The neem *Azadirachta indica* as a means to control soil nematodes and its application in vegetable cultures in Benin.

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Summary

The use of neem extracts for pest control is less common in nematology than in entomology. The purpose of this paper is to make a short review of the agronomical potential of the neem tree, with particular emphasis on its role for the control of deleterious nematodes. A specific case for the control of Meloïdogyne, *which was carried out in Benin, is presented.*

Résumé

L'emploi d'extraits de neem, arbre tropical à vertus insecticides-nématicides, est moins connu en nématologie qu'en entomologie. L'objectif de cet article est de faire un bilan de la potentialité de cette plante, et plus particulièrement pour le contrôle des nématodes nuisibles du sol. Une étude spécifique pour la lutte contre Meloïdogyne, *réalisée au Bénin, est présentée.*

Introduction

Several plant products are already used in agriculture for crop and post harvest control against insects. The most important chemicals are pyrethrum (from *Chrysanthemum cinerariaefolium*), nicotine (from *Nicotiana tabacum*) and rotenone (from *Derris elliptica, Lonchocarpus* sp. or *Pachyrrizus* sp.) (3,18).

Azadirachtin and the related molecules, extracted from the neem (*Azadirachta indica*), is another natural insecticide which shows real potentialities for the future. There is a considerable advance in laboratory research. The active ingredients are well characterized and their biological activity on insects or nematodes is well documented. Moreover, field research, particularly carried out in India, has shown for a long time that the neem is a potentially useful insecticide. The interest for such kind of crop protection agents is greatly justified taking into account the economical specificities and the ecotoxicological aspects of the tropical agriculture.

There is a need to develop simple methods requiring low levels of inputs for the vegetables growers in Africa. Intensive use of pesticides should not be encouraged because these products are generally too costly for the small growers and because these growers are not enough informed about the risks for the health (during the treatment, presence of toxic residues in food, ...) and for the environment (water pollution through the wells, ponds, etc...). Moreover, the choice of the chemical to be used, the application rate and frequency, the timing of the treatment with respect to the harvest, the risks to develop resistencies among the pests and the lack of selectivity towards the natural enemies are some examples of all the parameters to be taken into account in order to provide a safe and sustainable control.

The use of natural non-toxic pesticides could be an interesting alternative owing to their low cost and availability in numerous tropical countries. The neem tree, for instance, is already widespread and, in addition, can be easily multiplied. Its extracts have a large activity range against deleterious insects and nematodes. Neem products are, on the other hand, characterized by anti-feeding and systemic properties (translocation into plants) (19), which provide a better selectivity towards non-phytophageous insects or other useful organisms (20). Neem is not toxic for mammals (12) and has been used for ages in India in traditional medicine and as a local tooth brush. In addition, the quick degradation of the neem products in field conditions limits their accumulation in nature.

The applications of crude neem extracts, however, have a major disadvantage. The active ingredient concentration in the plant is far from being constant. It depends on the tree genotype, the environmental conditions or the extracts preparation methods. Consequently, the protective effect can be in some cases unsignificant and has to be confirmed under local conditions.

Description of the neem tree

The neem tree, also called margosa tree or Indian lilac (*Azadirachta indica*, syn.: *Antalaea azadirachta, Melia indica*) belongs to the Meliaceae (Mahogany) family. It occurs in tropical and subtropical areas of Africa, America, Australia and Asia, from where it originated. It is interesting for its numerous uses as afforestation of dry areas, «fuel» wood production, ornamental tree, traditional medicine and natural pesticide. This deciduous or evergreen tree can reach up to 25 meters height. The fruits appearing as drooping panicles are 1.4 to 2.4 cm long and are produced once a year, sometimes twice. Average fruit yield is about 20 kg/tree/year and can reach up to 50 kg/tree/year. The leaves of 30 cm length are unpaired binnates (7 to 17 asymetric serrate leaflets). The flowers are white and small (24).

Active compounds of the neem and preparation of neem products.

Azadirachtin, a triterpenoïd, is considered to be the most important active compound in *A. indica*. The quantity of this compound varies considerably within the plant parts but is

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also influenced by environmental factors, possibly for genetic reasons. The seeds contain the greatest concentrations of azadirachtin. In fact, this product is not a single chemical but includes a family of isomers (A to G) in which isomer A is the most important in terms of quantity, and isomer E the most effective as insect growth regulator (24).

Numerous other active compounds were isolated from the seed kernels, and most of them showed an antifeedant activity for insects. Among those various compounds, nimbin, deacetyl-nimbin and thionemon were identified as the active chemicals against soil nematodes (29).

The use of pure azadirachtin is not feasible in agriculture because it is too expensive. For trials or field applications, neem extracts can be obtained in using several methods of preparation. All plant parts have nematocidal properties. Their efficacy decreases in the following order: fruits, leaves, bark, flowers, gum and roots (25).

The first neem preparation is the aqueous extract. The latter is obtained from dried neem seeds and from dried or fresh leaves. Shelled or unshelled seeds are crushed at respectively single or dubble quantities and are mixed in more than twenty times their weight of water. After several hours of maceration, the mixture is filtered and sprayed on plants in large quantities. The same kind of preparation is obtained with the leaves of the neem. Soil treatments can also be done with those extracts.

Alcoholic extracts from seeds or leaves are used too, but they are more expensive and time consuming than the water extracts. Besides, there is an enriched formulation based on alcoholic extracts from seed which has been patented in the U.S. under the name of «Margosan-O».

Neem oil, obtained by pressing neem seeds, is used for insects control, most particularly in stored grains. Neem kernels contain up to 50% of oil. After addition of an emulsifier, a mixture of oil and water, at a low percentage of oil, can be sprayed on the plants.

The cake which is left after the pressing of neem seed kernels can be used as organic fertilizer. It has been proved that an effective control of soil nematodes is obtained by applying the neem cake to the soil at a rate of 1.000 kg/ha or even more.

A last mode of application of the neem products is by composting various parts of the neem tree and use them as soil amendments (26).

The use of neem extracts in agriculture

1. Protection against insects

The interest of neem for the control of plant pests was first demonstrated against insects. The potential of this material is mainly due to various biological effects on insects behaviour or development: settling, oviposition or feeding behaviours, metamorphosis, fecundity, egg-sterility or vigor of insects. The use of different neem extracts has found several applications in plant protection (foliar treatments with aqueous extracts) and post harvest protection (stored grains), most particularly against a wide range of caterpillars and the larvae of various beetles species (24,25).

2. Protection against nematodes

In nematology, the effects of neem treatments are not well documented but it has already been proved that the neem cake is in some cases highly deleterious for several nematodes species as: *Dorylaimoïdes sp. (28), Heliotylenchus erythrinae* (14,25), *Hoplolaimus indicus* (14), *Meloïdogyne arenaria* (20), *M. incognita* (4,25,28,29), *M. indica* (17), *M. javanica* (28,30), *Pratylenchus brachyurus* (9), *P. penetrans* (9), *Rotylenchulus reniformis* (4,25), *Tylenchorhynchus brassicae* (4,25), *T. elegans* (27), *Tylenchus filiformis* (25) and *Tylenchulus semipenetrans* (15).

The neem is effective against nematodes in different ways: global toxicity of the soluble water extract (4), decrease of the egg laying capability (29), reduction of larvae hatching (13), reduction of nematode mobility (28), reduction of nematodes populations (11), inactivation of the larvae and reduction in juveniles' penetration in the roots (1,7,8,29), reduction of root galling by root knot nematodes (10,11), and increase of resistance at the plant level to the nematodes' invasions (29).

3. Other favourable effects on plants

Neem cake is obtained from the seed kernels after extraction of the oil which is needed for soap production or other uses. Neem cake applications in soil have shown a stimulating effect on the blue-green algal growth, mainly by depressing predators' activity in soil. Algae biomass was higher in treated soil than in untreated situations (6). A similar effect is observed with *Azolla pinata* (a water fern used more particularly as natural soil amendment) by reducing the detrimental effect of soil salinity and enhancing the survival in summer conditions (18).

Populations of *Nitrosomonas* and *Nitrobacter* (ammonium and nitrite oxidizers) are reduced by neem cake applications and the treatments are beneficial in terms of nitrogen supply to the soil (22). It has also been proved that coating of urea with neem cake is effective in slowing ammonitrification and nitrification (21).

Chemical analysis of plant tissues has shown that plants growing in neem cake treated soils contain greater concentrations of phenol and frequently also amino-acids, proteins and carbohydrates. This consequently increases the resistance of plants to nematodes' attacks. In addition, the treatment with neem cake increases the content of phenolic compounds in the soil (1).

Furthermore, it seems that neem oil extracts can partially inhibit the degradation of carbofuran (systemic nematicide) (5) and also cause an increase of the mobility of other soil applied nematicides such as dazomet, oxamyl and dichlorfenthion (26).

Experiments against root knot nematodes in South Benin.

Root knot nematodes (*Meloïdogyne* sp.) are one of the greatest problems for the vegetables growers of South Benin. Neem cake has been shown to control this major pest in three different trials (2) and the main results are summarized in this section.

The crops selected in these studies were three leafy vegetables: jute (*Corchorus olitorius*), celosia (*Celosia argentea*)

and lettuce (*Lactuca sativa*). They were cultivated according to the local culture methods, on 2 m² bed with a standard spacing of 25 × 25 cm. Protective treatments were applied three days before transplanting by mixing the products (Table 1) with the upper level of soil. The treatments' design in the field was a complete randomized block with four replications of 15 or 24 plants by plot. Harvesting started 45 days after transplanting. The weight of the vegetables' leafy parts was measured and the galling rates were recorded. The latter were established using a gall scale at six levels starting from the lowest value (0) for healthy plants and giving the highest score (5) to the most affected plants (100% galling of the roots).

For the three crops, there were significant effects of the different protective treatments, with a maximum of 200% yield increase for lettuce (neem cake treatments) and with a reduction of the galling rates, generally around 30%. This is a clear demonstration of the interest of the neem cake for the soil nematodes control in South Benin conditions.

TABLE 1

Relative values of harvested leaves yields (Y) and galling rates (GR) of leafy vegetables protected against root knot nematodes with chemical and neem cake treatments at two concentrations.

Protective treatments (*)	Leafy vegetable species: jute (*Corchorus olitorius*)		Celosia (*Celosia argentea*)		Lettuce (*Lactuca sativa*)	
	Y	GR	Y	GR	Y	GR
Control	100	100	100	100	100	100
Carbofuran	124	66	124	65	170	73
Neem cake 1	129	71	117	70	285	62
Neem cake 2	165	50	125	96	306	63

(*): Control = soil without treatment, carbofuran at 0.6 g a.i./m², Neem cake 1 and 2 = respectively at 0.5 and 1.0 kg/m².

Discussion and Conclusion

In Benin, the research works on the neem have been carried out for several years and the results in local conditions have confirmed its interest as a protective tool against stored grain pests and field pests.

In this work, we have shown that neem cake was effective to protect three vegetables species growing in Benin and that this treatment could be favorably compared to carbofuran, a well known chemical insecticide-nematicide. It must be noted, however, that it is not clear whenever the increased yields could be attributed to the sole nematocidal effect of the neem cake or whether an additional effect, due to enrichment of the soil in organic matter, has to be taken into account. Nevertheless, it is quite encouraging to note that the neem cake, an easily available, cheap, and safe plant material could give a satisfactory protection against the nematodes' attacks under field conditions.

At present, the immediate purposes of further works are to offer to the farmers a natural product which cumulates the following advantages: a cheaper and easily prepared standard product, which has stable protective properties against soil pests and which is, last but not least, a good soil amendment.

Literature

1. Alam, M.M., Ahmad, M. & Khan, A.M., 1980. Effect of organic amendments on the growth and chemical composition of tomato, eggplant and chilli and their susceptibility to attack by *Meloïdogyne incognita*. Plant and soil, **57**: 231-236.
2. Agbakli, R., Maraite, H. & Colin, J., 1992. L'utilisation des extraits de neem (*Azadirachta indica* Juss) pour lutter contre les nématodes à galles (*Meloïdogyne* sp.) dans les cultures de légumes feuilles au Bénin. Med. Landbouw. Univ. Gent, **3**: 913-918.
3. Anonyme, 1979. Tropical legumes. Resources for the future. National Academy of Sciences (Washington DC), 331 p.
4. Alam, M.M., Khan, A.M. & Saxena, S.K., 1982. Relative toxicity of decomposed and undecomposed oilcakes to plant parasitic nematodes. Acta Botanica Indica **10**: 124-127.
5. Attri, P.S. & Prasad, R., 1981. Preliminary studies on the inhibition of carbofuran degradation in soil by neem cake and neem oil extractives. Pesticides **15**: 6-7
6. Aziz, T., 1981. Effect of differently applied rice straw and neem cake on nitrogen transformation in flooded soils. College Laguna (Phillipines) 122 p.
7. Bhattacharya, D. & Goswami, B.K., 1987. Comparative efficacy of neem and groundnut oil-cakes with aldicarb against *Meloïdogyne incognita* on tomato. Revue de Nématologie **10**: 467-470.
8. Bhattacharya, D. & Goswami, B.K., 1988. Effect of oil-cakes used alone and in combination with aldicarb on *Meloïdogyne* infecting tomato. Nematologia Mediterranea **16**: 139-141
9. Egunjobi, O.A. & Afolami, S.O., 1975. Effects of watersoluble extracts of neem (*Azadirachta indica*) on *Pratylenchus brachyurus* and on maize. Journal of Nematology **7**: 321
10. Gupta, D.C. & Kali Ram, 1981. Studies on the control of *Meloïdogyne javanica* infecting chickpea in different types of soil. Indian Journal of Nematology **11**: 77-80.
11. Haseeb, A., Pandey, R. & Husain, A., 1988. A comparison of nematicides and oilseed cakes for control of *Meloïdogyne incognita* on *Ocimum basilicum*. Nematropica **18**: 65-69.
12. Jotwani, M.G. & Srivastava, K.P., 1981. Neeminsecticide of the future. III-Chemistry, toxicology and future strategy. Pesticides **15** (12). 12-19.
13. Khan, A.M., Adhami, A., Siddiqui, Z.A. & Saksena, S.K., 1967. Effect of different oil cakes on hatching of larvae and on the development of root knot causing by *Meloïdogyne incognita*. Int. Symp. Pl. Path. New Delhi (India), 65 p.
14. Khan, M.W., Khan, A.M. & Saxena, S.K., 1973. Influence of certain oilcake amendments on nematodes and fungi in tomato fields. Acta Botanica Indica **1**: 49-54.

15. Mohammad, H.J., Husain, S.T. & Al-Zarari, A.J., 1980. Effect of oil-cakes amended soil on the growth of *Citrus reticulata* L. and the control of citrus nematode in Iraq. Phytopathologia Mediterranea **19**: 153-154.

16. Nandabalan, K. & Kannaiyan, S., 1986. Effect of soil amendments on summer growth and survival of *Azolla pinnata* (India). International Rice Research Newsletter (Philippines) **11**: 29.

17. Nanje Gowda, D., Shivanandappa, N. & Karegowda, C., 1985. Efficacy of soil amendments in control of root-knot nematodes in tobacco nursery. Journal of Soil Biology and Ecology **5**: 131-133.

18. Purseglove, J.W., 1987. Tropical crops. Dicotyledons. Ed. Longman Scientific and Technical (New York), 719 p.

19. Radwanski, S., 1977. Neemtree. 3: Further uses and potential uses. World Crops and Livestock. **29**: 168-169.

20. Rodriguez-Kabana, R., Morgan-Jones, G. & Chet, I., 1987. Biological control of nematodes: soil amendments and microbial antagonists. Plant Soil **100**: 237-247

21. Sarkunan, V. & Bidappa, C.C., 1981. Effect of the neem cake urea on the exchangeable ammonia and yield and uptake of nitrogen by lowland rice. Mysore Journal of Agricultural Sciences **15**: 540-554

22. Santhi, S.R., Palaniappan, S.P. & Purushothaman, D., 1986. Influence of neem leaf on nitrification in a lowland rice soil. Plant and Soil **93**: 133-135.

23. Schauer-Blume, M., 1988. Einfluss von Extraktionsrueckstaenden aus Niemsamen (*Azadirachta indica*) auf den Nematoden *Pratylenchus penetrans*. Gesunde Pflanzen **40**: 229-233.

24. Schmutterer, H., 1990. Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. Annu. Rev. Entomol. **35**: 271-297.

25. Schmutterer, H. & Hellpap, C., 1989. Effects of neem on pests of vegetables and fruit trees. In Focus on phytochemical pesticides. Vol I, The neem tree (Jacobson M., ed.) Boca Raton: CRC Press: 69-85.

26. Singh, R.P., Khan, A.M. & Saxena, S.K., 1979. Effect of castor, groundnut and neem oil cakes on the movement of three nematicides in soil. Indian Journal of Nematology **9**: 82.

27 Sitaramaiah, K., 1977. Effect of temperature of decomposition of margosa cake and on survival of *Tylenchorhynchus elegans*. Indian Journal of Nematology **7**: 167-169.

28. Sitaramaiah, K. & Singh, R.S., 1978. Role of fatty acids in margosa cake applied as soil amendment in the control of nematodes. Indian Journal of Agricultural Sciences **48**: 266-270.

29. Sitaramaiah, K. & Singh, R.S., 1978. Effect of organic amendment on phenolic content of soil and plant and response of *Meloïdogyne javanica* and its host to related compounds. Plant and Soil **50**: 671-679.

30. Zaiyd, M., 1977. Effect of organic soil amendments on the incidence of root-knot nematode (*Meloïdogyne javanica*) on bhindi plants. Proceedings of the Bihar Academy of Agricultural Sciences **25**: 23-26.

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