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Breakdown of Azadirachtin A in a Tropical Soil Amended with Neem Leaves and Animal Manures*[1]

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ABSTRACT

A field investigation was conducted to assess the breakdown of azadirachtin A in a tropical coastal savanna soil amended with neem leaves (NL) combined with poultry manure (PM) or cow dung (CD) using gas chromatography. Samples in polythene bags 15 cm long and 4.8 cm in diameter were randomly placed to a depth of 14 cm in the soil, and azadirachtin A concentration was assessed on days 0, 14, 28, 42, 56, 70, and 84. Azadirachtin A degradation in the soil followed first-order reaction kinetics with different half-lives obtained for varying combinations of the amendments. Higher neem amendment levels of 100 g gave shorter half-lives of azadirachtin A than the lower levels of 50 g. Within the 50 g NL group the additions of the poultry manure and the cow dung gave significantly shorter ($P < 0.05$) half-lives of azadirachtin A than the sole neem amendment, whereas in the 100 g NL group only additions of 10 g CD and 10 g PM were significantly less ($P < 0.05$) than the sole neem amendment. Different changes resulting from the kind and quantity of animal manure added were observed in the half-lives of azadirachtin A. The 100 g NL group had significantly higher ($P < 0.05$) moisture content, which, coupled with the likely differences in microbial biomass, could be the major factor responsible for variations in the half-life of the compound. Therefore, the quantity of the neem leaves applied and the addition of animal manure affected the breakdown of azadirachtin A in the soil amended with neem leaves.

Key Words: azadirachtin A breakdown, gas chromatography, manure, neem leaves, soil amendment

Synthetic pesticides have been used to control soil pests, leading to positive gains in agricultural production (Johnston *et al.*, 1995; Sharma and Sharma, 1995; Sultan *et al.*, 1995). Their use, however, has resulted in the disruption of ecosystems because of the effects on non-target species, accumulation of pesticide residues in the environment and in food, and build-up of pesticide resistance in the target species. Generally in developing countries, people suffer from short-term exposure to synthetic chemicals (including that resulting in suicide) and chronic effects of long-term exposure (Fening, 1999). These negative effects of synthetic pesticides on the environment have led to the search for alternative means of pest control (Powers *et al.*, 1993; Johnson *et al.*, 1995; Sarathchandra *et al.*, 1996; Kerry and Bourne, 1996). In this regard neem seed and leaf soil amendments have proved successful in the control of soil parasitic plant nematodes (Haseeb *et al.*, 1998; Musabyimana and Saxena, 1999). Neem contains a mixture of 3 or 4 related limonoids together with 20 or more others that are minor, but nonetheless active, with azadirachtin proving to be the main pesticidal agent (BOSTID, 1992). Azadirachtin A and B account for about 99% of the array of azadirachtins in the neem plant with azadirachtin A predominating (Isman *et al.*, 1996).

In the quest to control soil pests, information on the behaviour of azadirachtin as it breaks down in the soil is imperative. Studies on the degradation of azadirachtin A in the soil using high performance liquid chromatography (HPLC) have been carried out under controlled laboratory conditions (Stark and Walter, 1995) and in the greenhouse (Sundaram, 1996), both using different neem formulations as the source of the active ingredient. The present study, however, was designed to assess the breakdown

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of azadirachtin A in a soil amended with raw neem leaves combined with poultry manure or cow dung under tropical field conditions using gas chromatography (GC).

MATERIALS AND METHODS

The field experiment was conducted at the technology village of the University of Cape Coast, Ghana (5.11° N, 1.25° W). The soil belongs to the Benya series of the local classification system and is a Haplic Acrisol according to FAO (1988).

Dried neem leaves (NL), poultry manure (PM), and cow dung (CD) ground and passed through a 2-mm sieve were used in different combinations each added to one kilogram of soil as treatment: 50 g NL, 50 g NL + 10 g CD, 50 g NL + 5 g PM, 50 g NL + 10 g PM, 100 g NL, 100 g NL + 10 g CD, 100 g NL + 5 g PM, and 100 g NL + 10 g PM. The soils unamended and amended with animal manure only were taken as the controls. Each amendment was placed in a polythene bag of 4.8 cm diameter and 15 cm long leaving 1 cm of space at the top. The weight of the polythene bag and treatment was 300 g. There were three replicates. Each replicate represented a batch of treatments. Batches of replicates were placed in the soil on September 16, 2002, in a completely randomized design. One polythene bag per replicate was collected for azadirachtin extraction immediately after placement with subsequent samplings after 14, 28, 42, 56, 70, and 84 days. In the absence of rainfall watering was conducted twice weekly to keep the samples moist.

The highest soil temperature was measured at 15:00 hours and the lowest at 06:00 hours (Nathan and Malzer, 1994) twice each week throughout the study with a thermometer long enough to reach the depth of soil measured. The occurrence of vertical variations in azadirachtin concentration within samples as a result of temperature changes (Table I) within the soil environment was eliminated through thorough mixing of the individual representative samples before analysis.

TABLE I

Minimum and maximum soil temperatures for selected depths at the experimental site

Soil depth	Temperature	
	Minimum	Maximum
cm	°C	
1	25.4 ± 0.91	46.8 ± 8.69
14	28.2 ± 1.19	38.8 ± 2.69

For soil moisture, known weights (10 ± 0.01 g) of samples placed in 100 mL beakers were oven-dried overnight at 105 °C. The samples were reweighed and the moisture content calculated through the difference of the two readings (Rowell, 1994). To determine pH, sample weights of 10 ± 0.1 g were placed into 50-mL centrifuge tubes with 25 mL of distilled water being added to each sample. Tubes were capped and shaken by hand for 15 minutes and the pH was measured with a pH meter (Rowell, 1994).

The extraction of azadirachtin and gas chromatography (GC) analysis of extracts were conducted at the Chemistry Department of the Kwame Nkrumah University of Science and Technology, Ghana. The soil samples were air dried for 48 hours, and then, 50 g each was defatted with ether, followed by extraction with 200 mL methanol for 6 hours using a Soxhlet apparatus. Next, extracts were filtered with Whatman No. 1 filter paper and reduced to just dryness using a rotary evaporator at 20 °C. Reconstitution of extracts was made with 10 mL of GC grade methanol before the quantification of azadirachtin A with GC. A Perkin-Elmer gas chromatograph Model 1022 Plus equipped with a flame ionic detector (FID) was operated at oven, injection, and detector temperatures of 180, 265, and 265 °C, respectively, with an O-17 column being used for separation of the azadirachtin A. A flow rate of 15 mL per minute was utilized for the carrier gas (N_2), with sample injection volume of 5 μL. Standard analytical grade azadirachtin A was used as the standard to identify and quantify the unknown azadirachtin A in the

samples. The azadirachtin A concentrations in the neem leaves before soil amendment, unamended soil and soil amended with only animal manure as a background level were also found through the same processes of extraction and analysis.

The half-life (time required for 50% loss of the azadirachtin A in the soil) of azadirachtin A in the samples was calculated using the following equation (Stark and Walter, 1995):

$$t_{1/2} = \frac{0.6931}{k}$$

where $t_{1/2}$ = half-life and k = rate constant or slope, obtained from a regression analysis of the $\log_e$ azadirachtin A concentration in treatment (average of the three replicates) against incubation period. The data were subjected to analysis of variance (ANOVA) and the Duncan's multiple range test for the separation of means using the MSTAT-C statistical software (Freed, 1992).

RESULTS AND DISCUSSION

GC chromatograph of azadirachtin A

The graphical presentation of GC chromatograph in Fig. 1a showed that the retention of the standard analytical grade azadirachtin A occurred at 7.9 minutes. The unamended soil and soil amended with only the animal manure showed no peaks in the chromatograph (Fig. 1b). Therefore, peaks observed in the neem-amended soil sample chromatograph (Fig. 1c) were the results of the compounds in the neem leaves. The other peaks alongside azadirachtin A (Fig. 1c), which could be from some of the limonoids of neem, however, were not identified.

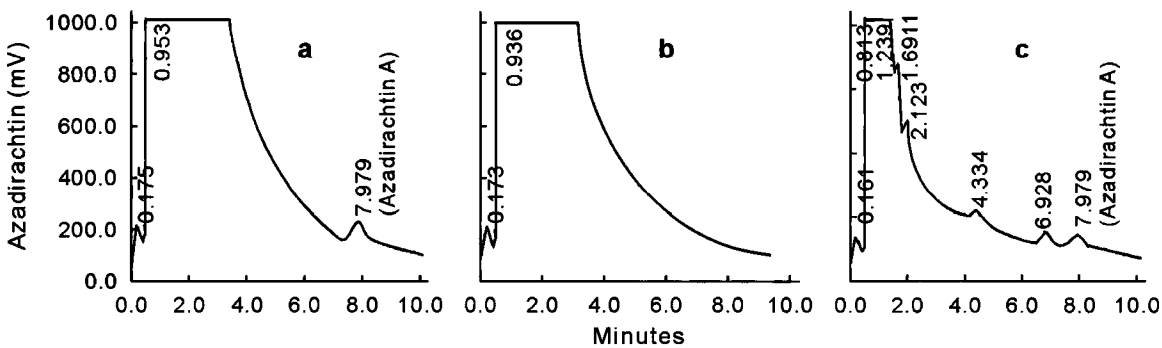

Fig. 1 GC chromatograph of standard analytical grade azadirachtin A (a), unamended soil and soil amended with animal manure only (b), and soil amended with neem leaves (c).

Azadirachtin A concentration in neem leaves and recovery from the soil

The concentration of azadirachtin A in neem leaves was found to be 3.31 μg g^{-1} neem leaves. Sundaram (1996) found the concentration of azadirachtin A in 100 g of neem leaves from India to be 0.59 mg (5.9 μg g^{-1} neem leaves), which was higher than the present concentration. This difference may be attributed to different environmental conditions, such as soil, climate and rainfall, where the neem was collected, and also the collection, processing and storage conditions of the neem leaves (Sundaram, 1996). The different analytical procedures in the current study (gas chromatography) and in that of the Sundaram (1996) (high performance liquid chromatography) might also contribute to the different azadirachtin A concentrations in the leaves.

Based on the current azadirachtin A concentration of 3.31 μg g^{-1} neem leaves, the percentage recovery of the compound in the neem-amended soil immediately after amendment was calculated (Table II). With no specific pattern observed among the treatments, the percentage recovery ranged between 54.26% and 85.98%, which compared favorably with that of Stark and Walter (1995). The percentage

recovery of azadirachtin A in soils obtained by Stark and Walter (1995) was found to be 80.4 ± 12.12% and was considered to be acceptable for analysis.

TABLE II

Percentage recovery of azadirachtin A extracted from soil treatments immediately after amendment of neem leaves and animal manure

Amendment[a)]	Expected recovery	Amount recovered	Percentage recovery
g kg^{-1} soil	mg g^{-1} soil		%
50 NL	0.1655	0.0898	54.26
50 NL + 5 PM	0.1655	0.1423	85.98
50 NL + 10 CD	0.1655	0.1275	77.04
50 NL + 10 PM	0.1655	0.1287	77.76
100 NL	0.3310	0.2299	69.46
100 NL + 5 PM	0.3310	0.2446	73.90
100 NL + 10 CD	0.3310	0.2618	79.09
100 NL + 10 PM	0.3310	0.2393	72.30

[a)]NL = neem leaves; PM = poultry manure; CD = cow dung.

Azadirachtin A breakdown in the soil

The regression curves of $\log_e$ azadirachtin A in the amended soil *versus* days of incubation are shown in Figs. 2 and 3, with the corresponding half-lives presented in Table III. It could be found that the breakdown of azadirachtin A followed first-order kinetics (Stark and Walter, 1995; Wan *et al.*, 1997). The half-life was inversely proportional to the rate constant and, therefore, the sharper the slope of the curve or the more negative the slope, the smaller the half-life. Treatments with higher neem amendment levels of 100 g kg^{-1} soil (Fig. 3) had steeper slopes with shorter half-lives of azadirachtin A ranging between 12.2 and 22.3 days (Table III) as compared to the lower amendment levels of 50 g neem leaves kg^{-1} soil with half-lives between 28.6 and 42.3 days. Within the 50 g NL group the additions of the poultry manure and the cow dung gave significantly shorter ($P < 0.05$) half-lives of azadirachtin A than the sole neem amendment, whereas in the 100 g NL group only additions of 10 g cow dung and 10 g poultry

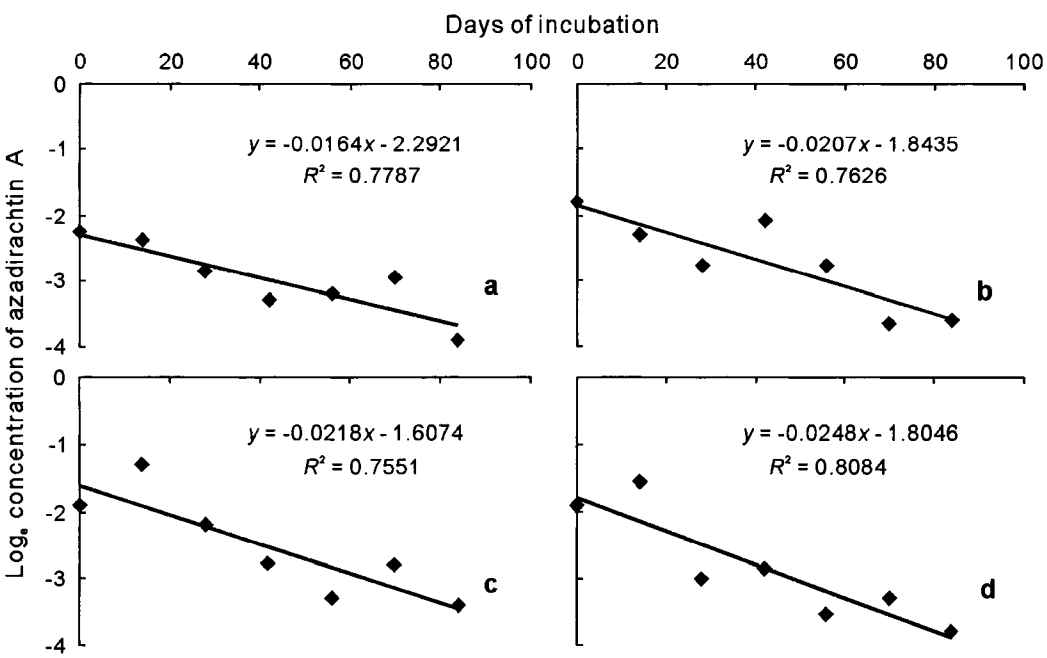

Fig. 2 Azadirachtin A breakdown in 1 kg of soil amended with 50 g neem leaves (a), 50 g neem leaves + 5 g poultry manure (b), 50 g neem leaves + 10 g cow dung (c), and 50 g neem leaves + 10 g poultry manure (d).

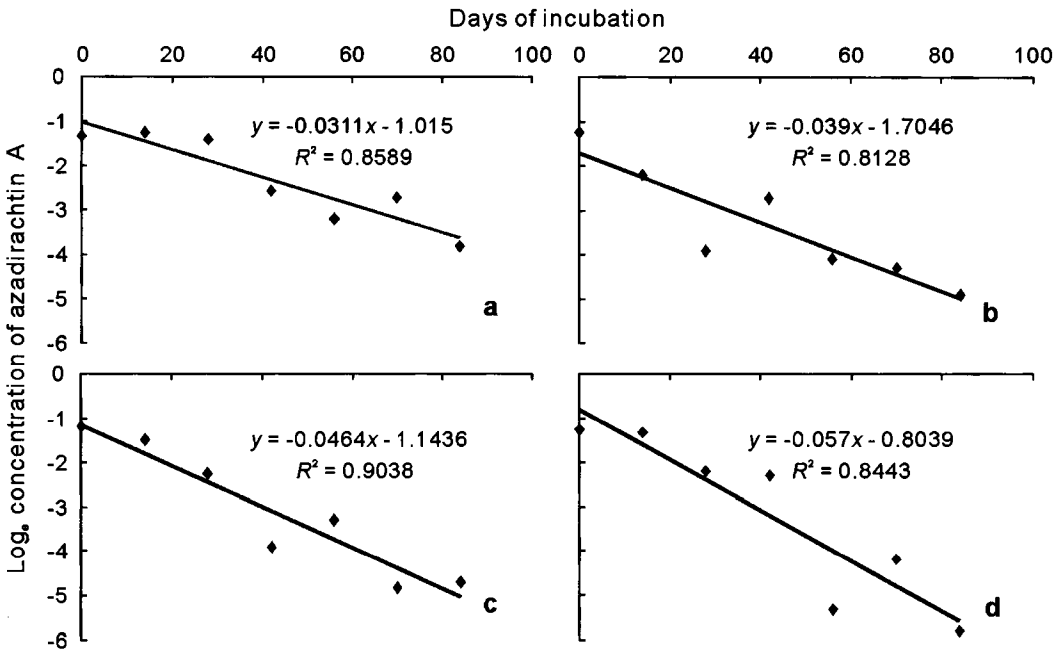

Fig. 3 Azadirachtin A breakdown in 1 kg of soil amended with 100 g neem leaves (a), 100 g neem leaves + 5 g poultry manure (b), 100 g neem leaves + 10 g cow dung (c), and 100 g neem leaves + 10 g poultry manure (d).

TABLE III

Half-life of azadirachtin A in different soil amendments of neem leaves and animal manure

Amendment[a)]	Half-life of azadirachtin A
g kg^{-1} soil	d
50 NL	42.26 a[b)]
50 NL + 5 PM	33.48 b
50 NL + 10 CD	31.79 b
50 NL + 10 PM	28.63 bc
100 NL	22.29 cd
100 NL + 5 PM	17.79 de
100 NL + 10 CD	14.32 e
100 NL + 10 PM	12.19 e

[a)]NL = neem leaves; PM = poultry manure; CD = cow dung.
[b)]Figures with the same letter are not significantly different ($P < 0.05$).

manure were significantly less ($P < 0.05$) than the sole neem amendment. Within the 50 g and 100 g groups, half-lives of azadirachtin A in the soil changed with the kind and the quantity of animal manure (cow or poultry) used, but the differences were not significant ($P < 0.05$) (Table III).

Other researchers have observed similar trends in the breakdown of azadirachtin A in the soil with changing soil conditions. Stark and Walter (1995) found the half-life of the compound at 25 °C for non-autoclaved soil to be 19.8 days and autoclaved soil at the same temperature to be 31.5 days. At 15 °C the half-lives in the non-autoclaved and autoclaved soils were 43.9 and 91.2 days, respectively. They attributed the differences in the half-life of the compound to faster degradation in non-autoclaved soil due to microorganisms as well as higher temperatures. Sundaram (1996) in a similar study found azadirachtin A at the same temperature conditions of 21 ± 2 °C and moisture content of 30 ± 2% to have a half-life of 25.8 days in a non-autoclaved soil and 35.6 days in an autoclaved soil. Sundaram (1996) also attributed the differences in the half-lives of the azadirachtin A to faster microbial degradation of the compound in the non-autoclaved soil. Stark and Walter (1995) and Sundaram (1996) used neem

formulations of RH-9999-20WP (Rohm and Hass experimental neem formulation) and Margosan-O, respectively. However, raw neem leaves were used in the current study. Since application of plant and animal manure in the soil increases soil microbial populations (Acea and Carballas, 1996), the different levels of the organic amendments in the current study could be the major factor in the half-life differences of azadirachtin A.

The quantity of organic matter in the soil affected the breakdown of organic compounds in soils (Morrill *et al*, 1982). Organic matter, in proportion to the amount added, provided substrates for many soil microbes (fungi, actinomycetes, and bacteria) and promoted their proliferation in the soil. These soil microbes, then, enhanced the breakdown of azadirachtin in the soil (Stark and Walter, 1995; Sundaram, 1996).

Moisture as a factor promoting the breakdown of organic compounds in the soil (Morrill *et al.*, 1982) could have contributed to the observed trend of the azadirachtin A breakdown in the soil as the higher levels of soil amendments (100 g NL) had significantly higher moisture content than the lower level (50 g NL) (Table IV). However, the contribution of pH to the breakdown of azadirachtin A in this study could not be considered as a factor as there were no significant differences in the pH of the amendments (Table IV). Also, samples were exposed to the same varying environmental field temperature conditions (Table I), thus eliminating temperature differences as a factor in the azadirachtin A breakdown.

TABLE IV

Moisture content and pH of soils amended with neem leaves and animal manure

Amendment[a)]	pH	Moisture content
g kg^{-1} soil		g kg^{-1}
50 NL	6.3 a[b)]	116.7 b
50 NL + 5 PM	6.6 a	104.5 b
50 NL + 10 CD	6.4 a	111.6 b
50 NL + 10 PM	6.6 a	121.0 b
100 NL	6.5 a	151.1 a
100 NL + 5 PM	6.4 a	169.0 a
100 NL + 10 CD	6.3 a	160.1 a
100 NL + 10 PM	6.6 a	160.0 a

[a)]NL = neem leaves; PM = poultry manure; CD = cow dung.
[b)]Figures with the same letter within columns are not significantly different ($P < 0.05$).

CONCLUSIONS

The breakdown of azadirachtin A in the soil varied with the amendment levels of neem leaves and animal manure. The higher level of neem leaves (100 g) had significantly ($P < 0.05$) shorter half-lives of azadirachtin A than the lower level of neem leaves (50 g) in combination with 5 g PM and 10 g CD. Furthermore, within its respective group (50 or 100 g neem leaves) the addition of poultry manure and cow dung in most cases significantly enhanced ($P < 0.05$) the breakdown of azadirachtin A. Therefore, the quantity of the neem leaves applied and the addition of animal manure affected the breakdown of azadirachtin A in the soil amended with neem leaves.

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REFERENCES

Acea, M. J. and Carballas, T. 1996. Microbial response to organic amendments in a forest soil. *Bioresource Technology.* **57**: 193–199.

Board on Science and Technology for International Development (BOSTID). 1992. Neem: A Tree for Solving Global Problems. CD-Rom Library 2.0, Global Help Projects & Humanity CD Ltd. Antwerp, Belgium.

Fening, J. O. 1999. Health hazards of pesticides usage in Ghana: The case of vegetable growers in the Akomadan district. *Journal of the Ghana Science Association.* **1**: 1–3.

Food and Agriculture Organization (FAO). 1988. Soil Map of the World. Revised Legend. World Soil Resources Report 60. FAO, Rome.

Freed, R. D. 1992. How to Use MSTAT-C to Analyze on-Farm Experiments. Farming Systems Conference Sept. 1992. Michigan State University, East Lansing.

Haseeb, A., Shukla, P. K. and Butool, F. 1998. Influence of hydrodistillation wastes of medicinal plants, oil cakes and pesticides on *Meloidogyne incognita* infesting *Ocimum canum. International Journal of Nematology.* **8**: 77–80.

Isman, M. B., Matsuura, H., MacKinnon, S., Durst, T., Neil Towers, G. H. and Arnason, J. T. 1996. Phytochemistry of the Meliaceae: So Many Terpenoids, So Few Insecticides. *In* Romeo, J. T., Saunders, J. A. and Barbosa, P. (eds.) Phytochemical Diversity and Redundancy in Ecological Interactions. Plenum Press, New York. 319pp.

Johnson, A. W., Burton, G. W., Wilson, J. P. and Golden, A. M. 1995. Rotations with coastal Bermuda grass and fallow for management of *Meloidogyne incognita* and soil borne fungi on vegetable crops. *Journal of Nematology.* **27**: 457–464.

Johnston, S. A., Probasco, P. R. and Phillips, J. R. 1995. Evaluation of soil fumigants and nematicides for the control of root-knot nematodes on carrot. *Fungicide and Nematicide Test.* **50**: 187.

Kerry, B. R. and Bourne, J. M. 1996. The importance of rhizosphere interactions in the biological control of plant parasitic nematodes—a case study using *Verticillium chlamydosporium. Pesticide Science.* **47**: 69–75.

Morrill, L. G., Mahilum, B. C. and Mohiuddin, S. H. 1982. Organic Compounds in Soils: Sorption, Degradation and Persistence. Ann Arbor Science Publishers, Inc., Collingwood. 326pp.

Musabyimana, T. and Saxena, R. C. 1999. Efficacy of neem seed derivatives against nematodes affecting banana. *Phytoparasitica.* **27**: 43–49.

Nathan, M. V. and Malzer, G. L. 1994. Dynamics of ammonia volatilization from turkey manure and urea applied to soil. *Soil Science Society of America Journal.* **58**: 985–990.

Powers, L. E., McSorley, R. and Dunn, R. A. 1993. Effects of mixed cropping on a soil nematode community in Honduras. *Journal of Nematology.* **25**: 666–673.

Rowell, D. L. 1994. Soil Science: Methods and Applications. Longman Scientific & Technical, UK. 350pp.

Sarathchandra, S. U., Watson, R. N., Cox, N. R., DiMenna, M. E., Brown, J. A., Burch, G. and Neville, F. J. 1996. Effects of chitin amendment of soil on microorganisms, nematodes, and growth of white clover (*Trifolium repens* L.) and perennial ryegrass (*Lolium perenne* L.). *Biol. Fertil. Soils.* **22**: 221–226.

Sharma, G. L. and Sharma, M. K. 1995. Management of root-knot nematodes *Meloidogyne incognita* and *Meloidogyne javanica* in tomato by bare root dip treatment. *Indian Journal of Nematology.* **29**: 174–176.

Stark, J. D. and Walter, J. F. 1995. Persistence of azadirachtin A and B in soil: Effects of temperature and microbial activity. *J. Environ. Sci. Health B.* **30**(5): 685–698.

Sultan, M. S., Sharma, S. K., Singh, I. and Iderjit, S. 1995. Effect of some chemicals and late sowing on the disease intensity of root-knot nematode *Meloidogyne incognita* and yield of black gram. *Plant Disease Research.* **1**: 86–88.

Sundaram, K. M. S. 1996. Azadirachtin biopesticide: A review of studies conducted on its analytical chemistry, environmental behaviour and biological effects. *J. Environ. Sci. Health B.* **31**(4): 913–948.

Wan, M. T., Rahe, J. E. and Wong, C. 1997. Persistence of azadirachtin-A in two biological systems used for culturing the mycorrhizal fungus *Glomus intraradices. J. Environ. Sci. Health B.* **32**: 929–953.