IN VITRO Evaluation Of Nematicidal Properties Of Neem Products On Egg Hatching Of A Phytonematode *Heterodera Cajani*.

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Abstract: - *In vitro* efficacy of five neem formulation was tested against a phytoparasitic nematode *Heterodera cajani.* The neem products viz. neem leaf, neembark,nimboli, Achook and Nimin were tested against egg hatching of *Heterodera cajani. The formulation was made in water and its various dilution was prepared i. e. S/30,S/45,S/60 and S/90. where S stands for stock solution. The maximum reduction (97. 6%) was observed at S/30 concentration of Nimin followed by Achook, Neem leaf, Nimboli and Neem bark treatments respectively at the same concentration. Maximum hatching of eggs or minimum inhibition in hatching was observed in S/90 neem bark(23). All the neem products show promising result in reducing hatching of Heterodera cajani larvae.* Cowpea (*Vigna unguiculata*L.) or Chawla is an important kharif crop of Rajasthan state due to its ability to grow and yield at relatively low level of fertility. Cow pea has relatively high lysine content (470-497mg/g N) which makes it an excellent improver of protein quality over cereal grains(Bressani,1985). Along with food and feed value it also helps in improving soil fertility by fixing atmospheric nitrogen in the soil with the help of root nodules inhabited by nitrogen fixing *Rhizobium* spp. (Rachie, 1985). During last twenty years the plant family meliaceae and especially its members *Azadirachta indica* and *Melia* was identified as one of the most promising member and source of compounds with pest control properties. Mishra and Prasad (1973)found water soluble fractions of neemcake (water extract) to be toxic to *Meloidogyne incognita*. Akthar and Alam(1993) used Nimin and Achook for seed coating for the control of various plant parasitic nematodes. Mojumdar and Mishra (1994) found that seed treatment of chickpea with neem seed kernel ,neem seed coat, achook, neemark and nimbicidine were effective in reducing 2nd stage juvenile penetration of *Meloidogyne incognita.* Basu and Mojumdar (1998) reported that the mortality percentage of2nd stage juvenile of *Meloidogyne incognita* was directly correlated with the concentration of neem based products and the period of exposure. Singh and Singh (1994) reported highly reduction in hatching of eggs, emergence of larvae from cyst,immobilization and mortality of *Heterodera cajani* juveniles in cowpea plants treated with aqueous neem cake extracts. Pandey and Trivedi (2000)reported significant reduction in *Heterodera cajani* population in cowpea plants treated with “Nimin” and “Achook” along with neem leaf powder ,Neem bark and nimboli. Sharma *et al* (2000) evaluated neemark and nimbicidine for management of root-knot nematode in okra. Sivkumar and Gunasekaran (2011) reported the nematicidal activity of three botanical formulation based on neem oil and Pongamia oil against *Meloidogyne incognita* infecting tomato, chilli and brinjal. Among three botanical formulation the formulation no 60 EC© was significantly superior and reduced the nematode population to 48. 6%. Now a days because the use of pesticides is subsidized in many countries due to pollution problems associated with the use of dangerous chemicals the environmental advantage of using safe material such as neem for pest control and different parts of neem viz. neem leaf, neem bark, neem seed and neem kernel as well as neem based pesticides is gaining importance in the present decade. In the present study the ‘*in vitro*’ efficacy of five types of neem products viz. neem leaf, neembark,nimboli, Achook and Nimin were tested against egg hatching of *Heterodera cajani.*

Materials and Methods:

For *in vitro* evaluation aqueous extracts of neem leaf, neem bark was prepared by grinding (2gm) each of the product in grinder with distilled water. This was filtered through four ply muslin cloth and stored in the refrigerator for 12 hrs (Stock solution –S). Water extract of nimboli was left in water for overnight. After 24 hours the extract was filtered through four ply muslin cloth and stored in refrigerator for 24 hours. (Stock solution –S) Nimin and Achook was also mixed with distilled water to obtain an emulsive concentration (S). Each formulation was diluted with water to get emulsion with strength of S/30,S/45,S/60, and S/90. For hatching experiment special polyvinyl chloride (P. V. C) tubing (4cm diameter 0. 5mmHigh) were cut and 26micronmeter stainless screen was sealed to each ring. Four PVC legs were attached to elevate each ring which allowed the juveniles to pass through and it also made possible to transfer of eggs. Eggs of *Heterodera cajani* were collected from the heavily infected roots of cowpea. 200 eggs of *H. cajani* were used per replicate sample and each treatment was replicated thrice. The experiment was conducted at room temperature.

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The number of hatched juveniles were counted after 24,48 and 72 hrs. After every 24 hours test solution was discarded after counting the number of hatched larvae and the unhatched eggs in the sieve were placed in freshly made test solution . This was done to eliminate the effect of bacterial action on the unhatched eggs. After 72 hours the unhatched eggs were removed and transferred to distilled water for 24 hours to ascertain the hatching if any.

Results and discussion

The data revealed in (Table -1) indicates that there was gradual enhancement in hatching of eggs of *Heterodera cajani* as they were transferred from highest concentration to lowest concentration. The maximum reduction (97. 6%) was observed at S/30 concentration of Nimin followed by Achook, Neem leaf, Nimboli and Neem bark treatments respectively at the same concentration. At higher concentration reduced hatching was very clearly observed after 24 hrs. While at lower concentration it appears after 72hrs. After resuspension of unhatched eggs in distilled water for 24 hours reduced hatching was observed showing the nematotoxic properties of neem compounds. Kumar and Khanna (2006) reported a reduction in root knot nematode,*Meloidogyne incognita* population by the five neem based nematicide product on the growth of tomato. The most effective formulation was neem seed kernel extract and Econeem. Hatching of unhatched eggs were enhanced in distilled water which were previously kept in S/60 and S/90 concentration of neem products. Maximum hatching was observed in S/90 neem bark(23). On contrary

at S/30 concentration an inhibition in hatching was observed with maximum being in S/30 Nimin and S/30 Neem leaf (Table :1). The direct toxicity of neem products can be attributed to some of the chemical extracted from neem like Nimbin, Quercetin,Salanin, Kaemferol,Thionine, Thionemone,Aza and Nimbidine limnoids(Khan *et al*. , 1974;Devkumar *et al.* , 1985, 1986;Alam,1993). Parveen *etal*(1994)reported the efficacy of neem products in the order Neem oil>Neem leaf>Nimin>Neem cake which are contrary to our findings where Nimin was proved best on cowpea plant. Rao and Parmar (1984) reported the presence of 34 bitter principles in neem. The fresh extract of fruit, leaf, bark root and gum of neem inhibited hatching of *Meloidogyne incognita* (Siddiqui and Alam,1985a). Basu and Mojumdar(1998) also favoured our present findings where they reported the toxicity of all the five aqueous neem products(neem seed kernel, neem seed coat, Achook, Neemark, and Nimbicidine) against 2nd stage juvenile of *Meloidogyne incognita.* Water soluble fraction of Neemcake were found toxic to *M. incognita* (Mojumdar and Mishra1992; Lanjewar and Shukla,1986). In the present investigation higher concentration of Achook was proved effective in reducing hatching of *Heterodera cajani* eggs *in vitro*. This was supported by Paruthi *et al* (1996) who reported *in vitro* efficacy of Achook against *M. incognita*. Thus it is concluded from the studies that all the five tested compounds show high degree of promises in controlling the hatching of *Heterodera cajani* eggs. But the variation in their effectivity *in vitro* conditions can be attributed due to lack of standard composition of all bioactive compounds compared.

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Table 1 : *in vitro* efficacy of different neem products against egg hatching of *Heterodera cajani*.

Treatments	Mean Larval hatch after various duration (in hours)			Total hatch	% Reduction in hatching over check
	24	48	72		
1. Nimin					
S/30	3. 30	2. 00	0. 00	5. 30	97. 60
S/45	5. 00	4. 00	0. 70	9. 70	95. 60
S/60	25. 00	20. 00	3. 00	48. 00	78. 50
S/90	27. 30	20. 60	6. 30	54. 20	75. 70
2. Achook					
S/30	4. 00	3. 00	1. 00	8. 00	96. 40
S/45	7. 20	4. 90	2. 00	14. 10	93. 60
S/60	26. 00	18. 60	5. 00	49. 60	77. 10
S/90	28. 00	20. 00	8. 00	56. 00	74. 90
3. Neem leaf					
S/30	4. 00	3. 20	0. 90	8. 10	96. 30
S/45	6. 60	5. 60	3. 60	15. 80	92. 90
S/60	24. 00	22. 00	6. 00	52. 00	76. 70
S/90	30. 20	28. 00	7. 00	65. 00	70. 80
4. Neem bark					
S/30	7. 00	6. 00	0. 60	13. 60	93. 80
S/45	8. 90	8. 80	3. 60	21. 30	90. 40

S/60	27. 70	26. 40	3. 60	57. 70	74. 00
S/90	33. 40	28. 00	8. 60	70. 00	68. 60
5. Nimboli					
S/30	5. 80	4. 50	1. 40	11. 70	94. 74
S/45	8. 00	7. 30	2. 60	17. 90	91. 90
S/60	25. 00	23. 20	7. 00	55. 20	75. 20
S/90	32. 60	27. 00	8. 00	67. 60	69. 70
6. Water	32. 30	45. 00	12. 60	89. 94	-

(The observation are mean of three replicates.)

Table 2: Percentage juvenile hatching of *Heterodera cajani* eggs after resuspension in water (after 24hrs)

	Number of juveniles hatched in various concentration of neem products			
Treatments	**S/30**	**S/45**	**S/60**	**S/90**
Nimin	-	3. 5	13	14
Achook	2. 6	4. 6	11	12. 3
Neem leaf	1	2. 1	10	11
Nimboli	2	4	10	10. 3
Neem bark	4. 5	7. 1	19	23
Water	18	18	18	18