AATCC Review

23 November 2022: Received
28 March 2023: Revised
06 June 2023: Accepted
16 July 2023: Available Online

www.aatcc.peerjournals.net

Review Article | Open Access

Streptomyces avermitilis – A bionematicide of microbial origin against root-knot nematode, *Meloidogyne incognita*

Jaya Kumar, J[1], C. Harisudan[2], S.Thiruvarassan[3] V.Ravichandran[4] and S.Ganapathy[5]

[1]Krishi Vigyan Kendra, TNAU, Vridhachalam, Tamil Nadu, India
[2] Regional Research Station, TNAU, Vridhachalam, Tamil Nadu, India
[3]Oil seed Research Station, TNAU, Tindivanam, Tamil Nadu, India
[4]Tapioca and Castor Research Station, TNAU, Yethapur, Tamil Nadu, India
[5] Agricultural College and Research Institute, TNAU, Vazhavachanur, Tamil Nadu, India

ABSTRACT

In recent years, microbial bionematicide against plant parasitic nematodes has assumed great importance after realizing the drawbacks of exclusively relying on chemicals for nematode management. In the context of increasing worldwide awareness of environmental pollution, the development of bionematicides of microbial origin or plant products suitable to all cropping systems and agro-climatic situations, biological methods of nematode suppression and evolving new bioactive molecules of microbial origin have been recognized as the safest approaches to nematode management. The production of avermectins is a big challenge with commercial importance in the bio-pesticide market, because its output is remarkably inadequate for human consumption as of now.

Keywords: *Streptomyces avermitili, bionematicide, root-knot nematode, vegetable crops*

INTRODUCTION

Over the past 50 years, there has been much activity directed to chemical work on the isolation and identification of a wide array of biologically active natural products that in some way affect the behavior, development, and reproduction of pests such as insects, diseases, and nematodes. Even though various biologically active compounds have been identified, only a limited percentage of compounds focused on commercial applications due to a limited percentage of chemical guidance with regard to screening of their properties. As environmental concerns are constricting synthetic chemical pesticide usage and also the growing resistance of pests to present pesticides added urgency to search for better safer compounds. The range of synthetics available presently against the plant parasitic nematodes in the Indian market is limited to carbofuran and phorate and their field efficacy in different crops has been reported to be quite variable, besides, their adverse impact on the soil and groundwater flora and fauna. In the present scenario of the limited choice of effective nematicides, lack of commercially registered nematode-effective bio-control agents on the side, and endangered environmental sustenance on the other side, natural metabolites toxic to target organisms are becoming major targets of research. Metabolites from soil microbes offer promising solutions to the above dilemma and become an important component of sustainable agriculture. The versatility in structure and activity, biodegradability, and environmental-friendly properties make these proposed microbial metabolites, avermectins, and agro-active agents of future generation.

**Corresponding Author: Harisudan C*
Email Address: harisudan@tnau.ac.in

DOI: https://doi.org/10.58321/AATCCReview.2023.11.03.385

Avermectins.

In recent years the use of avermectins, a new class of metabolites, obtained from *Streptomyces avermitilis* was found to be the most promising approach to control nematodes that infest animals and plants. Avermectins are unique macrocyclic lactones with excellent nematicidal and insecticidal properties (27, 3). These metabolites are produced by *S.avermitilis* which is a commonly occurring soil-borne actinomycetes.

Biology of the organism

Streptomyces avermitilis have sporophore-forming spirals as side branches on aerial mycelia (5). The spirals are compact but become open as the culture ages. Spores are in chains of more than 15 and are usually spherical to oval. The spore surface is smooth as revealed by the electron microscope. Sporulation occurred on oatmeal agar glycerol asparagine agar and inorganic salts starch agar. They contain brownish grey spore mass color, and produce melanoid pigments in nutrient agar medium.

Avermectins represents a novel class of 16-membered macrocyclic lactones that have demonstrated nematicidal (3,31) acaricidal (30) properties derived from a soil actinomycetes *Streptomyces avermitilis* (5, 22) at the Kitasato Institute from a soil sample collected at Kawana City, Japan. Avermectin characteristically showed extremely high efficacies at low rates of application. The anthelminthic, insecticidal, and acaricidal activity of the avermectins is well known (6,8,12,19,32). The discovery of the avermectins from this organism in 1976 has greatly influenced the arsenal of chemicals available for the control of household and agricultural arthropod pests as well as parasites of mammals. Avermectin potent, broad-spectrum anthelmintic activity of avermectin when administered at 10-300 ppm (body weight) to sheep, cattle, dogs, and poultry infected with various common gastrointestinal parasites (2, 9). Avermectins have a nematicidal action different from that of the organophosphates and organocarbamates (10). They are antagonists of -aminobutyric acid (GABA), as opposed to the acetylcholinesterase inhibition by the organophosphate and organocarbamate, and their potential efficacy, is greater than the current nematicides.

Effect of Avermectin on nematodes

The available literature on avermectins indicated that the root-knot nematodes including *Meloidogyne incognita*, *M. javanica,* and *M. arenaria* were the commonly used test organisms to assess the nematicidal activity of these metabolites. The other nematodes experimented on were *Tylenchulus semipenetrans* and *Bursaphelenchus lignicolous*. Further, the host crops included in the experiments were tobacco, tomato, cucumber, and citrus. In the greenhouse test, avermectin B2a exhibited excellent control of the root-knot nematode *M. incognita* in sandy loam soil at 0.16 to 0.24 kg a.i/ha which is about 10-30 times as potent as several commercial contact nematicides (3). Similar levels of activity have been reported for a derivative of 2, 3-ketone (29). Application of novel experimental compounds such as avermectin B1a, avermectin B2a and avermectin B2a 2,3-Ketone at rates ranging from 0.05 to 0.5 kg a.i/ha suppressed root galling due to *M. incognita* in tobacco and also reduced *M. incognita* eggs/plant to the extent of 21 to 86 percent depending on rate of application and compound used. The present study findings were in close agreement with the study of *Streptomyces avermitilis* tested under glasshouse conditions on the management of reniform nematode, *Rotylenchulus reniformis* in okra with avermectin, revealed that the plants treated with avermectin (100%) as seed treatment recorded maximum shoot length, fresh shoot weight, dry shoot weight, and fruit yield. The highest reduction in *R.*reniformis population was observed in plants treated with avermectin 10% as seed treatment. Avermectin-treated plants also recorded significantly increased total phenol content and enzymatic activities such as peroxidase and indole acetic acid compared to control plants (15). Levels of control achieved by the avermectins were comparable to those of the registered compounds Ethoprop and Fenamiphos at 6.73 kg a.i/ha. Avermectins significantly reduced the number of *M. incognita* juveniles in cucumber roots and the proportion of juveniles that developed to a saccate stage (31,36). The author reported that avermectins rapidly impaired the locomotor activity of *M. incognita* second-stage juveniles as its mode of action. The rate of oxygen uptake of freshly hatched juveniles of *M. javanica, M. arenaria,* and *M. incognita* after exposure to 0.05 ppm avermectin B2a, and compared to untreated control (23). The average oxygen uptake was reduced to the extent of 61.1 percent by avermectin B2a for the three root-knot nematode species. Similarly, the report interprets that in different types of avermectin treatments, the highest reduction in root-knot index, soil nematode population, number of females/g root, egg masses/g root, eggs/egg mass ere recorded by avermectin 100% as seedlings root dip (17).

A preliminary test carried out to explore the nematicidal properties of one avermectin, abamectin B1 commercialized under name Vertimec against the root-knot nematode, *M. arenaria*, on tomato, showed that 1 mg/ litre avermectin B1 solution inhibited hatching of *M. arenaria* after 12 days inhibition and juveniles paralyzed after 24 hr exposure when dipped in a low concentration (0.3 mg/lit). Direct application of avermectin B1 to the soil induced a significant reduction in nematode penetration. However, avermectin B1 may be only used as an effective nematicide in sandy or low organic soils because of its rapid decomposition in high organic soils. Avermectin B1 when incorporated into the soil at rates of 0.3, 1.1 and 3.3 kg/ha, it was 10 to 30 times more potent than several organophosphate and carbamate nematicides against *M. incognita* (7, 30). The residual activity of avermectin also can be increased from 30 to 60 days by the rapid addition of ketones by microbes. A research study reports a reduction in a number of galls produced by *Meloidogyne* species when dipping 14 days old tomato seedlings in 1 mg/litre of avermectin B1 (26). The treatment delayed nematode invasion and development up to 20 days.

Avermectin application resulted in a reduction of chemicals required to control root-knot nematodes. Their water insolubility and rapid degradation in the soil also suggest that avermectins will not pose any contamination problems in agricultural groundwater (1, 3). On an evaluation study avermectin B1, isazafos and fenamiphos under greenhouse conditions for their bioefficacy against *Hoplolaimus galeatus*, and *Tylenchorhynchus dubious* (4).

Exposure of egg masses of *Meloidogyne javanica, M. arenaria,* and *M. incognita* to 1 mg/lit (1.2 μM) and 1/4, 1/16, 1/64 of AVM B2 aqueous solution, at 28°C for 5 days, completely suppressed egg hatching (25). The inhibitory effect on all three species noted was more with AVM B2 than with phenamiphos > ethoprophos > aldicarb at 10 mg/lit, oxamyl at 100 mg/lit, and carbofuran > carbosulfan at 10 mg/lit. The inhibitory effect was more marked in AVM B2a–23-ketone, a metabolite derivative, which suppressed egg hatching within 24 h in 0.1 mg/lit aqueous solution but rinsed in water 4 days later, the hatching resumed at a greater rate than in the control (35). This may indicate that embryogenesis proceeded normally and that hatching was halted by the immobilization of the juveniles by the avermectin. AVM B1 and B2 have shown equally high activities under *in vitro* tests against *M. incognita*, on tobacco grown in microplates or in greenhouse experiments where granulated formulations of these entities were incorporated (at 0.2 to 1.5 kg a.i/ha), AVM B2 was slightly more potent than B1 (29,30) and was about 10-40 times more potent than several organophosphorus and carbamate nematicides. (30,31,24). AVM B2a was rapidly converted by soil microbes (half-life of 2-5 days) to B2a – 23-ketone, having a soil half-life of about 30 days (30). The greater nematicidal potency of B2a seemed to be the result of its own nematicidal property combined with that of its metabolites.

Injections (1 ml) of > 100 μg a.i. of abamectin into banana pseudostems were effective at controlling *M. javanica* and *R. similis*, and were comparable to control achieved with a conventional chemical nematicide, fenamiphos (12). Abamectin injections of 250 and 500 μg a.i/plant were effective in reducing nematode infections ranging from 28 to 56 days after inoculation. Several researchers have shown that root dip, bulb dip, and soil applications of avermectins were effective at controlling plant parasitic nematodes on certain crops (4,7,11). However, use rates needed to achieve satisfactory control were

cost-prohibitive. Because avermectins are highly toxic to nematode parasites of animals and plants (6,30,33). Studies have focused on discovering other applications for these compounds for the control of plant parasitic nematodes.
Results of studies conducted to determine the potential of two avermectin compounds, abamectin and emamectin benzoate, applied by three methods; foliar spray, root dip, and pseudostem injection, against *Meloidogyne incognita* on tomato, *M. javanica* on banana, and *Radopholus similis* on banana revealed that foliar applications of both avermectins to banana and tomato were not effective for controlling any of the nematodes evaluated. Root dip of banana and tomato were moderately effective for controlling *M. incognita* on tomato and *R. similis* on banana. Injections (1 ml) of avermectins into banana pseudostems were effective for controlling *M. javanica* and *R. similis*, and were comparable to control achieved with a conventional chemical nematicide, fenamiphos. Injections of 125 to 2,000 μg/plant effectively controlled one or both nematodes on banana; abamectin was more effective than abamectin benzoate for controlling nematodes (14). Injection of Ivermectin into the stem of tomato seedlings was effective in the control of root-knot nematode *M. incognita* (18). Root dip treatment of abamectinreduced *M. arenaria* penetration into tomato roots (7) and application of avermectins via drip irrigation and by soil incorporation reduced *M. incognita* in Chrysanthemum (28). A compatibility study of avermectin with carbofuran 3G, *Pseudomonas fluorescens,* and *Trichoderma viride* for the management of *Meloidogyne incognita* in tomato revealed that combination of seedling root dip with avermectin and soil application of carbofuran 3G @ 1 kg a.i/ha recorded maximum shoot length, fresh shoot weight, dry shoot weight and fruit yield (16).

Toxicity and environmental aspects of avermectins used in crop protection

Environmental considerations regarding abamectin use in crop protection including the compound effects on non-target organisms etc. Abamectin is environmentally acceptable because it is used in low rates, is rapidly last from the environment, and does not bioaccumulate in the biological systems (34). Although abamectin is toxic to target pests its physical property and use patterns reduce exposure to nontarget organisms.

Effect of light

Abamectin undergoes rapid photolysis by oxidative and photooxidative mechanisms when it was exposed to light. After exposure to sunlight in water and soil the half-life of abamectin is less than 12 and 21 hours respectively. Avermectin B1 a and B1b are also susceptible to degradation when present as a thin film even in the absence of light. However, the presence of light accelerates degradation resulting in a half-life of 4 to 6 hours (21).

Bio-accumulation in soil

In soil binding studies that show the bioavailability of a compound to biological systems, abamectin was found to bind tightly to soil particles making it an immobile pesticide lacking bioavailability in the environment. The half-life of abamectin in the soil ranges from 20 to 47 days.

Effect on aquatic and terrestrial nontarget organisms

Field studies designed to evaluate the fate of abamectin in the aquatic environment by stimulating pesticide drift and runoff demonstrated that abamectin degrades rapidly both in soil and in the water. Avermectins degrade easily in water and therefore will not accumulate as natural waste and adversely affect the environment (34). Abamectin neither accumulates nor persists in fish because of the large size of the molecule which prevents it from being a truly lipophilic compound.

Effect on mammals

The toxicity of abamectin varies considerably between species and methods of application. Abamectin is moderately toxic to mammals in case of acute oral exposure ($LDss_{50}$ values for rate and mice vary from 10 to 20 mg/kg) but it is much less toxic in instances of dermal exposure LD_{50} more than 2000 mg/kg because it does not readily penetrate the skin (20).

Conclusions

Avermectins offer an outstanding alternative to any of the available synthetic pesticides as they showed excellent nematicidal, insecticidal, and miticidal action. Further, they do not leave residues and are relatively less persistent in the environment. Semi-synthetic derivatives of this fermented product have wide scope for industrial exploitation. However, viable and effective formulations need to be developed for wide-scale application. The talc, charcoal, and coir pith formulations examined were effective for short-term/immediate use for nematode control. Keeping the cost of production and commercial production, they can be recommended in high-value and polyhouse-grown crops.

Future scope of study

The application of avermectin as a bio-nematicide and the new discovery of nematicide activity will increase its requirement in various horticultural crops with root-knot nematode incidence.

Acknowledgment

I acknowledge the co-workers associated in this study and I further acknowledge Department of Plant Pathology, TNAU, Coimbatore and Sugarcane Breeding Institute, ICAR, Coimbatore for providing laboratory support to carry out the research work.

Conflict of interest

There is no conflict of interest. The authors had full access to all set of data and take complete responsibility for the accuracy of the data analysis.

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