Abamectin and Azadirachtin as Eco-friendly Promising Biorational Tools in Integrated Nematodes Management Programs

Mohamed S Khalil*

Central Agricultural Pesticides Laboratory, Agricultural Research Center, El-Sabaheya, Alexandria, Egypt

Abstract

Despite the usefulness of nematicidal compounds in agricultural practices, but it cause environmentally problems, which lead to seek about safe and alternative agents as tools to be used in the plant nematodes management programs.

Abamectin is one of the suggested alternative biocidal tool that belongs to avermectin group, which belong to macro cyclic lactones metabolites produced by a natural fermentation of the bacterium Streptomyces avermitilis. Abamectin mixture contains more than 80% avermectin B1a and less than 20% avermectin B1b. Meanwhile, abamectin used as an insecticide, acaricide and nematicide on vegetables, fruits and field crops.

On the other hand, neem oil is a vegetable oil pressed from the fruits and seeds of the neem tree, Azadirachta indica. The neem tree was recognized for its unique properties against insects, and in improving human health. It is grown in most tropical and sub-tropical areas. Its leaves, bark, and seed kernels extracts, or cake, can be utilized in phytomycicides management.

Various components such as nimbins, nimbidins, azadirachtins, salannins, thionemons, and meliantriol occur in the seeds, leaves, and the bark of neem with high concentrations. The effect of neem against plant parasitic nematodes was noticed and recorded in many reports, as well as its insecticidal, fungicidal and bactericidal efficacy.

Keywords: Avermectins; Abamectin; Neem oil extract; Azadirachtin; Plant parasitic nematodes

Introduction

Plant parasitic nematodes found to be one of the most widespread diseases in the last two decades that attract the attention of researchers, especially those in the field of plant protection. There are thousands of species of plant parasitic nematodes (PPN), which cause damages in quality and quantity of yields in varied crops; moreover, increasing the costs of production. The most famous and destructive genus around the world is the root-knot nematodes (Meloidogyne spp.), because of its wide host range which included more than 2000 hosts, such as vegetables, fruit trees, oil crops, fiber crops, grains crops and feeding crops, in addition, weeds which are considered secondary host to nematodes.

Hence, the most researches are concerned by the root-knot nematodes, which are found to be the most important and being responsible for at least 90% of all damage caused by nematodes [1], as well as plant parasitic nematodes, causes an estimated $118b annual loss to world crops [2].

In 1976, scientists at Merck & Co. Inc. discovered a complex of eight closely related natural products, named avermectins in a culture of Streptomyces avermitilis, MA-4680 (NRRL 8165), obtained from isolate by the Kitasato Institute from soil samples collected at Kawana, Ito city, Shizuoka Prefecture, Japan [3]. The avermectins are closely related to another group of pesticidal natural products, the milbemycins, which was the first examples described by Japanese workers, but later found to be more abundant in nature than the avermectins [3].

Avermectin B1 (abamectin), is the major component of the fermentation which showed the ability to control mites and insect pests on a variety of agricultural and horticultural crops worldwide. Abamectin has shown low toxicity to non-target beneficial arthropods that help its acceptance into Integrated Pest Management (IPM) programs, besides supporting the safety to man and the environment [4].

Certain reports unanimously recorded that abamectin has nematicidal efficient against the root-knot nematode and other genus in several crops [5-10].

The Neem tree (Azadirachta indica) originated in India and Myanmar, but is now found throughout the Indian subcontinent, and can be grown in sub-tropical and tropical areas [11]. Neem oil contains many triterpenoid compounds, the main component is azadirachtin, whose final synthesis was completed in 2007 and published in 2008 [12].

Neem components have attracted global attention for their insecticidal, fungicidal, bactericidal and nematicidal properties [13]. Crude neem extracts have been used at a local and small-farm level for some time in countries where neem grows. In the major countries such as USA, Canada and Europe, the commercial neem insecticides have reached the markets.

Azadirachtin is one of the most interesting constituents of neem which is derived from seed kernels, that has been shown influence on insect feeding behaviour and insect developments, and indicated highly activity against a number of insect pests [14,15]. Also, the toxicological profile of azadirachtin is generally favorable [16-18].

*Corresponding author: Mohamed S Khalil, Central Agricultural Pesticides Laboratory, Agricultural Research Center, El-Sabaheya, Alexandria, Egypt, E-mail: melonemia@gmail.com

Received February 12, 2013; Accepted April 19, 2013; Published April 24, 2013

Citation: Khalil MS (2013) Abamectin and Azadirachtin as Eco-friendly Promising Biorational Tools in Integrated Nematodes Management Programs. J Plant Pathol Microb 4: 174 doi:10.4172/2157-7471.1000174

Copyright: © 2013 Khalil MS. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
Azadirachtins contains many isomers from A up to I, and the most studied includes azadirachtin A, azadirachtin B, nimbin and salannin [19]. It has been demonstrated that neem products are very effective in reducing the phytoparasitic nematodes and improve plant health. In same time, the ability of eggs to hatch and mobility of juveniles is reduced by various neem products.

The interest in developing pesticides of natural origins has increased during recent years, because of the adverse effects of synthetic chemical pesticides, like impact on environment, toxicity to non target organisms including humans, and resistance development in insect population.

Regarding the integrated nematodes, management need many efforts from the researchers to find new tools that could be used in IPM programs. In spite of this, there are varied methods to manage plant nematodes, but the chemical nematicides are still the most used and widespread method. Usage of biorational agents as one of new trends in IPM programs, still need to develop. Several strategies have been proposed against plant parasitic nematodes that included fungi, bacteria, entomopathogenic nematodes, plant extracts, soil amendments, as well as metabolites of fungi and bacteria.

Therefore, this work aimed to through a light on alternative and effective tools that could be used in integrated nematodes management programs, as a safe method on human health and the surrounding environment.

**Abamectin**

Avermectins were discovered and developed by the scientists from Merck Sharp Dohme Research Laboratories [20,21]. The avermectins contain four pair’s compounds which contain four major components \( A_1, A_2, B_1, \) and \( B_2 \), and four minor components \( A_1, A_2, B_1, \) and \( B_2 \).

The macrocyclic lactones (avermectins and milbemycins) are products or chemical derivatives of soil microorganisms belonging to the genus Streptomyces. The avermectins are a family of 16-membered macrocyclic lactones, such as avermectin, abamectin, doramectin, eprinomectin, and selamectin [22].

Abamectin is a natural fermentation product of the soil bacterium Streptomyces avermitilis [23,24]. Furthermore, abamectin is a blend of avermectin \( B_1 \) and \( B_2 \), which contain at least about 80% avermectin \( B_1 \) and 20% avermectin \( B_2 \). These two components, \( B_1 \) and \( B_2 \), have very similar biological and toxicological properties.

Abamectin is not only used to control insect and mite pests of citrus, pear, and nut tree crops, but also leafy vegetables, fruiting vegetables, plum, prune, avocado, mint, and basil. It is used by home owners to control the fire ants, and as cockroach toxic baits. Meanwhile, abamectin proved his activity against plant parasitic nematodes, therefore, there is a new formulation used as seed treatment under trade name Avicta®, to protect the plants in the initial growing phase from insects and plant parasitic nematodes which attack root system.

Abamectin has shown low toxicity to non target beneficial arthropods, which was considered a motivation to use it in IPM programs. Abamectin was evaluated against plant parasitic nematodes as seed treatment, soil drench application, and root dipping against several genus of nematodes like Meloidogyne incognita, M. arenaria, M. javanica, Tylenchulus semipenetrans and Rotylenchulus reniformis.

The persistence of abamectin in the environment is moderately. The reported laboratory soil aerobic half-life was 115 days, and the reported field dissipation half-life was 31 ± 6 days. Abamectin is relatively stable to hydrolysis, but may undergo direct photolysis with half-life in surface soil =21 hours. Also, it was found that abamectin toxicity increased with increasing the temperature from 17 to 37°C [25].

Moreover, in a soil rhizosphere, abamectin is bind tightly to soil particles, and has a low water solubility, resulting in poor movement of the product through the soil profile, as well as limited plant systemic activity [26-29]. The water solubility of avermectin \( B_1 \) is approximately 6-8 ppb, and its leaching potential through many types of soil is extremely low.

Abamectin is highly unstable to light, and found to be degraded readily by soil microorganisms, hence, residues in or on crops are very low. These physical properties also confer many advantages upon the use of avermectins as pesticides. Their rapid degradation in soil and poor leaching potential suggest that field applications would not result in persistent residues or contamination of ground water.

**The effect of Abamectin on plant parasitic nematodes**

Avermectin \( B_1 \) found to be significantly reduce Hoplolaimus Galeatus and Tylenchorhynchus dubius after 14 and 28 days of treatment [30]. The gall rating and egg counts of \( M. \ javanica \) were reduced when different concentrations of abamectin were injected in to the pseudostem of banana [5].

Abamectin was more effective than emamectin benzoate, for controlling Meloidogyne incognita on tomato, \( M. \ javanica \) and Radopholus similis on banana [31]. Abamectin, in certain doses, were effective against Ditylenchus dipsaci in garlic, which decreased the nematodes per cm² of tissue [32].

It was recorded that abamectin \( B_1 \) reduced both Aphelenchoides fragariae and Ditylenchus dipsaci populations in Lamium maculatum and Phlox subulata, especially after repeated applications [33]. Young and Maher [34] proved the activity of abamectin against bud and leaf nematodes (Aphelenchoides ritzenhodis) in vitro and in vivo. While abamectin as seed treatment to coat cucumber seeds (cv. Kahina), reduced the penetration of Meloidogyne incognita juveniles within the roots at 0.3 mg a.i./seed (approximately 20 g/ha) [35].

Abamectin (Vertemic 1.8% EC) as soil application proved its nematicidal activity, that suppress the root-knot nematodes, Meloidogyne spp., on different vegetables crops [9,10,36-40].

Emamectin benzoate, which follow avermectins group, were effective against root-knot nematodes, but it was less effective than abamectin [41,42]. Seed treatment with abamectin decreased the penetration of the second stage juveniles into the roots, resulting in lower colonization and reproduction of \( M. \ incognita \), on cotton plants [43]. Meanwhile, abamectin recorded an increase in plant growth parameters such as plant shoot and root systems length and weight [9,39,44-47].

The avermectins mode of action is blocking the transmittance of electrical activity in nerves and muscle cells, by stimulating the release and binding of gamma-amino butyric acid (GABA) at nerve endings [48-52]. This causes an influx of chloride ions into the cells (activate or opining glutamate-gated chloride channel), which lead to hyper polarization and subsequent paralysis of the neuromuscular systems [50,53,54], and then death. This unique mode of action is effective on insect pests that are resistant to other insecticides, such as organophosphates, pyrethroids and other acaricides. In nematodes, GABA receptors are found at the neuromuscular junctions and the
Azadirachtin

The Indian Neem tree (*Azadirachta indica*, Aturs) was widely distributed in India and some regions of Asia, Africa and Australia. Neem tree is an evergreen tree that is part of the Meliaceae (mahogany) family and known as margosa or Indian lilac, this group of trees that is characterized by the widespread occurrence of bitter triterpenoids [11]. Neem is perhaps, the most useful traditional medicinal plant in India [56]. Each part of the neem tree such as leaves, neem seed, neem oil and neem cake are traditionally used by the farmers to hundred years for its unique properties for insect and fungi control, in addition to cure certain human diseases.

The neem tree has been described in 1830 by De Jussieu as *Azadirachta indica*, and its taxonomic position is as follows:

Kingdom: Plantae
Division: Magnoliophyta
Order: Sapindales
Family: Meliaceae (mahogany family)
Subfamily: Melioideae
Genus: Azadirachta
Species: indica

The earliest report on the isolation of nimbin, which is considered the first compound isolated from neem oil was published by Siddiqui [57]. There are more than 300 compounds that have been characterized from neem seeds, with over 50 different bioactive constituents from various parts of neem tree, and certain reports have been published the chemistry and structural diversity of these compounds [58].

Neem is available in simple homemade formulations like seed powder, seed kernel powder, seed cake powder, dry leaf powder and aqueous extracts made from them [59]. Azadirachtin is found widespread throughout the Neem tree, along with other similar triterpenoids, but is collected primarily from the oily extract of the tree's seeds, where 40-90 g of azadirachtin are found for every 1 kg of extract [11,60].

The isolated triterpenoid compounds from neem have been divided into two major classes: isoprenoids and non isoprenoids [63]. The isoprenoids included diterpenoids and triterpenoids, which contain protomelacins, limonoids, azadiron, gedunin and its derivatives, vilasinin and C-secomeliacins, such as nimbin, salanin and azadirachtin. While the non isoprenoids included proteins (amino acids) and carbohydrates (polysaccharides), sulphur compounds, polyphenolics such as flavonoids and their glycosides, dihydrochalcone, coumarin, tannins and aliphatic compounds [56,58,63].

Azadirachtin has not come to commercial use because it is expensive to isolate it in a pure form from the need seed/kernel extract, and it is a very complex molecule for an economical chemical synthesis. But, now the total synthesis of azadirachtin was done after nearly 30 years of work. This highly complex molecule, which contains 16 stereocenter, and is nevertheless one of the most highly oxidized limonoids known [64]. Despite these difficulties, the total synthesis of azadirachtin was finally published in 2008 [12]. These synthesis masters built upon the past three decades of successes and failures to piece together the compound that had eluded chemists for so long [12].

A large number of compounds have been isolated from various parts of neem, a few of them have been studied for their biological activity. Nimbidin, a major crude bitter principle extracted from the oil of seed kernels of *A. indica* demonstrated several biological activities. From this crude principle, some tetranortriterpenes, including nimbin, nimbinin, nimbidinin, nimbolide and nimbidic acid have been isolated [56,57,65].

Azadirachtin, a complex tetranortriterpenoid limonoid from the neem seeds, is the main component responsible for both antifeedant and toxic effects in insects. Other limonoid and sulphur containing compound with repellent, antiseptic, contraceptive, antipyretic and antiparasitic properties are found elsewhere in the tree, e.g. leaves, flowers, bark, roots [66].

The Neem tree has attracted the attention of many chemists and biologists all over the world during the past two decades, because of its efficacy against certain pests. Neem products have revealed that some of them are effective against insects and nematodes [67-71].

A large part of the high level of enthusiasm for the use of azadirachtin as a natural and eco-friendly pesticide is due to the compound's potential safety to humans and other warm-blooded vertebrates [72]. In fact, it is believed that azadirachtin and related neem triterpenoid extracts may have beneficial effects on humans [11]. A study clarified that pure azadirachtin is not toxic to humans [73], while a more recent study estimates at least 15 mg of azadirachtin per kg of body weight could be taken safely by humans each day, which is well within range for use as a pesticide [16].

Neem oil extract is not only used in pests management, but also neem cake, which is utilized as a natural and environmental friendly fertilizer, soil conditioner, nitrogen saver and manure in farming and agriculture [74], as well as some reports mentioned that neem cake was effective against phytonematodes when blended with the soil.

Azadirachtin has been found to degrade rapidly due to environmental factors such as UV radiation in sunlight, heat, air moisture, acidity and enzymes present in foliar surfaces.

There are instances of toxic effects of residues of some of the synthetic insecticides for the consumers of the product due to poor biodegradability. Therefore, there is a need for environmentally compatible insecticides, possessing activities at low concentration and selective toxicity to targeted pests, in addition, low toxic to plants and mammals, desired stability and economic viability. The best performance for formulated neem was obtained under warm temperature conditions [15,75].

Azadirachtin is considered the only relative safe pesticides, which could not cause environmental risk, and would not cause an ecological problem in the microbial community in soil [76]. The potential for mobility of formulated azadirachtin in soil is very low and the accumulation in the environment is not expected [77]. Moreover, it was reported that the formulated azadirachtin breaks down rapidly in 100 hours in water or light, and will not cause long-term effects [77].
The activity of azadirachtin on plant parasitic nematodes

In addition to insects, other pests, including mites [78,79], snails [80] and plant nematodes [9,81-83] have been reported susceptible to neem components.

Azadirachtin (Achook® 0.15% EC and Nimbecidine® 0.03% EC) proved highly active against Meloidogyne incognita in tomato plants, which reduced galls, egg masses and juveniles by 69.31 and 64.48%, 62.25 and 40.37, and 60.15 and 63.71, respectively. Meanwhile, azadirachtin (Achook® 0.15% EC) recorded reduction in the presence of galls on plant roots and juveniles in soil [10,40,82,83].

Neem based formulations and azadirachtin significantly suppress root-knot nematode (Meloidogyne incognita), on cucumber [84], and cyst nematode (Globodera rostochiensis), on potato [85].

The seed and leaf extracts of neem (Azadirachta indica A. Juss) caused 100% juvenile mortality of the root-knot nematodes and some free-living nematodes on potato [86-88].

Two concentrations of aqueous extracts of the neem leaves and seeds were evaluated on root-knot nematode, Meloidogyne incognita, on tomato plants. The neem extracts recorded reduction in nematodes population between 38 and 50% [89]. Moreover, the use of dry neem leaves as incorporated into the soil reduced the root-knot nematode Meloidogyne incognita significantly and enhanced the weight of fruits/plant by 19% of eggplants [90]. Also, the neem leaf extract was effective against root-knot nematode M. incognita and inhibit the eggs hatching [91].

Using formulated neem oil as seed treatment and seedling root dip against root knot nematode Meloidogyne incognita on tomato, chili and brinjal was effective and reduced nematode population [92]. Besides, seed coatings with neem oil, neem formulations and products obtained from different plants, have also been used for the control of plant parasitic nematodes [59,92-96].

Several reports found that azadirachtin, and/or neem extracts enhanced the plant growth, and increased the yield in different crops [9,10,83,85,91].

On the other hand, soil amended with oil-cakes of neem and other plant products have been successfully used for the control of plant-parasitic nematodes [92,97-103]. Whilst, fewer juveniles penetrated the roots of plants raised in neem cake amended soil compared to untreated plants [104]. The numbers of Pratylenchus penetrans and Meloidogyne hapla in tomato roots grown in 1% neem cake were reduced by 67 and 90%, respectively [105].

The anti-feedant effects of neem were described scientifically for the first time in 1959 by the German entomologist, Heinrich Schmutterer who recorded desert locusts (Schistocerca gregaria Forskal), refusing to feed on neem trees during locust migratory in Sudan [66].

There are numerous examples reporting the insect anti-feedant and insect growth inhibitory properties of azadirachtin for a variety of insect pests, and may also have a useful role to play in resistance management. Azadirachtin has also been reported to be non mutagenic, and it appears to have no apparent mammalian toxicity.

The actions of azadirachtin as insecticide are based on multiactions pathways such as toxicity, anti-mitotic effects, anti-feedant activity, insect growth regulator, fecundity suppression, sterilization, oviposition repellency, including harmful effects on endocrine system and damages of the cuticle of larvae, preventing them from moulting [106,107]. Also, reduced levels of detoxification enzymes due to its blockage of protein synthesis [108].

Azadirachtin is considered strong anti-feedant because of its effects on the insect's chemoreceptors, which deter the insect from consuming the plant. Moreover, azadirachtin not only blocks peptide hormone release that cause molting abnormalities, but also cause damage in insect’s tissues, including muscle, fat and gut cells [11,109].

Active neem constituents can be absorbed through plant roots and systemically move upward through the plant’s xylem tissues [110-113], which mean that it could be used to manage plant parasitic nematodes as soil application, especially against those plants’ root feeders.

On the other hand, in certain reports, the nematocidal mechanisms of neem were suggested and concluded that the involvement of phenolic compounds absorbed systemically by the roots of tomato plant might have induced tolerance against nematodes [103]. The narcotic effect of neem formulations could be due to by-products (ammonia, formaldehyde, phenols and fatty acids), released during their decomposition [114]. It was claimed that direct toxicity of neem formulations due to nimbin, salanine, thionemone, azadirechtin and nimbidine [114,115]. The neem leaf extract inhibited the eggs hatching of root-knot nematode in vitro [81].

Accordingly, it could be one of possibilities that Neem play a role as plant nematodes as shown as insects. It was suggested that the efficacy of neem constitutes on plant parasitic nematodes could be refer to chemoreceptors (Amphids and Phasmids), which are responsible for recognizing the host plant and work as repellent compound.

On the other hand, there are evidences that molting and exsheathment in nematodes are under neurosecretory and endocrine control. Ecdyson, and/or its active metabolite, 20-hydroxyecdysone, or substances similar to ecdysteroids have been detected in several nematodes [116-119].

Acknowledgments

I am grateful to Emeritus Prof. Dr. Abdel-Fattah S.A. Saad, Professor of Pesticides Chemistry and Toxicology, Plant Protection Department, Faculty of Agriculture, Saba Bacha, Alexandria University, for his help, support and advice.

References

1. Castagnone-Sereno P (2002) Genetic variability in parthenogenetic root knot nematodes, Meloidogyne spp., and their ability to overcome plant resistance genes. Nematology 4: 605-608.
2. Atkinson HJ, Lilley CJ, Urwin PE (2012) Strategies for transgenic nematode control in developed and developing world crops. Curr Opin Biotechnol 23: 251-256.
3. Fisher MH (1990) Recent advances in avermectin research. Pure Appl Chem 62: 1231-1240.
4. Lasota JA, Dybas RA (1990) Abamectin as a pesticide for agricultural use. Acta Leiden 59: 217-225.
5. Jansson RK, Rabatin S (1997) Curative and residual efficacy of injection applications of avermectins for control of plant-parasitic nematodes on banana. J Nematol 29: 695-702.
6. Monfort WS, Kirkpatrick TL, Long DL, Rideout S (2006) Efficacy of a novel nematicidal seed treatment against meloidogyne incognita on cotton. J Nematol 38: 245-249.
7. Faske TR, Starr JL (2007) Cotton root protection from plant-parasitic nematodes by abamectin-treated seed. J Nematol 39: 27-30.
8. Ibrahim HS, Saad ASA, Massoud MA, Khalil MSH (2010) Evaluation of certain agrochemical and biological agents against Meloidogyne incognita on tomatoes. Alexandria Science Exchange Journal 31: 10-17.
9. Khalil MSH, Allam AFG, Barakat AST (2012a) Nematicidal activity of some biopesticide agents and microorganisms against root-knot nematodes on tomato plants under greenhouse conditions. Journal of Plant Protection Research 52: 47-52.

10. Saad ASA, Massoud MA, Ibrahim HS, Khalil MS (2012) Activity of nemathorin, natural product and bioproducts against root-knot nematodes on tomatoes. Archives of Phytopathology and Plant Protection 45: 955-962.

11. Aerts RJ, Mordue AJ (1997) Feeding deterrence and toxicity of neem triterpenoids. J Chem Ecol 23: 2117-2133.

12. Veitch GE, Boyer A, Levy SV (2008) The azadirachtin story. Angew Chem Int Ed Engl 47: 9402-9429.

13. Gajalakshmi S, Abbasi SA (2004) Neem leaves as a source of fertilizer-cum-pesticide vermicompost. Bioreso Technol 92: 291-296.

14. Ascher KRS (1993) Nonconventional insecticidal effects of pesticides available from the neem tree, Azadirachta indica. Arch Insect Biochem Physiol 24: 433-449.

15. Schmutterer H (1990) Properties and potential of natural pesticides from the neem tree, Azadirachta indica. Ann Rev Environ Toxicol 35: 271-297.

16. Boeke SJ, Boersma MG, Alik GM, van Loon JJ, van Huis A, et al. (2004) Safety evaluation of neem (Azadirachta indica) derived pesticides. J Ethnopharmacol 94: 25-41.

17. Stark JD (2007) Ecotoxicology of Neem. ACS Symposium Series 947: 275-286.

18. Thompson DG, Kreutzweiser DP (2007) A review of the environmental fate and effects of natural “reduced-risk” pesticides in Canada. Crop Protection Products for Organic Agriculture: Environmental, Health, and Efficacy Assessment. ACS Books, American Chemical Society, Washington, USA 245-274.

19. Silva JCT, Gulab NJ, D’are R, Oliveira L, Brown L (2007) Purification of seven tetraterpenoids in neem (Azadirachta indica) seed by counter-current high-performance liquid chromatography. Journal of Chromatography A 1151: 203-210.

20. Burg RW, Miller BM, Baker EE, Birnbaum J, Currie SA, et al. (1979) Avermectins, new family of potent antihelmintic agents: producing organism and fermentation. Antimicrob Agents Chemother 15: 361-367.

21. Miller TW, Chaiet L, Cole DJ, Cole LJ, Flor JE, et al. (1979) Avermectins, new family of potent antihelmintic agents: isolation and chromatographic properties. Antimicrob Agents Chemother 15: 368-371.

22. Jayakumar J (2009) Bio-efficacy of Streptomyces avermitilis culture filtrates against root knot nematode, Meloidogyne incognita and reniform nematode, Rotylenchulus reniformis. Karnataka Journal of Agriculture Science 22: 567-571.

23. Omura S, K Shiomi (2007) Discovery, chemistry, and chemical biology of microbial products. Pure Appl Chem 79: 581-591.

24. Pitterna T, Cassayre J, Hüter OF, Jung PM, Maenfisch P, et al. (2009) New microbial products. Pure Appl Chem 79: 581-591.

25. Boina DR, Onagbola EO, Salyani M, Stelinski LL (2009) Influence of posttreatment temperature on the toxicity of insecticides against Diaphorina citri (Hemiptera: Psyllidae). J Econ Entomol 102: 865-691.

26. Bull DL, Ivie W, MacDonnel JG, Gruber VF, Ku CC, et al. (1984) Fate of avermectin B1a in soil and plants. J Agric Food Chem 32: 94-102.

27. Bull DL (1985) Environmental fate of avermectin. South-Western Entomologist Supplement 7: 2-11.

28. Mrozik H (1994) Advances in research and development of avermectins. Natural and Engineered Pest Management Agents. American Chemical Society, Washington DC, USA 54-73.

29. Chuakwudebe AC, Feely WF, Burnett TJ, Crouch LS, Wislocki PG (1996) Uptake of emamectin benzoate residues from soil by rotational crops. J Agric Food Chem 44: 4015-4021.

30. Blackburn K, Alm SR, Yeh TS (1996) Avermectin B1, isazofos, and fenamiphos for control of Hoploaimus galeatus and Tylenchorhynchus dubius infesting poa annua. J Nematol 28: 687.

31. Jansson RK, Rabatin S (1998) Potential of foliar, dip, and injection applications of avermectins for control of plant-parasitic nematodes. J Nematol 30: 65-75.

32. Becker WF (1999) The effect of abamectin on garlic infected by Ditylenchus diplocir. Nematol Bras 23: 1-8.

33. Lamondia JA (1999) Efficacy of insecticides for control of Aphelenchoides fragariae and Ditylenchus dipsaci in flowering perennial ornamentals. J Nematol 31: 644-649.

34. Young JEB, Maher HM (2000) Evaluation of abamectin against bud and leaf nematodes in hardy ornamentals. The BCPC Conference: Pests and diseases, Proceedings of an international conference, Brighton, UK 309-314.

35. Becker JO, Becker JS, Morton HV, Hofer D (2006) Early protection against root-knot nematodes through nematicidal seed coating provides season-long benefits for cucumbers. Cucurbitaceae, Asheville, North Carolina, USA 395-402.

36. Hamido AO, El-Gindi AY, Hoda HA, Youssef MM, Asmahan ML (2006) Evaluation of the nematicidal effects of a biotechnological product (Abamectin) on Meloidogyne incognita, root-knot nematode infecting cowpea plants. Pakistan Journal of Nematology 24: 75-79.

37. Khalil MSH (2009) Influence of nematicides and certain natural products on the infestation of nematodes attacking tomato plants (Lycopersicon esculentum, Mill.). M. Sc. Thesis, Alexandria University, Alexandria, Egypt.

38. Shahid M, Rehman AU, Khan SH, Mahmood K, Khan AU (2009) Management of root-knot nematode infecting blijal by biopesticides, chemicals, organic amendments and bio-control agent. Pakistan Journal of Nematology 27: 159-166.

39. Saad ASA, Massoud MA, Ibrahim HS, Khalil MS (2010) Nematicidal effect of biological control agents and other chemical compounds on Meloidogyne incognita infecting tomato plants. Alexandria Science Exchange Journal 31: 240-247.

40. Khalil MS (2012) A comparison study with alternative biorational agents to suppress the root-knot nematode populations and galls formation in tomato plants. International Journal of Nematology 22: 112-116.

41. Abbas W, Anwar SA, Zia A, Javed N (2008) Response of four tomato cultivars to Meloidogyne incognita infection and its chemical management. Pakistan Journal of Nematology 26: 37-43.

42. Rehman AU, Javed N, Ahmad R, Shahid M (2009) Protective and curative effect of bio-products against the invasion and development of root-knot nematodes in tomato. Pakistan Journal of Phytopathology 21: 37-40.

43. Bessi R, Sujimoto FR, Inomoto MM (2010) Seed treatment affects Meloidogyne incognita penetration, colonization and reproduction on cotton. Câncin Rural 40: 1428-1430.

44. Korayem AM, Mahmoud MAY, Moawad MMM (2008) Effect of chitin and abamectin on Meloidogyne incognita infecting rape seed. Journal of Plant Protection Research 48: 365-370.

45. Cabrera JA, Kiewnick S, Grimm C, Dababat AA, Sikora RA (2009) Effective concentration and range of activity of abamectin as seed treatment against root-knot nematodes in tomato under glasshouse conditions. Nematology 11: 909-915.

46. López-Pérez JA, Edwards S, Ploeg A (2011) Control of root-knot nematodes on tomato in stone wool substrate with biological nematicides. J Nematol 43: 110-117.

47. Qiao K, Liu X, Wang H, Xia X, Ji X, et al. (2012) Effect of abamectin on root-knot nematodes and tomato yield. Pest Manag Sci 68: 853-857.

48. Bloomquist JR (1996) Ion channels as targets for insecticides. Annu Rev Entomol 41: 163-190.

49. Bloomquist JR (2003) Chloride channels as tools for developing selective insecticides. Arch Insect Biochem Physiol 54: 145-156.

50. Burkhardt CN (2000) Ivermectin: an assessment of its pharmacology, microbiology and safety. Vet Hum Toxicol 42: 30-35.

51. Campbell WC, Fisher MH, Stapley EO, Albers-Schönberg G, Jacob TA (1983) Ivermectin: a potent new antiparasitic agent. Science 221: 823-828.

52. Roder JD, Stair EL (1998) An overview of ivermectin toxicity. Vet Hum Toxicol 40: 369-370.
53. Bloomquist JR (1993) Toxicology, mode of action and target site-mediated resistance to insecticides acting on chloride channels. Comp Biochem Physiol C 106: 301-314.
54. Cully DF, Vassilatis DK, Liu KK, Paress PS, Van der Ploeg LH, et al. (1994) Cloning of an avermectin-sensitive glutamate-gated chloride channel from Caenorhabditis elegans. Nature 371: 707-711.
55. Stewart GR, Perry RN, Wright DJ (1994) Immunocytochemical studies on the occurrence of gamma-aminobutyric acid (GABA) in the nervous system of the nematodes Panagrellus redivivus, Meloidogyne incognita and Globodera rostochiensis. Fundamental and Applied Nematology 17: 433-439.
56. Biswas K, Chattopadhyay I, Banerjee RK, Bandyopadhyay U (2002) Biological activities and medicinal properties of neem (Azadirachta indica). Curr Sci 82: 1336-1345.
57. Siddiqui S (1942) A note on isolation of three new bitter principles from the neem oil. Curr Sci 11: 278-279.
58. Kraus W (1995) Source of unique natural products for integrated Pest Management, medicine, industry and other purposes. The Neem Tree 35-68.
59. Javed N, Gowen SR, El-hassan SA, Imam-ul-Haq M, Shahina F, et al. (2008) Efficacy of neem (Azadirachta indica) formulations on biology of root-knot nematodes (Meloidogyne javanica) on tomato. Crop Prot 27: 36-43.
60. Saxena RC (1989) Insecticides from neem. ACS Symp Ser 387: 110-135.
61. Mongkolkhajomsip D, Supaporn D, Douglas PL, Elkamel A, Teppaatoon W, et al. (2005) Supercritical CO2 extraction of nimbin from neem seeds-a modelling study. Journal of Food Engineer 71: 331-340.
62. Kurose K, Yatagi M (2005) Components of the essential oils of Azadirachta indica A. Juss, Azadirachta siamensis Velton and Azadirachta excels (Jack) Jacobs and their comparison. Journal of Wood Science 51: 183-188.
63. Devakumar C, Dev S (1996) Chemistry. In: Randhawa, NS, Parmar BS (Eds.), Neem (2nd Edn), 77-110.
64. Ley SV, Denholm AA, Wood A (1993) The chemistry of azadirachtin. Nat Prod Rep 10: 109-157.
65. Mitra CR, Garg HS, Pandey GN (1971) Identification of nimbidic acid and nimbinidin from Azadirachta indica. Phytochemistry 10: 857-864.
66. Mordue AJ, Nisbet AJ (2000) Azadirachtin from the Neem Tree Azadirachta indica: its action against insects. An Soc Entomol Brasil 29: 615-632.
67. Holyoke CW, Reese JC (1987) Acute insect toxicants from plants. Handbook of Natural Pesticide, Insect Growth Regulators. CRC Press, Boca Raton, Florida, USA 67-118.
68. Byomakesh D, Padhi NN, Dash R (1998) Relative efficacy of neem products against root knot nematode on tomato. Indian J Nematol 28: 163-167.
69. Khanna AS, Sharma RK (1998) Phytotherapeutic effect of some indigenous plants/nematicides on Meloidogyne incognita infesting tomato. In: Proceedings of National Symposium on Rational Approaches in Nematode Management for Sustainable Agriculture, GAU, Anand Gujarat, India 4-6.
70. Nanjegowda D, Naik BG, Ravi K, Reddy PP, Kumar NKK, et al. (1998) Efficacy of neem products and a nematicide for the management of root-knot nematode Meloidogyne incognita on tomatoes as feeding-habitate and Artisbicular Mycorrhiza Fusiga. Journal of the Advanced in Agricultural Research 16: 137-147.
71. Khan MR, Solanki RD, Bohra B, Vyas BN (2012b) Evaluation of Achook (Azadirachtin 1500 ppm) against root knot nematode (Meloidogyne incognita) infecting okra. South Asian Journal of Experimental Biology 2: 149-156.
72. Lynn OM, Song W, Shin J, Kim J, Lee K (2010) Effects of azadirachtin and nematode-based formulations for the control of sweet potato whitefly and root-knot nematode. J Korean Soc Appl Biol Chem 53: 598-604.
73. Trifonova Z, Atlasov A (2011) Control of potato cyst nematode Globodera rostochiensis with some plant extracts and neem products. Bulgarian J Agric Sci 17: 623-627.
74. Akhtar M, Alam MM (1991) Integrated control of plan-parasitic nematodes on potato with organic amendments, nematicide and mixed cropping with mustard. Nematol Medit 19: 169-171.
75. Khurma UR, Singh A (1997) Nematicidal potential of seed extracts: in vitro effects on juvenile mortality and egg hatch of Meloidogyne incognita and M. javanica. Nematol Medit 25: 49-54.
76. Upadhyay KD, Dwivedi K, Uttam SK (2003) Effect of some plant extracts on the mortality and hatching of Meloidogyne incognita and Heterodera cajani infesting pigeon pea. Nematol Medit 31: 28-32.
77. Taye W, Sukhija PK, Tefera T (2012) Evaluation of plant extracts on infestation of root-knot nematode on tomato (Lycopersicon esculentum Mill). J Agric Res Dev 2: 86-91.
78. Khan MR, Mohiddin FA, Ejaz MN, Khan MM (2012a) Management of root-knot disease in eggplant through the application of biocontrol fungi and dry neem leaves. Turk J Biol 36: 161-169.
79. Agbenin NO, Ernchebe AM, Marley PS, Akpa AD (2005) Evaluation of nematicidal action of some botanicals on Meloidogyne incognita in vivo and in vitro. J Agric Rural Dev Trop Subtrop 106: 29-39.
80. Sivakumar M, Gunasekaran K (2011) Management of root knot nematodes in chili and brinjal by neem oil formulations. J Biopesticides 4: 199-200.
81. Leela NK, Khan AM, Reddy PP, Nidiery ESJ (1992) Nematicidal activity of essential oil of Pelargonium graveolens against the root-knot nematode, Meloidogyne incognita. Nematol Medit 20: 57-58.
82. Akhtar M, Mahmood I (1995a) Evaluation of a neem-based product against root-knot nematode Meloidogyne incognita Tests Agrochemicals and Cultivars. Ann Appl Biol 16: 6-7.
83. Akhtar M, Mahmood I (1995b) Control of root knot nematode Meloidogyne incognita in tomato plants by seed-coating with Achook and neem oil. International Pest Control 37: 86-87.
84. Ayeni LS, Adeluye EO (2011) Soil nutrient status and nutrient interactions as influenced by agro wastes and mineral fertilizer in an incubation study in the South West Nigerian. International Journal of Soil Sciences 6: 60-68.
97. Alam MM (1990) Neem in biocontrol. Nematode Biocontrol (Aspects and Prospects). CBS Publishers and Distributors, Delhi, India 17-40.
98. Akhtar M, I Mahmood (1996) Organic amendments in relation to nematode management with particular reference to India. Integrated Pest Management 1: 201-215.
99. Tejada M, Garcia C, Gonzalez JL, Hernandez MT (2006) Organic amendment based on fresh and composted beet vinasse: Influence on physical, chemical and biological properties and wheat yield. Soil Science Society of America Journal 70: 900-908.
100. Tejada M, Hernandez MT, Garcia C (2009) Soil restoration using composted plant residues: Effects on soil properties. Soil Tillage Research 102: 109-117.
101. Wani AH (2006) Management of root-knot nematode, Meloidogyne incognita, on okra and mung by soil amendment with oil cakes and leaves of different plants. Nematol Medit 34: 83-87.
102. Deepa SP, Subramanian S, Rama KS (2011) Biomanagement of citrus nematode Tylenchulus semipenetrans Cobb on lemon, Citrus limon L. Journal of Biopesticides 4: 205-207.
103. Mohan K (2011) Comparison of inorganic and organic nematicides on the population of soil nematodes in hybrid of Saccharum species. J Biopesticides 4: 201-204.
104. Alam MM, Ahmad M, Khan AM (1980) Effect of organic amendment on the growth and chemical composition of tomato, eggplants and chili and their susceptibility to attack by Meloidogyne incognita. Plant Soil 57: 231-236.
105. Abbasi PA, Riga E, Conn KL, Lazarovits G (2005) Effect of neem cake soil amendment on reduction of damping-off severity and population densities of plant parasitic nematodes and soil borne plant pathogens. Can J Plant Pathol 27: 38-45.
106. Mulla MS, Su T (1999) Activity and biological effects of neem products against arthropods of medical and veterinary importance. J Am Mosq Control Assoc 15: 133-152.
107. Howard AF, Adongo EA, Hassanali A, Omlin FX, Wanjoya A, et al. (2009) Laboratory evaluation of the aqueous extract of Azadirachta indica (neem) wood chippings on Anopheles gamabiae s.s. (Diptera: Culicidae) mosquitoes. J Med Entomol 46: 107-114.
108. Lowery DT, Smirle MJ (2000) Toxicity of insecticides to oblique banded leaf roller, Choristoneura rosacea, larvae and adults exposed previously to neem seed oil. Entomol Exp Appl 95: 201-207.
109. Mordue AJ, Blackwell A (1993) Azadirachtin: an update. J Insect Physiol 39: 903-924.
110. Gill JS, Lewis CT (1971) Systemic action of an insect feeding deterrent. Nature 232: 402-403.
111. Larew HG (1988) Limited occurrence of foliar-, root-, and seed-applied neem seed extract toxin in untreated plant parts. J Econ Entomol 81: 593-598.
112. OsmanMZ, Port GR (1990) Systemic action of neem seed substances against Myzus persicae. Entomol Exp Appl 54: 297-300.
113. Nisbet AJ, Woodford JAT, Strang RHC, Connoly JD (1993) Systemic antifeedant effects of azadirachtin on the peach-potato aphid Myzus persicae. Entomol Exp Appl 68: 87-98.
114. Khan AM, Alam MM, Ahmad R (1974) Mechanism of the control of plant-parasitic nematodes as a result of the application of oilcakes to the soil. Indian Journal of Nematology 4: 93-96.
115. Devakumar C, Goswami BK, Mukherjee SK (1985) Nematicidal principles from neem (Azadirachta indica) part 1, Screening of neem keman fraction against Meloidogyne incognita. Indian Journal of Nematology 15: 121-124.
116. Dennis RD (1977) On ecdysone-binding proteins and ecdysone-like material in nematodes. Int J Parasitol 7: 181-188.
117. Franke S, Kaiser G (1989) Occurrence and hormonal role of ecdysteroids in non-arthropods. In: Koolman J (Ed), Ecdysone. From chemistry to mode of action, Georg Thieme Verlag, Stuttgart, Germany 296-307.
118. Barker GC, Rees HH (1990) Ecdysteroids in nematodes. Parasitol Today 6: 384-387.
119. Barker GC, Chitwood DJ, Rees HH (1990) Ecdysteroids in helminths and annelids. Invertebr Reprod Dev 18: 1-11.