NEMATICIDAL PRINCIPLE FROM THE LEAVES OF *Daemia extensa*

[1]Debnarayan Roy
[2]Dinesh kumar Das

[1]*Principal, S B S S Mahavidyalaya, P.O. Goaltore, Paschim Medinipur, West Bengal, India 721128*
[2]*Research Scholar, Jhargram Raj College, P.O. Jhargram, Paschim Medinipur, West Bengal, India 721507*

Abstract

We are running towards the era of biotechnology and this millennium is progressing towards the goal to achieve a control measure of pest in a sustainable manner to make the world free from pesticidal toxicity. A routine search of medicinal plants was done frequently in a random fashion in the local as well as forest areas of Paschim Medinipur District. After getting information from rural peoples we have collected different plants, which are commonly used by them for ailment. Our collection was mainly based on bark, leaves, roots, fruits etc. of different herbs and trees. The objective of our study is to find out suitable control method against noxious nematodes by applying plant products. The nematicidal activity of different plant products of over locality screened out in a random fashion. In vitro as well as in vivo test of a few plant products was done. We prepared aqueous extract in our laboratory from collected plant material and tried to determine their biological activity through in vitro test. After observing the positive effect from aqueous extract we further prepare alcoholic extract from a few plant products. Again we are trying to determine their biological activity of alcoholic extracts through in vitro test. Among them leaf extract of Daemia extensa is found to be most effective and highly promising in respect to future prospect of production of potential botanical pesticide.

Keyword : *Nematode Control, Meloidogyne incognita, Daemia extensa*

1.Introduction:

Plant parasitic nematodes are responsible for 10–40 % loss yields of various kinds of field and fruit crops. Among them Root-knot nematode, *Meloidogyne incognita* Kofoid and White (Chitwood) (Tylenchida: Heteroderidae) is one of the major plant-parasitic nematode species affecting the quantity and quality of the crop production in many annual and perennial crops. Different symptoms are observed in severely infested plants like root galls, stunting growth of plants and nutrient deficiency, particularly nitrogen deficiency (Siddiqui *et al.*, 2001). The yield losses of cotton production caused by *M. incognita* in 2002 were estimated to be between 18.0-47.3% (Davis and May, 2005). Several approaches have been attempted from time to time to minimize the population of plant-parasitic nematodes in the field by using natural enemies (Khan and Kim, 2007), enhancing cultural practices (Okada and Harada, 2007) cultivating resistant cultivars (Williamson and Kumar, 2006) and applying pesticides (Taniwiryono *et al*.,2007). A large number of plant species were selected based on their availability and potential use as botanical pesticide, are tested for nematicidal activity. In the present study we evaluate and trying to establish the nematicidal potency of extracts from *Daemia extensa* plant species, which may have nematicidal activity against *M. incognita.* Though effective in reducing nematode population are not always cost effective. Moreover, they are often phytotoxic (Rodriguez–Kabana *et al.*, 1988) and cause environmental pollution, endangering the life of many animals including fish (Landau & Tucker, 1984) and even contaminate ground water (Loria *et al.*, 1986). Nematodes infest almost all kinds of crops and the amount of damage they inflict on plants, constitutes 10 – 40% crop losses annually. Overall, 50% global yield loss is guessed to be due to root-knot nematodes, which may be much higher in the tropics and the subtropics. Sasser (1989) estimated losses of 17 - 29%, 18-33% and 24-38% on eggplant, melon and tomato respectively throughout the tropics. In India losses have been variously reported, such as, 35% death in eggplant to 60-75% in tomato etc. In India over 350 plant species are known as the hosts of *M. spp.* among them *Meloidogyne incognita* alone infecting about 250 and *M. javanica* infects about 150 genera of plant. Frequency of *M. spp.* in soil ranges between 17- 44% in different surveys. Applications of chemical nematicides, though effective in some cases, are not always cost effective and moreover their use involves some problems. These include phytotoxicity (Roberts *et al.*, 1988; Baujard *et al.*, 1989), ground water contamination (Loria *et al.*, 1986) and environmental pollution (Landau and Tucker, 1984). Another hazard is residual toxicity. Aldicarb at 1 ppm level in drinking water could suppress immune function in mice (Olsen *et al.*, 1987). Residues of aldicarb were reported in coconuts (Singh *et al.*, 1983) and carrots (Lue *et al.*, 1984). Traditional chemical control using chemical nematicides

available for the last few decades is in a declining status internationally (Osman and Viglierchio, 1988). Chemical nematicides, though effective in reducing nematode population, are not always cost effective. Moreover, they are hazardous and more difficult to apply. Therefore, it has become an important issue to find alternative control strategies, which are not only as effective as synthetic pesticides and safer to farmers but also which would be cheap, non-phytotoxic, cost effective, relatively less persistent in soil and relatively easily available to consumers (Fernandez *et al.*,2001). Thus there remains a need for the development of effective nematicides,. The prospects of finding such nematicide from among plant parts are favourable. One of possible alternatives is the utilization of pesticides from plant origin, known as botanical pesticides (Javed *et al.*, 2006). The botanical pesticides are generally biodegradable under field conditions as they are readily transformed by light, oxygen and microorganisms into less toxic products. Thus there is no residual effect in the environment (Ujvary, 2001).

2.Materials and Methods:

2.1 Preparation of ethanol extract from plant product:

The leaves of *Daemia extensa* were air dried in room temperature (28 ± 2^0C) for a week. The dust of air-dried leaves was prepared in grinder machine. The leaf dust was then taken in conical flask and rectified spirit was poured over it. The flask mouth was sealed by parafilm and kept for seven days. After that period the solution was filtered and the filtrate was placed in petridish for evaporation at room temperature for two to three days. The residue obtained after removal of the solvent under reduced pressure, was dried over anhydrous calcium chloride. The sticky materials were then scrap out for the preparation of test compound.

2.2 Preparation of test compounds:

The test solution was prepared for two concentrations viz. 5mg/ml and 10mg/ml by dissolving the crude extract of *Daemia sp.* in distilled water. Before the *in vivo* tests, phytotoxicity of the test compound was determined by cut shoot experiments as well as by foliar spray.

2.3 Collection of juveniles of *Meloidogyne incognita* :

Juveniles of *M. incognita* were collected from freshly hatched egg masses obtained from the *M. incognita* female. The *M. incognita* was maintained in the host plants in the experimental garden. A garden with different vegetable plants was maintained for this purpose. An isolated plot of the garden containing some vegetable plants (Brinjal plant, tomato plant etc.) was infected with harmful plant parasitic nematodes (*Meloidogyne incognita)*. The purpose is that to collect frequently harmful nematodes for observing the bioactive principle of the plant products during *in vitro* test as well as to inoculate the plant during *in vivo* test (pot experiment).

2.4 Application of test compound:

Active juveniles were placed in sterilized sand (2g) medium kept in a series of cavity blocks (3-4cm in diameter) and divided into two groups. One group serves as the control and the other are treated with test compound. Each block contained more than 100 nematodes. The cavity blocks were kept at room temperature (35 ± 2^0C). The test solutions were poured into the sand medium immediately after transferring the nematodes, at the rate of 2ml per cavity block. All cavity blocks except the control received treatments. Control received sterile tap water. After a fixed time interval the nematodes were shifted to sterile tap water and observed for four hour to see if any revival occurred or not. The experiment was replicated ten times for each treatment. Nematode mortality was obtained at varying lengths of time with a fixed concentration of the solution as well as by varying the concentration of the emulsion with a fixed time of treatment (Chatterjee and Sukul, 1981). Nematode mortality was determined by the prolonged immobility of nematodes after their transfer from the test medium to water (Das and Sukul, 1986). The regression of time mortality was calculated from the data.

2.5 Pot experiment :

Seeds of experimental plant were surface sterilized, allowed to germinate and sown in clay pots containing sterilized soil mixed with compost manure. The pots were divided into 4 batches, each consisting of at least 10 pots in the following manner:

Batch I – Uninoculated & untreated control plant.

Batch II – Inoculated & untreated.

Batch III – Inoculated and treated with the test compound by soil drench.

Batch IV – Inoculated and treated with the test compound by foliar spray to see the systemic effect, if any of the test compound.

The inoculation was done at four leaf stage. Each plant was inoculated by *M. incognita juvenile* @ 2500 ± 40. The test solution for the treatment was prepared by dissolving the test compound in distilled water. Each plant receives a treatment of 10 ml test solution. The first treatment was done after ten days of inoculation. The second treatment was done after 20 days of first treatment. The plants of Batch I receives 10 ml of distilled water at the time of both treatment. Forty-five days after second treatment, the plants were harvested and their shoot weight, shoot length, root length and root weight were measured. Root galls of the inoculated batches were counted. Root protein content was estimated by Folin phenol method (Chatterjee and Sukul, 1981). Nematode population in soil as well as in root

was determined by the modified Baermann method (Christie and Perry, 1951). All the batches were compared with each other by the analysis of variance and the mode of treatment showing maximum reduction in disease symptoms were ascertained.

3.Results :

The *in vitro* result shows 100% mortality of the nematodes at 5mg/ml and 10mg/ml concentration within 3 hrs and 2 hrs respectively. The *in vitro* test results shows that the alcoholic leaf extract of *Daemia extensa* have strong nematicidal property. This plant is very common in our rural locality and may have effectively used in agricultural field with a very low cost of their production. The water extract of this plant is also effective potentiality against noxious nematodes. The alcoholic extract prepared from the plant products can be stored for a long time for future use. The application of those compounds showed that the application of compound in the form of soil drench is more effective than that of the foliar spray. One of the interesting findings of the field trial was that the plants treated with above compound are less susceptible to insect attack. These observations indicate that the compound could be applied against insect pest also. The result of the *in vivo* test is given in following table.

Table 1: Effect of crude ethanol leaf extract of *Daemia extensa* on the growth and root knot disease symptoms in tomato *(Lycopersicon lycopersicum)* plants infected with *Meloidogyne incognita*.

Treatments*	Shoot length (cm.)	Shoot weight (g)	Root length (cm.)	Root weight (g)	Number of galls/ plant		Juveniles/ 2 g roots	Juveniles / 200 g soil	Root protein (mg/ g)
					Large	Small			
Uninoculated untreated	62.22 ± 2.42a	92.20 ± 5.29a	18.32 ± 1.44a	10.62 ± 2.84a	-	-	-	-	10.32 ± 0.05a
Inoculated untreated	36.72 ± 2.32b	48.42 ± 3.96b	14.44 ± 2.42b	27.92 ± 1.54b	17.60 ± 0.78a	560.42 ± 10.69a	1480 ± 7.51a	520.80 ± 8.28a	15.92 ± 0.03b
Inoculated & treated with crude extract (10 mg/ml) by soil drench	58.92 ± 3.23a	80.45 ± 4.82ab	16.23 ± 3.02ab	14.42 ± 2.23ac	8.20 ± 1.28b	192.21 ± 4.78b	370.60 ± 4.57b	142.21 ± 2.69b	12.24 ± 0.04c
Inoculated & treated with crude extract (10 mg/ml) by foliar spray	48.82 ± 2.78b	62.98 ± 6.22ab	17.92 ± 3.27a	18.20 ± 2.48c	14.22 ± 1.08ab	320.42 ± 5.42c	420.52 ± 5.82c	192 ± 6.41c	13.26 ± 0.02d
C. D. at 5% level	15.52	36.48	5.74	5.23	3.40	48.24	49.49	39.52	0.11

Dashes (-) indicate absence; * - 10 replicates for each group

a, b, c, d – Different small letters indicate significant difference ($P < 0.05$) by the analysis of variance

4.Conclusions:

Although the crude plant products were applied a little bit high dose in field trial because the active principle of the compound is not establish from the crude plant product in our project work. But interestingly it was observed that during *in vitro* test the compound could effectively kill the nematodes at a very low concentration with a long time of (about 4 hrs.) exposure.

It is evident from the results of *in vivo* test that compound can reduce nematode infection in tomato plants and augment their growth. Since the compounds are soluble in water and not phytotoxic it could be used with profit on

standing crops on a large scale. With an approach of mass propagation by cultivation of such plants, the problem of crop losses can be solve in relation to the damage caused by harmful nematodes.

These types of phytoremedial approach of pest control can boost up the rural economy. These products can be utilized for the remediation of diseases of human beings and other animals caused by various animal parasitic nematodes. So, this plant product commonly available in the local area of the forest of Jhargram district of West Bengal which is non-phytotoxic and application of such plant compound as nematicide may reduce ground water contamination. In field application these compounds will be less expensive in comparison to chemical nematicide.

5.References:

1. Baujard, P., Chambrier, C., Martini, B., Meunier, L., Pariselle, A. and Sarr, E. 1989. Comparison of seven nematicides and studies on the use of dibromochloropropane in groundnut culture in the Sahelian area of Senegal. Rev. de Nematol. 12: 293-299.

2. Chatterjee, A. and Sukul, N.C. 1981. Total protein content of galled roots as an index of root-knot nematode infestation of lady's finger plants. Phytopathol. 71: 372-374.

3. Christie, J.R. and Perry, V.G. 1951. Removing nematodes from soil. Proc. Helminthol. Soc. Washington 18: 106-108.

4. Das, S. and Sukul, N.C. 1986. Biocchemical changes in some crop plants due to root-knot nematode infestation. Proc. Sym. New Dimension on Parasitol., Allahabad: 122-125.

5. Davis, R. F. and May, O. L. 2005. Relationship between yield potential and percentage yield suppression caused by the southern root-knot nematode in cotton. Crop Sci., 45(6) : 2312-2317.

6. Fernandez, C., Rodriguez-Kabana, R., Warrior, P. and Kloepper, J. W. 2001. Induced soil suppressiveness to a root-knot nematode species by a nematicide. Biol. Control, 22(2), 103-114.

7. Javed, N., Gowen, S. R., Inam-ul-Haq, M. and Abdullah, K. Shahina, 2006. Systemic and persistent effect of neem (*Azadirachta indica*) formulations against root-knot nematodes, *Meloidogyne javanica* and their storage life. Crop Protect. 26(7), 911-916.

8. Khan, Z. and Kim, Y. H. 2007. A review on the role of predatory soil nematodes in the biological control of plant parasitic nematodes. Appl. Soil Ecol., 35(2), 370-379.

9. Landau, M. and Tucker, J.W. Jr. 1984. Acute toxicity of EDB in Aldicarb to two young estuarine fish species. Bull. Environ. Cont. Toxicol. 33: 127-132.

10. Loria, R., Eplea, R.E., Baier, J. H., Martin, T.M. and Moyer, D.D. 1986. Efficacy of sweep-shank fumigation with 1, 3-dichloropropene against *Pratylenchus penitrans* and subsequent ground water contamination. Plant Dis. 70: 42-45.

11. Lue, L.P., Lewis, C.C. and Melchor, V.E. 1984. The effect of aldicarb on nematode population and its persistence in carrots, soil and hydroponic solution. J. Environ. Sci. Heal. B19: 343-354.

12. Okada, H. and Harada, H. 2007. Effects of tillage and fertilizer on nematode communities in a Japanese soybean field. Appl. Soil Ecol., 35(3), 582-598.

13. Olsen, L.J., Erickson, B.J., Hinsdill, R.D., Wymam, J.A., Forter, W.P., Binning, L.K., Bidgodd, R.C. and Nordheim, E.V. 1987. Aldicarb immunomodulation in mice: An inverse dose-response to parts per billion levels in drinking water. Arch. Environ. Cont. Toxicol. 16: 433-439.

14. Osman, A.A. and Viglierchio, D.R. 1988. Efficacy of biologically active agents as nontraditional nematicides for *Meloidogyne javanica*. Ibid. 11: 93-98.

15. Roberts, P.A. Magyarosy, A.C., Matthews, W.C. and May, D.M. 1988. Effect of metam-sodium applied by drip irrigation on root-knot nematodes, *Phythium ultimum* and *Fusarium sp.* in soil and on carrot and tomato roots. Pl.dis. 72(3): 213-217.

16. Rodriguez-Kabana, R., Weaver, C. F., Robertson, D.G. and Ivey, H.1988. Bahiagrass for the management of *Meloidogyne arenaria.* Ann. Appl. Nematol. 2: 110-114.

17. Sasser, J.N. 1989. Plant parasitic nematodes: Dept. Pl. Pathol., NCSU, Consortium Int.Crop Prot.,Releigh. pp. 15.

18. Siddiqui, Z. A.; Iqbal, A.; Mahmood, I. 2001. Effects of *Pseudomonas fluorescens* and fertilizers on the reproduction of *Meloidogyne incognita* and growth of tomato. Appl. Soil Ecol., 16(2) : 179-185.

19. Singh, K.P. Pandey, S.Y. Khan, M.M. and Singh, S. 1983. Residues of aldicarb in coconut. Pesticide Sci. 14: 441-443.

20. Taniwiryono, W.D., Brink, P. V. D., Rietjens, I. M. C. M. and Murk, J. 2007. A case study in Bangka Island, Indonesia on the habits and consequences of pesticide use in black pepper plantations. J. Environ. Toxicol., 22(4), 405-414.

21. Ujvary, I. 2001. Pest Control Agents from Natural Products, Handbook of Pesticide Toxicology. 2nd ed.; Academic Press: San Diego.

22. Williamson, V. M. and Kumar, A. 2006. Nematode resistance in plants: The battle underground. Trends Genet., 22(7), 396-403.