applied to unpaired banana plants kept the nematode population below the economic threshold.

KEY WORDS: Neem; *Azadirachta indica*; banana; *Musa* spp.; nematodes; *Meloidogyne* spp.; *Pratylenchus goodeyi*.

### INTRODUCTION

Banana (*Musa* spp.) is a popular and widely consumed tropical fruit that provides carbohydrates, proteins, vitamins and minerals to more than 400 million people worldwide (7). In eastern Africa it is a staple food and source of family income for more than 60% of the population (14,18). Banana yields are adversely affected by a number of crop pests (3,6). Nematodes that infest banana roots and cause toppling result in significant yield losses (3,16). Globally, banana yield losses due to nematodes average US\$178 million annually (17).

In the highlands of eastern Africa, the root-lesion nematode, *Pratylenchus goodeyi* Sher & Allen, and the root-knot nematodes, *Meloidogyne* spp., are the economically most important pests, and often found in mixed populations in banana fields (3). The burrowing nematode, *Radopholus similis* Cobb, is economically important at altitudes below 1000 m (23). Most of the highland banana cultivars belonging to the *Musa acuminata* genotype of Eastern Africa (AAA-EA) are highly susceptible to nematode infestations (14). The annual losses under optimal growing conditions in fertile soil may reach 30–35%, and exceed 75% in poor soil (18).

The economic threshold level (ETL) for chemical control of plant parasitic nematodes is 10,000 per 100 g of root (4). While chemical control can reduce the impact of nematode infestations, effective nematicides are too expensive for poor farmers with limited resources. In addition, their prolonged use can be hazardous to human health and the environment and may lead to pest resistance.

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Bioactive products from the neem tree, *Azadirachta indica* A. Juss. (family Meliaceae), are effective in controlling approximately 16 species of plant parasitic nematodes (2) and more than 400 species of arthropod pests of important food crops (21,22). Neem is widespread in Asia and Africa and has found varied uses in pest control, herbal medicine, toiletries, as a source of timber and fuel, and in reforestation programs (21). Neem products may provide an alternative, sustainable and inexpensive means of managing banana pests.

The bioactivity of neem materials against pests is attributed to the presence of an array of complex compounds, triterpenes, or more specifically limonoids (9). Alam (2) has reviewed the bioactivity of neem materials against some phytonematodes. However, little information exists on the efficacy of neem products against the key banana parasitic nematodes. The purpose of this study was to determine the efficacy of neem seed derivatives against *P. goodeyi* and *Meloidogyne* spp. affecting banana plants.

## MATERIALS AND METHODS

Experiments were conducted at ICIPE's Mbita Point Field Station (MPFS) in western Kenya, an important banana growing area. MPFS is located at an altitude of 1240 m. The annual rainfall averages 1100 mm, the temperature ranges from 18–33°C, and the soil is primarily sandy.

*Planting Material.* 'Nakyatengu' (AAA-EA), a nematode-susceptible banana cultivar popular for cooking, was planted. The suckers were collected from nematode-infested farmers' fields near the research site.

*Neem Seed Derivatives.* Fresh neem seeds, collected from ripe fruits harvested from 30-year-old neem trees growing at Garissa, northeastern Kenya, were cleaned and dried in the shade to 12–14% moisture content. Whole seeds or dehulled seeds were manually pounded in a large steel mortar with pestle to produce a fine neem seed powder (NSP) or neem kernel powder (NKP). Neem cake (NC) and neem oil (NO) were obtained simultaneously by cold-pressing whole seeds in a single-screw vegetable oil expeller (IBG Monforts, GmbH & Co., Mönchengladbach, Germany). The azadirachtin A content of the neem preparations, as determined by high pressure liquid chromatography, was found to be 4,000 ppm in NSP, 5,500 ppm in NKP, 5,800 ppm in NC, and 850 ppm in NO.

*Pot Experiment.* One-month-old, 50-cm-tall banana suckers, obtained from banana fields which had an infestation level of ~10,000 nematodes per 100 g of roots, were planted in 6-l-capacity pots, containing soil mixed with composted cow dung (1:1). Four weeks later, each plant was inoculated with approximately 500 banana parasitic nematodes in mixed population (mainly *P. goodeyi* and *Meloidogyne* spp.) by pouring a nematode suspension onto the soil around the plant. Nematodes for inoculation were extracted from fresh banana roots obtained from infested plantations having *P. goodeyi* as the predominant species (>70%) by a maceration-sieving technique (10,15), as attempts to rear them on carrot discs were unsuccessful. Two weeks after inoculation, NSP, NKP, NC, or Furadan 5G (carbofuran) was applied at 5 g/plant. Plants inoculated with nematodes, but not treated with neem or Furadan, served as the control. Potted plants were placed in a completely randomized design at 40 × 40 cm spacing in a screened greenhouse; each treatment was replicated four times and each potted plant constituted a replicate.

The experiment was terminated 45 days after treatment applications, *i.e.*, 90 days after planting. Plants were gently removed from pots and shaken to clear soil from the roots.

The roots of respective plants were evaluated for necrosis on a 0 to 4 scale. The necrosis index indicates the amount of cortical root tissues necrosed as a percentage of total root tissues (0 = no damage; 1 = slight damage, *i.e.*, <25% of total root cortex with necrosis; 2 = moderate damage, *i.e.*, 25–50% of the total root cortex with necrosis; 3 = severe damage, *i.e.*, 51–75% of total root cortex with necrosis; 4 = very severe damage, *i.e.*, >75% of total root cortex with necrosis (3).

For each treatment, the roots were bulked and the nematodes were extracted in a blender by maceration-sieving technique (10,15). To determine relative nematode populations, 1-ml aliquots were taken from respective nematode suspensions and placed in counting cells (Sedgewick Rafler S 50) and nematodes were counted using a compound microscope. The soil population of nematodes was not assessed because this is of little value for endoparasitic nematodes (19). Throughout the duration of the experiment, the daily temperature in the greenhouse ranged between 22 and 34°C.

*Outdoor Experiment.* A second experiment was conducted outdoors using 100-l-capacity oil-drums (40 cm high, 55 cm diameter) with a spacing of 3 × 3 m. Each drum contained soil mixed with ~5 kg of composted cow dung in which 3–4-month-old, ~1-m high, pared or unpared banana suckers were planted. The soil used was virtually free from endoparasitic banana nematodes as it was dug from a grassy fallow. Plants were irrigated and manually weeded. NC, NSP or NSK was incorporated into the soil around the plant at 100 g/plant at planting time and again at 3-month intervals. In the NO group, suckers were dipped in a 5% NO emulsion for 10–15 min prior to planting. In the nematicide treatment, Furadan was applied at 40 g/plant at planting time and then after 6 months.

The drums were arranged in a randomized design. Ten treatments, comprising pared and unpared suckers with various neem treatments or Furadan, and the control (unpared and untreated), were replicated four times. Each drum constituted a replicate. Forty days after planting, the plants were inoculated with a mixed nematode population by pouring a suspension containing approximately 2000 nematodes into the soil around each plant. The nematode populations were recorded for each treatment at 3 and 6 months after planting before applying neem or Furadan.

Eight months after planting, plants were uprooted and the root necrosis index and the nematode population per 100 g roots was recorded. The roots for nematode extraction were collected from an excavation extending 20 cm outward from the corm and a bulked sample of 25 g of roots was taken for each treatment. Data were analyzed by ANOVA and Tukey's Test was used to compare means (20).

## RESULTS

The nematode population in bananas planted in either pots or drums was: ~60% *P. goodeyi*, ~35% *Meloidogyne* spp. and <5% *R. similis*, *Helicotylenchus multicinctus* Golden and *Hoplolaimus* spp.

In potted plants treated with neem, the root damage, as determined by the necrosis index, was significantly less than that in untreated plants; Furadan-treated plants also had less root necrosis but not significantly different from control (Table 1). Two months after inoculation, the nematode population in the neem treatments was 5% (NSP), 15% (NKP) and 30% (NC) of that in untreated plants and, in the Furadan treatment, 15%. In addition, in neem-treated plants, 75% of the nematodes were juveniles, while in the control, 90% of the nematodes were adults (not given in Table).

TABLE 1. Efficacy of neem seed powder (NSP), neem kernel powder (NKP) and neem cake (NC) against banana parasitic nematodes as compared with Furadan in potted plants in a screened greenhouse at 2 months after nematode inoculation with a mixture of *Pratylenchus goodeyi* and *Meloidogyne* spp. (500 nematodes per plant) (Mbita Point Field Station, 1996)

Treatment	Necrosis index (0–4 scale) <sup>z</sup>	Nematode population (no./100 g of roots) <sup>z</sup>
NSP	0.00a	500a
NKP	0.25a	1500a
NC	0.25a	3000a
Furadan 5G	0.50ab	1500a
Control (untreated)	1.25b	10000b

<sup>z</sup> Within columns, means followed by a common letter do not differ significantly ( $P < 0.05$ ; Tukey's test); averages of four replicates.

In the outdoor experiment, 2 months after treatment, the nematode population in neem-treated and in Furadan-treated plants was significantly less than in untreated plants (Table 2). Eight months after planting, this significant difference in the nematode population between neem- or Furadan-treated plants and the untreated controls remained. Even unpaired suckers treated with NC or NSP had 95 and 32 times fewer nematodes, respectively, than the untreated, unpaired suckers.

Classification of the total nematode population in the 8-month drum experiment observation (Table 2) according to species showed that *P. goodeyi* and *Meloidogyne* spp. were the predominant species infesting banana plants (Table 3). *P. goodeyi* and *Meloidogyne* spp. appeared to be highly sensitive to treatment with NC or NSP.

TABLE 2. Effect of soil application of neem seed powder (NSP), neem cake (NC), neem kernel powder (NKP), or of treatment with neem oil (NO) on population of banana nematodes at 2 and 8 months after treatment of pared or unpaired suckers planted in drums outdoors (Mbita Point Field Station, 1997)

Treatment	Nematode population (no./100 g of roots) $\pm$ SEM <sup>z</sup>	
	at 2 months	at 8 months
Pared	1200 $\pm$ 489a	22200 $\pm$ 3747a
Pared + Furadan	0 $\pm$ 0a	16800 $\pm$ 2135a
Pared + NC	0 $\pm$ 0a	12000 $\pm$ 2135a
Pared + NSP	0 $\pm$ 0a	22500 $\pm$ 2265a
Pared + NKP	0 $\pm$ 0a	81600 $\pm$ 23510b
Unpared + NC	300 $\pm$ 300a	1200 $\pm$ 0a
Unpared + NSP	300 $\pm$ 300a	3600 $\pm$ 490a
Unpared + NO	0 $\pm$ 0a	5700 $\pm$ 1025a
Unpared + NKP	125 $\pm$ 125a	27600 $\pm$ 3730a
Unpared (untreated)	25050 $\pm$ 4057b	114000 $\pm$ 4673b
CV%	95.7	50.4
Difference <sup>y</sup>	**	**

<sup>z</sup> Within columns, means followed by the same letter do not differ significantly ( $P < 0.05$ ; Tukey's test); averages of four replicates.

<sup>y</sup>\*\*\* =  $P < 0.01$  (Tukey's test).

TABLE 3. Effect of soil application of neem cake (NC), neem seed powder (NSP), neem kernel powder (NKP) or of treatment with neem oil (NO) on relative distribution of nematode species in banana roots 240 days (8 months) after treatment and 200 days after inoculation with 2000 nematodes per plant (Mbita Point Field Station, 1997)

Treatment	Nematode population (no.)/100 g root				
	<i>Radopholus similis</i>	<i>Pratylenchus goodeyi</i>	<i>Meloidogyne</i>	Others	Total population (no.)
Pared	1111	11544	7560	1985	22200
Pared + NC	1200	3600	6024	1176	12000
Unpared + NC	28	960	180	32	1200
Pared + NSP	19	2976	19125	380	22500
Unpared + NSP	36	2880	620	64	3600
Pared + NKP	6528	58752	15312	1008	81600
Unpared + NKP	1656	21528	4014	402	27600
Pared + Furadan	1176	3360	6132	6132	16800
Unpared + NO	513	4734	206	508	5700
Unpared (untreated)	4560	79800	24500	5140	114000

## DISCUSSION

Neem products have been shown to be effective against nematodes attacking vegetables and legumes (1). Neem cake alone was found to be an effective nematicide and gave even better control when combined with synthetic nematicides (2). Neem oil and Nimin (containing neem triterpenes) as urea coating agents, and root-dip or seed treatment with neem extracts, have also been found to be nematicidal against several species of parasitic nematodes in vegetables. However, soil amendment with neem seems to be the most practical method for nematode control (2). The roots of plants raised in neem-cake-amended soil appear to undergo physiological changes that render them unsuitable for nematode penetration and development, thus inducing a certain degree of resistance in plants against nematode infestation (2). The bioactive principles in crude extracts of neem seed have also been reported to inhibit the penetration, hatchability and development of nematodes (13).

In the present study, the efficacy of soil applications of azadirachtin-rich NC or NSP in reducing especially *P. goodeyi* and *Meloidogyne* spp. infestation in banana plants was shown. The results support earlier reports of decreased infestations of *Meloidogyne incognita* Chitwood and *Tylenchorhynchus brassicae* Siddiqi following NC application in vegetables and legumes (1,12,13). In addition, NC application was shown to reduce *Pratylenchus zae* Graham infestation in sugarcane (11). However, similar studies of the control of the banana lesion nematode, *P. goodeyi*, were lacking.

Although 8 months after planting, paring alone became ineffective in reducing nematode population, unpared neem-treated suckers suppressed the nematode population well below the ETL of 10,000 nematodes per 100 g roots. This finding could have practical applications in the integrated management of banana nematodes. By applying neem, the labor-intensive practice of paring and hot water treatment of banana corms before planting (to reduce the banana parasitic nematode load) could be avoided, leading to substantial saving of time and money.

The finding that NC application caused a reduction in nematode populations similar to

that achieved with Furadan 5G concurs with another recent report, according to which NC application was as effective as Furadan 3G in reducing the *P. zeae* population in sugarcane fields (11).

After 8 months of incorporation into the soil, NC or NSP application was still effective against banana nematodes, while the nematicidal activity of Furadan 5G seemed to decline. Davide (5) also reported that Furadan 3G controlled *R. similis* for 3 months but thereafter its effectivity gradually declined.

Dipping the rhizomes of banana suckers in a nematicide solution has been considered as one way of disinfecting the planting material (18). In our tests, dipping the banana suckers in 5% NO emulsion did reduce the banana nematode populations, but the NO treatment was unsatisfactory, as it was phytotoxic. The NKP treatment also caused banana plants to dry up within 5 months due to high oil content in the kernel, which may have interfered with water absorption and assimilation of nutrients.

NC- or NSP-treated banana plants appeared healthier than the untreated controls. The observed enhanced vigor of neem-treated banana plants might have been due to reduced nematode infestations and possibly also to the nutritive value of NC or NSP. Neem cake is rich in organic matter (N, 5.5-7.1%; P, 1.1%; K, 1.5%) and has been used as a manure by farmers in India (8).

The high level of nematode infestation observed in untreated control plants in this study indicates that sandy soils are ideal for parasitic nematode multiplication, as has been observed previously by others (10,18). The effectiveness of neem seed derivatives for banana nematode management is of practical significance since, unlike chemical methods, the use of NC or NSP requires no special equipment or extra capital investment by farmers. In addition, the broad spectrum of biological activity of neem materials against parasitic nematodes and insect pests would confer dual benefits on the users. Trials using neem derivatives for the management of banana nematodes are in progress in farmers' fields in Kenya.

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