NEMATICIDAL PRINCIPLE FROM THE LEAVES OF

Polygonum barbatum

[1] Dr. Debnarayan Roy
[2]Dinesh kumar Das
[3]Moumita Sahoo

[1]Officer in charge, Jhargram Raj College, P.O. Jhargram, West Bengal, India 721507
[2] Research Scholar, Jhargram Raj College, P.O. Jhargram, West Bengal, India 721507
[3]Part-time Teacher P.K College,Contai P.O. Contai, Purba Medinipur, West Bengal, India 721401.

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 Polygonum barbatum 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, Polygonum barbatum .***

Introduction:

Medicinal plants are future source of herbal medicine for various human disease management **(Shakya, 2016)**. *Polygonum barbatum* (family: Polygonaceae), common name 'panimarich' is an annual herb, with knotted stem and spicy, pungent aroma leaf distributed in wet places specially beside the river, stream and canals of India, Bangladesh, Thiland, china and some other oriental regions **(Research wing)**. Phytochemical screening of *P. barbatum* ethanolic extract shows the presence of Carbohydrate, Saponin, Gums, Phenolic compounds,Flavenoids, Alkaloids and Phytosteroid **(Mazid et al., 2011;Sheela et al., 2011; Ezhilan and Neelamegam, 2015**). Aqueous and ethanolic extract of *P. barbatum shows cholinergic*, spasmolytic, larvicidal, antifungal, antibacterial activity and significant effect in curing gastric ulcer in rat **(Choudhryi et al, 2003; Gouri et al, 2008)**. Antitumor activity and control over hispa beetle which cause huge loses to rice crop was studied with Polygonum species in Bangladesh **(Haque et al, 2002; Mazid et al, 2011)**. Beside the phytochemical derived from various plants, synthetic drug like Albendazole, Praziquantel, Niclosamide are known to eleminate completely intestinal flat worm infection with 100% efficacy. Recently genetic diversity of *P. barbatum* was analysed using RAPD molecular marker and large variation seen in samples **(Prabudha and Tiwari, 2014)**.

MATERIALS AND METHODS

A. Screening of heterocyclic compounds for their nematicidal activity

Considering the bioactivity of different heterocyclic compounds, we have synthesized different derivatives of heterocyclic compounds form mother compounds like carbazole, pyridine, pyrimidine and thiazole heterocycles. Thenematicidal properties of heterocyclic compounds of plant origin like triterpenoid saponins was also studied. The nematicidal properties have been studies by both in vitro and in vivo tests. In in vitro test juveniles of M. incognita were exposed to the aqueous solution of different compounds and their efficacy was recorded in terms of percentage mortality of nematodes. The host plant selected for the in vivo test was tomato (L. lycopersicum) .

B. Synthesis of compounds

Different workers have tried to synthesized different types of heterocyclic compounds from mother . We have synthesized the following compounds and tested their activity on root-knot nematodes :

C. In vitro test for nematode mortality with the test compound

i. Preparation of test compound

Some of the compounds were water soluble and others not. For the compounds soluble in water, test solutions were prepared by dissolving the compounds in water at different dilutions. The emulsion of water insoluble compounds were prepared by sonication. In the sonication method, the compounds at different doses are put in distilled water and then emulsified in a sonicator (Labsonic 2000, Brawn)for 3 min. at 20 Hz frequency in intermitant episodes each of 20 seconds duration.

ii. Collection of juveniles of M. incognita

Juveniles of M. incognita were collected from freshly hatched egg masses obtained from the female. M. incognita was maintained in the host plants in the experimental garden. Active juveniles were placed in sterilized sand (2g) medium kept in a series of cavity blocks (3-4 cm in diam.) and divided into two groups. One group served as the controls and the others are treated. Each blocks contained more than hundred nematodes. The cavity blocks were kept at room temperature (35 + 2°c).

iii. Application of test compounds

The test solutions or emulsions of the test compounds was poured into the sand medium immediately after transferring the nematodes, at the rate of 2 ml per cavity block. The compounds were tested in different concentrations. The send was soaked with the solution or emulsion up to a limit at which it became saturated. Al cavity blocks except the controls received treatments. Controls received sterile tap water. Each cavity block was then covered with a lid and kept for a fixed time. After a fixed time interval the sand medium from the treated as well as control groups were poured into a beaker containing sterile tap water. Sand particles settled down at the bottom of the beaker and the water containing nematodes were transferred to another beaker. Finally, nematodes from the beakers were transferred to a cavity block containing sterile tap water and observed for 4 hrs to see if any revival occurred (Ghosh and Sukul, 1990). The experiment was replicated ten times for each treatment.

iv. Analysis of results

A time mortality regression was drawn and the correlation coefficient calculated for each compound.

v. Phytotoxicity of the compounds

The test compounds were sprayed at 10 mg/ml concentration on the experimental plants at the rate of 10 ml/plant, 15 days after emergence. The treated plants were observed for 15 days.

D. In vivo tests

i. Arrangements of pots

Forty earthen pots of 32-cm diam. And 32-cm height were taken and divided into four batches, each consisting of ten pots. Pots were arranged in the following manner:

a) 1st batch - Uninoculated untreated control.
b) 2nd batch - Inoculated and untreated control.
c) 3rd batch - Inoculated and treated with test compound by soil drench.
d) 4th batch - Inoculated and treated with test compound by foliar spray.

ii. Denematization

All the earthen pots were filled up with a mixture of clay soil, compost manure and rice mill ash in the ratio of 2: 1: 0.25 (v/v). Decomposed cow dung was used as compost manure. To maintain the light texture and to increase the porosity of the soil, rice mill ash was added to it (Sinhababu, 1983). Each pot contained an average of 3 kg of soil. Soil mixture was denematized by purring boiling water into the pots. Holes present at the bottom of the post were sealed thereby allowing hot water to stand in the pot for 2 hrs. Hot water was applied to the soil in such a way that every portion of the soil was thoroughly covered with water. Finally the water was removed by opening the holes of the pots at the bottom. This hot water treatment was repeated twice with an interval of 4 hrs.

iii. Seedling

Seeds were surface sterilized with 0.1% $Hgcl_2$ for 15 min. and then repeatedly washed in sterile tap water for 15 min. Sterilized repeatedly seeds were then kept on a filter paper soaked in sterile water and put in a sterilized petridish for germination. After germination 3-4 seeds were sown in each pot.

iv. Inoculation

Before inoculation, each pot contained one healthy seedling having equal growth. Three batches were inoculated with second stage juveniles of M. incognita simply by pouring the water containing juveniles around the roots. Juveniles used as inoculums were collected from the freshly hatched egg masses of female M. incognita. The juveniles were extracted by the modified Baermann technique (Christie and Perry, 1951). The inoculation was done when the plant was at four-leaf stage. Uninoculated and inoculated pots were arranged in 4 rows, maintaining a distance of 3‘ x 3‘ in between two rows to provide sufficient light and to prevent contamination. Inoculation was given at the rate of 2 juveniles/g of soil.

v. Treatment

Ten days after inoculation, inoculated batch-III received a soil drench of the test compound and batch-IV received the same compound by foliar spray. The remaining plants of batch-I and II served as uninoculated untreated and inoculated controls, respectively. During foliar spray each plant was covered at the base with polyethylene sheeting. Spraying was done by hand sprayer. In soil drench, treatment was given around the base of the stem by using glass pipette. The concentration of the test compound was same in both soil drench and foliar spray. The treatment both in the form pouring into soil and spraying on leaves, were given at dusk and repeated twice at an interval of seven days. The remaining two batches that served as an uninoculated and untreated and inoculated and untreated controls, respectively, received same amount of distilled water sprayed on the leaves and poured over the soil at the time the other batches received treatment.

vi. Maintenance of plants

The experimental gardens as well as plants were properly maintained to prevent the attack of insects and other pests. All the plants were irrigated twice daily, once in the early morning and next time in the late afternoon.

vii. Harvesting

Plants were harvested carefully after 30-45 days of planting. The plants were uprooted gently to prevent damage of the root system. The main root system was then cut out and washed in running tap water. The roots were wiped in towel and tissue paper simultaneously to remove water adhering to it.

viii. Assessment of final growth

After harvesting, the results were analysed by observing and measuring different plant growth parameters like shoot length, root length, shoot weight and root weight. Stems and root of all the plants were taken separately and their length and weight were measured using centimeter scale and a physical balance.

IX. Assessment of root nodules

In case of tomato *(L. lycopersicum)* bacterial nodules were counted and classified into two categories i.e Large(above 5 mmin diam.) and small (bellow 5 mm diam,).

x. Assessment of root nodule

In case of tomato *(L. lycopersicum)* bacterial nodules were counted and classified into two categories i.e. Large (above 5 mm in diam.) and small (below 5 mm in diam.).

xi. **Assessment of nematode population**

Nematode population in the rhizospheric soil (200g) of different treatments was estimated by modified Baermann technique. The number of juveniles for 2gm roots of different treatments were also estimated by the same technique.

E.Scanning Electron Microscopy: Specimens were prepared for scanning electron 40 microscopical study to know the changes in surface ultra structural organization of treated 41 cestodes, following the method as described by **Ash et al., 2012,** with slight modification. 42 Both the controlled and treated worms were fixed in 10% neutral buffered formalin and kept 43 **6 |** P a g e at 4℃ for 24 hours. Then the worms were washed in PBS and dehydrated firstly with 1 ascending grades of alcohol and then ascending grade of acetone to pure dried acetone. 2 Specimens were then critical point dried (CPD) using liquid carbon dioxide as transitional 3 fluid followed by air drying at 25℃. Samples were mounted in a metal stub, coated with 4 platinum (Pt) and examined within ZEISS EVO-MA 10 model scanning electron microscope. 5

In vitro tests:

The aqueous solution of crude extract *of Polygonum barbatum* prepared by dissolving it into distilled water . Three different concentrations was prepared i.e. 15mg/ml, 10 mg/ml and 5mg / ml .The procedure adopted for in vitro tests has been described earlier. In efficacy tests *M.incognita* were exposes at different concentration death and paralysis times were noted. In phytotoxicity tests *L. Lycoperscium* was used as the host plant. The method has been mentioned earlier.

In vivo test :

The host plant used for the in vivo test was *L. Lycoperscium* Inoculation was given at 5920 ±30 juveniles/pot. The method was mentioned earlier in page --. The experiment was replicated for 3 times.

Results

Invitro Tests;

In-vitro efficacy test with all applied doses effects on the mobility of worms. In each repeated experiment all worm shows paralysation following by death. During efficacy, light microscopical observation, suggest scolaces and strobilae of nematodes move faster when they are exposed to crude extract. Scolaces of worms shows immobility when paralyzed but their highly segmented body proper, strobila shows little movement. Proglotids of long segmented strobila shows maximum movability during first contact with ethanolic extract. As the time increases movability becomes low. Three different doses of 5mg/ml,10mg/ml, and15mg/ml concentration shows mean paralyzed time 20min ,17. min,15min and mean death time 29min,28min,25min respectively. Graph no. 1 shows maximum effect in 15 mg/ml concentration in which mean paralyzed and death time are 15min and 25min respectively. Morphological observation shows changes in tegument color and alteration in shape and size of the parasites. Petri dishes containing controlled parasite in 0.9% PBS get attached to each other forming many knuckles and nodes, on contrary treated cestodes remains free from each other minimizing their body size and shape. Statistical analysis of mean death time of both the test material and reference drug compared to control group shows null hypotheses to be rejected and difference between the mean death time is considered significance at ($P<0.05$) **(Roy et al, 2007)**.

Invivo Tests;

The test compound augmented the plant growth in terms of shoot length and shoot weight as compare to inoculated untreated plants (table-5).The numbers of juveniles in 200g soil and 2g roots also significantly reduced in treated plants.

Table 1:Comparisons of paralysis time at different concentration of *Polygonum barbatum*

Doses	**Mean paralysis time**
15ng/ml	**15**
10mg/ml	**17**
5mg/ml	**20**

Table 2:Comparisons of at death time different concentration of *Polygonum barbatum*

Doses	Mean death time
15ng/ml	25
10mg/ml	28
5mg/ml	29

Table 3:Comparisons of paralysis time between Carbofuran (commercial nematicide) and *Polygonum barbatum* at different concentration

Doses	Paralysis time in minutes	
	Carbofuran	*Barbantus sp*
15ng/ml	8	15
10mg/ml	10	17
5mg/ml	11	20

Table 4:Comparisons of Death time between Carbofuran (commercial nematicide) and *Polygonum barbatum* at different concentration

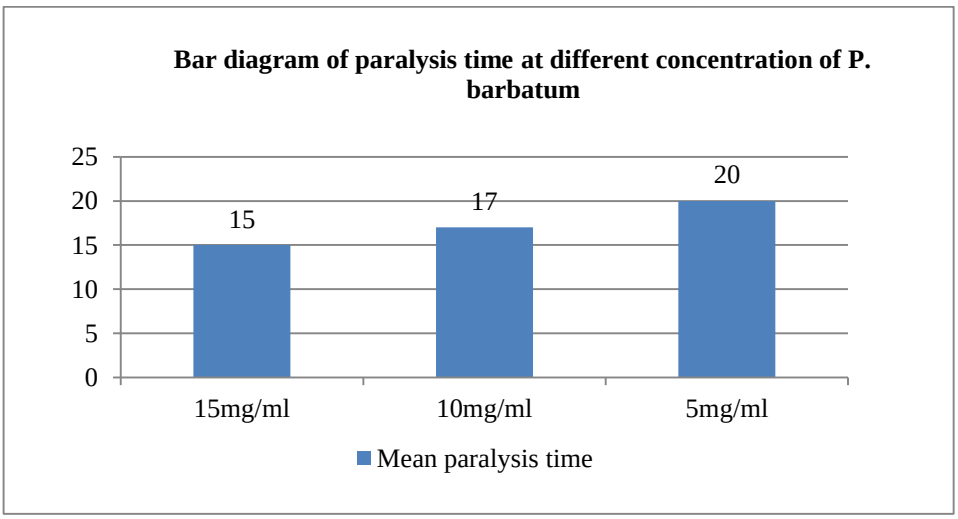
Doses	Death time in minutes	
	Carbofuran	*Barbantus sp*
15ng/ml	17	25
10mg/ml	20	28
5mg/ml	22	29

Table 5: Effect of crude ethanol leaf extract of *Polygonum barbatum* on the growth and root knot disease symptoms in tomato *(L. lycopersicum)* plants infected with *Meloidogyne incognita.*

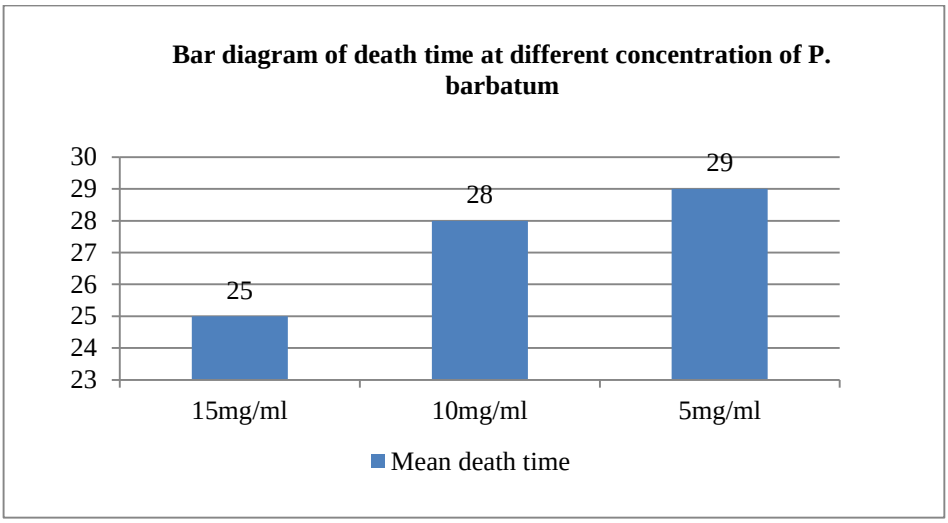
Treatments*	Shoot length (cm.)	Shoot weight (g)	Root length (cm.)	Root weight (g)	Number of galls/ plant		Juveniles / 2 g roots	Juveniles / 200 g soil	Root protein (mg/ g)
					Large	Small			
Uninoculated untreated	64.14 ± 2.41a	95.42 ± 5.42	18.42 ± 1.42a	10.22 ± 2.91a	-	-	-	-	10.22 ± 0.05a
Inoculated untreated	36.71 ± 2.32b	47.24 ± 3.94b	26.43 ± 2.20b	24.79 ± 1.45b	16.7 ± 0.62b	5.20 ± 10.21	1420 ± 7.51a	510.8 ± 8.42a	15.32 ± 0.02b
Inoculated & treated with crude extract (10 mg/ml) by soil drench	56.24 ±3.32a	79.14 ± 4.28ab	22.31 ± 3.20ab	14.24 ± 2.23ac	12.4 ± 0.45b	172.1 ± 4.56b	380.6 ± 4.84b	162.2 ± 2.17b	12.42 ± 0.05c
Inoculated & treated with crude extract (10 mg/ml) by foliar spray	40.56 ± 2.22b	62.33 ± 6.2ab	19.56 ± 3.77a	19 ± 0.48c	14.2 ± 0.32ab	245.4 ± 5.20c	630 ± 5.82c	210.8 ±6.41c	13.62 ± 0.04d
C. D. at 5% level	15.42	37.99	5.84	5.13	3.3	48.74	39.99	39.45	0.11

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

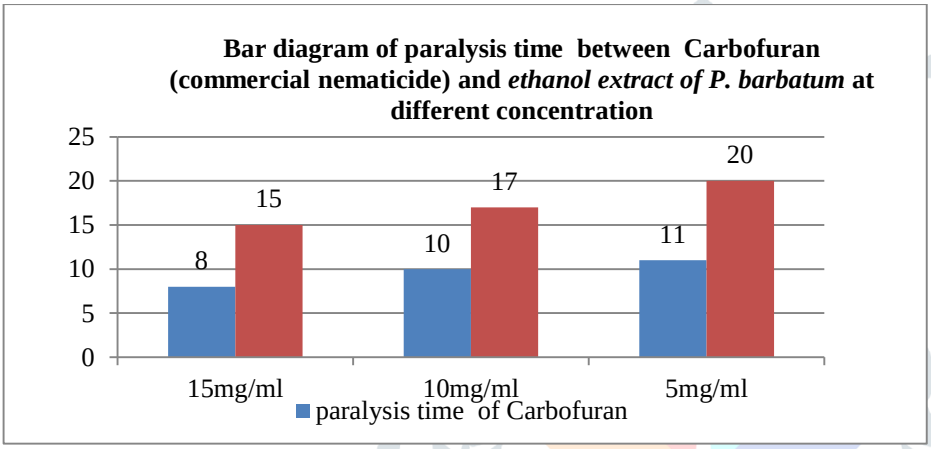
a, b, c, d – Different small letters indicate significant difference ($P < 0.05$) by the analysis of variance



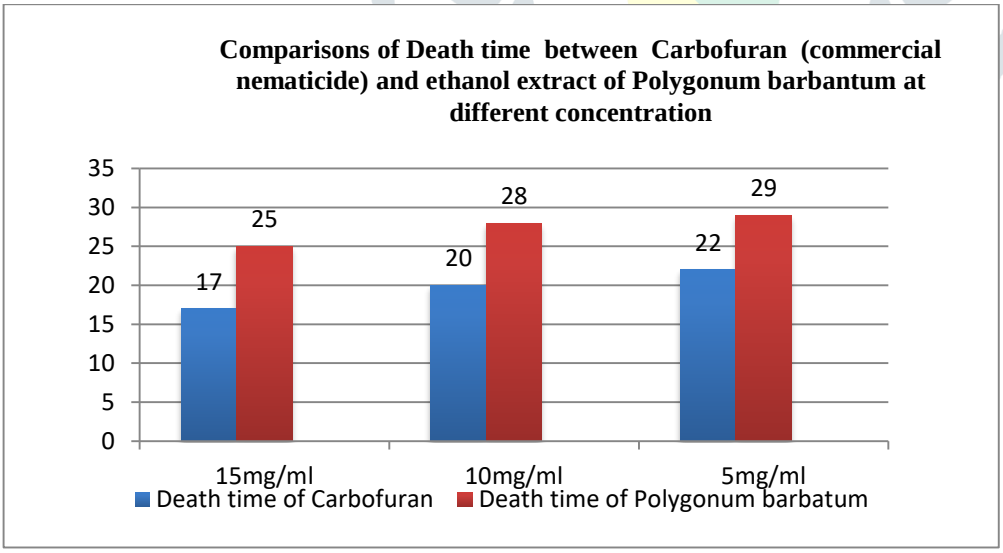
Graph: 1



Graph: 2



Graph: 3



Graph: 4

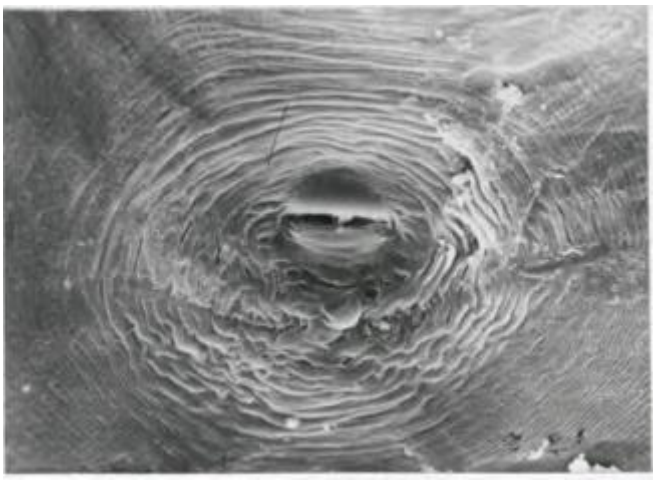


Fig: 1 Perineal structure of Meloidogyne incognita(SEM) Controled

(A) (B) (C)

Fig: 1 (A), (B), (C) Perineal structure of Meloidogyne incognita(SEM) after treatment with Ethanol of extract *Polygonum barbatum*

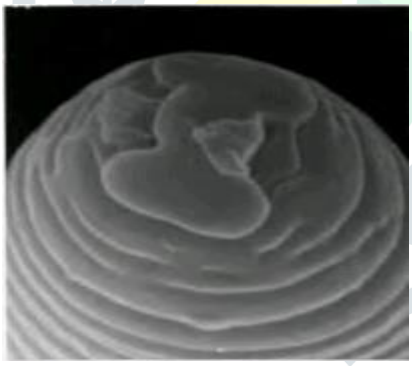
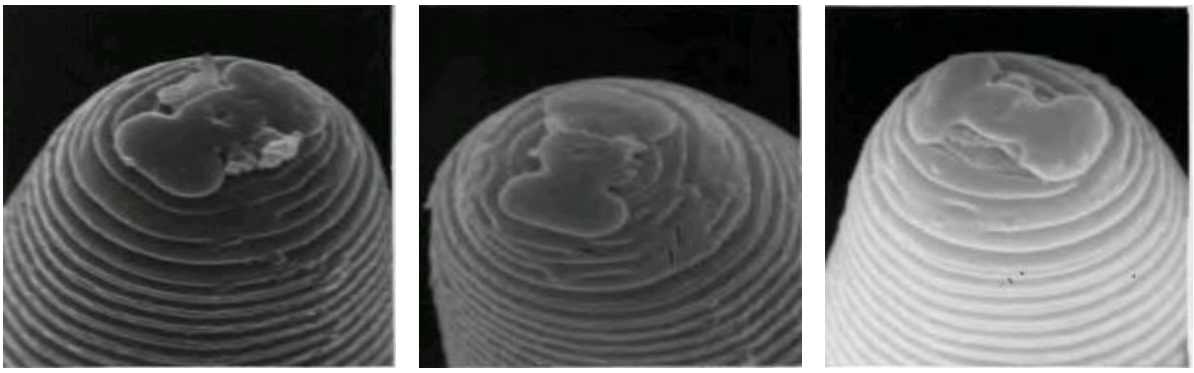


Fig: 2 Head structure of Meloidogyne incognita(SEM) Controled



(A) (B) (C)

Fig: 2 (A), (B), (C) Head structure of Meloidogyne incognita(SEM) after treatment with Ethanol of extract *Polygonum barbatum*

Resut discussion

Comparison of paralysis time at different concentration of ethanolic extract of *Polygonum barbatum* is shown in table.. The graph(1) reveals that lower the dose increases paralysis time. In 10mg/ml & 5mg/ml concentration the paralysis time 17min & 20 min respectively ,which is different . So we used lower level of dose,that is 5mg/ml concentration of ethanolic extract of *Polygonum barbatum* to control nematode population.

Comparison of death time at different concentration of ethanolic extract of *Polygonum barbatum* is shown in table.. The graph(2) reveals that lower the dose increases death time. In 10mg/ml & 5mg/ml concentration the death time 28min & 29 min respectively ,which is more or less same . So we used lower level of dose,that is 5mg/ml concentration of ethanolic extract of *Polygonum barbatum* to control nematode population

Carbofuran , a commercial nematicide, which is used to control nematode population. The mean paralysis time of carbofuran at different doses are shown in table 3 .The comparison of mean paralysis time of carbofuran and ethanolic extract of *Polygonum barbatum* is shown in table3. The graphical representation of these two are in graph no3 ,in which we show that the *Polygonum barbatum* take more time to paralyse nematode ,in contrast it is a plant product, which have no hazardous for health

Carbofuran , a commercial nematicide, which is used to control nematode population. The mean death time of carbofuran at different doses are shown in table4 .The comparison of mean death time of carbofuran and ethanolic extract of *Polygonum barbatum* is shown in table4. The graphical representation of these two are in graph no 4 ,in which we show that the *Polygonum barbatum* take more time to control nematode ,in contrast it is a plant product, which have no hazardous for health.

The test compound augmented the plant growth in terms of shoot length and shoot weight as compare to inoculated untreated plants (table-5).The numbers of juveniles in 200g soil and 2g roots also significantly reduced in treated plants.

Microscopic examination of perineal structure of M. incognita shows that(fig:1) they are roughly oval with a high arch and lateral lines inconspicus or inter-rupted by stirae.Forking of the striae frequently occurred at the lateral lines.

Perineal structure of Meloidogyne incognita(SEM) after treatment with Ethanol extract of *Polygonum barbatum*. has been changed with slight modification of striae in some cases it shown to be destruction of striae in Fig: 1 (A), (B), (C)

Microscopic examination of head structure of M. incognita shows that(fig:2) the head region are composed with annules, lateral lip, and dorsal lip which was cleft by an incisures.

Head structure of Meloidogyne incognita(SEM) after treatment with Ethanol extract of *Polygonum barbatum*. has been changed with almost or completely devoid of annules, lateral lips were disfigured which has been seen in fig: 2 (A), (B), (C)

Conclusion

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 environment pollution (Landau and Tucker, 1984). Another hazards is residual toxicity. Aldicarb at 1ppm level in water could suppress immune function in mice (Olsen et al., 1987). Residues of aldicarb were report changing the enzyme like oxidase and esterase, nematodes were known to develop resistance aganst aldicarb and oxamyl after a long time exposure (Below et al.,, 1987). Carbofuran is reported to inhibit seed germination and reduce seedling height in some plants (Benjamini,1986),reduce nodulation in beens(Karanja et al...,1982) affects the lands and in biotic community (Broder,1987) and also cause human sterility(Termoto and shirasu,1989) Traditional chemical control using chemical nematicides available for the last few decades is in declining status internationally (Osman and Viglierchio,1988).

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. Benjamini,1986, Below et al.,, 1987, Baujard et. al. ,1989

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. Haque et al, 2002; Mazid et al, 2011

8. 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.

9. 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.

10. 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.

11. 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. Labsonic 2000, Brawn

12. 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.
13.Mazid et al., 2011;Sheela et al., 2011; Ezhilan and Neelamegam, 2015
14. 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.
15. Osman, A.A. and Viglierchio, D.R. 1988. Efficacy of biologically active agents as nontraditional nematicides for *Meloidogyne javanica*. Ibid. 11: 93-98.
16.Prabudha and Tiwari, 2014).
17 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. Roberts et al., 1988;
18. 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. Roy et al, 2007
19. Sasser, J.N. 1989. Plant parasitic nematodes: Dept. Pl. Pathol., NCSU, Consortium Int.Crop Prot.,Releigh. pp. 15. Shakya, 2016
20. 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.
21. Singh, K.P. Pandey, S.Y. Khan, M.M. and Singh, S. 1983. Residues of aldicarb in coconut. Pesticide Sci. 14: 441-443.
22. 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.
23. Ujvary, I. 2001. Pest Control Agents from Natural Products, Handbook of Pesticide Toxicology. 2nd ed.; Academic Press: San Diego.
24. Williamson, V. M. and Kumar, A. 2006. Nematode resistance in plants: The battle underground. Trends Genet., 22(7), 396-403.