Efficacy of the fruit extract of *Citrullus colocynthis* (l.) on the root-knot nematode *Meloidogyne incognita* infecting *Vigna ungiculata* (L.)

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ABSTRACT

Root – knot nematodes (*Meloidogyne* spp.) are one of the most wide spread pests limiting world agricultural productivity and their control in future will largely depend on the continued development of resistant varieties as well as careful management practices which rely less on chemicals and more on plant inbuilt strategies to fight pathogens. So far, the conventional systemic nematicides were readily used by previous workers in several crops for the management of root-knot nematode. However, adverse effect on environment and human health is limiting the use of such nematicides in India. Therefore, in the present investigation, the bio-pesticide property of the fruit extract of *Citrullus colocynthis* on the root-knot nematode *Meloidogyne incognita* infecting *Vigna ungiculata* was carried out. The total carbohydrate content, total chlorophyll content and root gall index present in the leaves of control, inoculated with root- knot nematode and in the inoculated plants treated with fruit extract of *Citrullus colocynthis* were analyzed and the root gall index that was an indirect measure of nematode population density after treatment was also studied.

Key words: Root – knot nematodes, *Meloidogyne incognita*, *Vigna ungiculata* and *Citrullus colocynthis.*

INTRODUCTION

Among the different ecological groups of the nematodes, the terrestrial nematodes (plant and soil inhabiting nematodes) form a highly diversified group and play an important role either in restricting the crop yields or in maintaining a natural balance in soil(Baqri, 2000). Phytophagous nematodes are microscopic organisms which are generally soil dwellers and infest underground parts, mostly roots of cultivated plants. The economic importance of nematodes is well recognized all over the world for centuries, as most of the agricultural crops are damaged by their continuous feeding on roots, buds, stems, crowns, leaves and even seeds, resulting in low yields and poor quality of crops. The population of plant parasitic nematodes is mainly influenced by new technological development in agriculture involving different cropping sequences, introduction of new culture, changes in fertilizer level and type of pesticides being used in these days. The degree of damage caused by nematodes depends upon the population density of nematodes, susceptibility of the crop, environmental conditions, such as, soil fertility, moisture and also the presence of other pathogenic microorganisms which may interact with nematodes as they are known to cause complex plant diseases in association with fungi, bacteria and viruses (Mishra and Nageswari, 2000). The root-knot nematodes (*Meloidogyne spp.*) are sedentary endoparasites and are among the most damaging agricultural pests, attacking a wide range of crops including green gram (Sikora and Greco, 1993). Although *M.incognita* has a wide host range among crop plants and weeds, mango was considered to be a non-host (Saka and Carter, 1987). Application of chemicals is one of the effective methods of pest management. However their use has been restricted due to high cost, environmental problem and non-availability of potent nematicides. Likewise, the use of organic matters as soil amendments to control nematode pests has some limitations (Das and Sinha, 2005). Chemical nematicides very often lead to environmental pollution and even depletion of stratospheric ozone (Wheeler *et al.*, 1979). So far, the conventional systemic nematicides were readily used by previous workers in several crops (tomato, egg plant, chilli and cardamom) for the management of root-knot nematode. However, adverse effect on environment and human health is limiting the use of such nematicides in India (Ahuja, 1982; Jain and Gupta, 1985; Ali, 1986). Prakash *et al.* (2008) proposed inter trap crops for the management of this nematode. Hence the present study, efficacy of the fruit extract of *Citrullus colocynthis* on the root-knot nematode, *Meloidogyne incognita* infecting *Vigna ungiculata*.

MATERIALS AND METHODS

Surface sterilized *Vigna ungiculata* seeds were sown in plastic pots of one litre capacity containing autoclaved sterilized river soil, garden soil and red soil (2:1:1). The egg masses of root-knot nematode, *M. incognita* were collected from the root galls infected plants of *Acalypa indica* and experimental plants were inoculated with 5 and 10 egg masses of the nematode by pouring into four holes and were closed with top soil. Distilled water was poured for three days after inoculation. Thereafter the nutrient solution and plant extract were added alternatively. The air dried *Citrullus colocynthis* fruits were prepared by extracting 25g of fruit material in 200 ml acetone (55^0C) in soxhlet apparatus (Peach and Tracey, 1956). Different concentrations of plant extract such as, 2, 4, 6, 8 and 10 ppm were prepared from stock solution using distilled water. After 25 days of treatment, the biochemical characteristics, such as total carbohydrate content (Jayaraman, 1981) and Cholorophyll (a, b and total) were estimated according to the method of Wellburn and Litchenthaler (1984).

RESULTS AND DISCUSSION

The effect of the root-knot nematode, *M. incognita* and the fruit extract of *C. colocynthis* on biochemical constituents, such as, total carbohydrate (mg/g) of the cowpea, *V. ungiculata* after 25 days of treatment was estimated and presented in table 1. The total carbohydrate content (mg/g) in the leaf of the experimental plants was found to be fluctuating. In the control plants, the total carbohydrate (mg/g) was found to be 79.50 ± 1.8 mg/g and inoculated plants after 25 days of treatment the total carbohydrate content was found as, 125.33 ± 1.1 mg/g at five egg mass inoculum level and 121.76 ± 0.8 mg/g at ten egg mass inoculum level. Nutman (1958) found that nodulation depended upon the supply and translocation of certain materials particularly carbohydrates from the shoot. Therefore, the reduced nodulation in the nematode infested plant might also be due to the interruption of translocation and or consumption of host plant materials during gall formation or directly by the nematodes. Vaitheeswaran *et al*, (2005) noticed a reduction in dehydrogenase of glucose and alcohol and â-amylase activities both in root and shoot systems of the host plant during nematode infection, the maximum reduction being in root system for all enzyme activities might be due to reduced substrate concentration namely sugar contents in the systems under infection stress. The total chlorophyll content (mg/g) in the leaf of the experimental plants after 25 days of treatment was found to be 3.695 ± 0.0 mg/g, 4.103 ± 0.0 mg/g, 4.727 ± 0.0 mg/g, 6.056 ± 0.0 mg/g and 7.330 ± 0.0 mg/g at 2, 4, 6, 8 and 10 ppm, respectively in the 5 egg mass inoculum level and 0.927 ± 0.0 mg/g, 3.891 ± 0.1 mg/g, 4.413 ± 0.0 mg/g, 6.709 ± 0.0 mg/g and 7.858 ± 0.0 mg/g at 2, 4, 6, 8 and 10 ppm, respectively in the 10 egg mass inoculum level.

The total chlorophyll content (mg/g) in the leaf of the experimental plants after 25 days of treatment was found to be 3.393 ± 0.0 mg/g, 3.544 ± 0.1 mg/g, 3.645 ± 0.0 mg/g, 3.816 ± 0.0 mg/g and 4.012 ± 0.1 mg/g at 2, 4, 6, 8 and 10 ppm, respectively in the 5 egg mass inoculum level and 3.426 ± 0.0 mg/g, 3.566 ± 0.0 mg/g, 3.652 ± 0.0 mg/g, 3.755 ± 0.0 mg/g and 3.955 ± 0.3 mg/g at 2, 4, 6, 8 and 10 ppm respectively in the 10 egg mass inoculum level. Ramakrishnan (1997) reported that the leaf chlorophyll content was lowered with an increase in nematode densities independently and concomitantly. Melakeberhan *et al*. (1985) reported that the total chlorophyll and chlorophyll a content, photosynthetic nitrogen basis decreased significantly with increase in level of nematode infection. The chlorophyll b content and photosynthetic rate on a total chlorophyll basis did not significantly decrease with increasing nematode infection. However, respiration rate increased with nematode infection.

The effect of the root-knot nematode *M. incognita* and the fruit extract of *C. colocynthis* on the nematode density after 25 days of treatment in the form of root gall index of the cowpea, *V. ungiculata* was recorded and presented in table 3. The root gall index of the experimental plants was found to be decreased with the increasing concentrations of *C. colocynthis* treatment. The root gall in the control plants was found to be absent, but in the case of inoculated

Table 1. Effect of the root-knot nematode *M. incognita* and the fruit extract of *Citrullus colocynthis* on total carbohydrate content (mg/g) in the leaf of cowpea, *Vigna unigiculata* after 25 days of treatment.

Inoculum Egg masses / plant	Total carbohydrate content (mg/g)						
	25 days of treatment						
	Control	Inoculated control	2ppm	4ppm	6ppm	8ppm	10ppm
5	79.50±1.8	125.33±1.1	94.35±0.5	169.93±1.4	135.43±0.5	129.41±1.3	145.02±3.8
10		121.76±0.8	136.18±1.0	26.47±0.8	211.79±0.9	112.23±0.9	169.33±0.9

Note : Data are the average value of three replicates.

Table 2. Effect of the root-knot nematode *Meloidogyne incognita* and the fruit extract of *Citrullus colocynthis* on chlorophyll content a, b and total (mg/g of fr.wt.) in the leaf of the cowpea, *Vigna ungiculata* after 25 days of treatment.

Egg masses inoculated / plants	Chlorophyll content (25th day mg/g of fr.wt.)																				
	Control			Inoculated Control			2ppm			4ppm			6ppm			8ppm			10ppm		
	a	b	Total	a	b	Total	a	b	Total	a	b	Total	a	b	Total	a	b	Total	a	b	Total
5	0.224 ± 0.0	3.222 ± 0.0	3.446 ± 0.0	0.090 ± 0.0	2.695 ± 0.0	2.785 ± 0.1	0.409 ± 0.0	3.286 ± 0.0	3.695 ± 0.0	0.413 ± 0.0	3.691 ± 0.0	4.103 ± 0.0	0.413 ± 0.0	4.314 ± 0.0	4.727 ± 0.0	0.0453 ± 0.0	5.603 ± 0.0	6.056 ± 0.0	0.465 ± 0.0	6.866 ± 0.0	7.330 ± 0.0
10				0.215 ± 0.0	2.337 ± 0.0	2.552 ± 0.1	0.831 ± 0.0	0.096 ± 0.0	0.927 ± 0.0	0.093 ± 0.0	3.797 ± 0.0	3.891 ± 0.1	0.047 ± 0.0	4.366 ± 0.0	4.413 ± 0.0	0.085 ± 0.0	6.629 ± 0.0	6.709 ± 0.0	0.097 ± 0.0	7.76 ± 0.0	7.858 ± 0.0

Table 3. Effect of the root-knot nematode *M. incognita* and the fruit extract of *Citullus colocynthis* on root gall index of the cowpea, *Vigna ungiculata* after 25 days of treatment.

Egg masses inoculated / plant	Control	Inoculated Control	Root Gall Index		6 ppm	8 ppm	10 ppm
			2 ppm	4 ppm			
5	0	3.33±1.3	2.88±1.3	2.83±1.3	2.66±0.6	2.66±2.1	0.66±1.1
10		4.33±1.1	3.00±1.0	2.33±0.5	1.66± 0.6	1.66±0.6	1.33±0.6

control with 5 egg masses was recorded as 3.33 ± 0.1 and in the inoculated control plants with 10 egg masses, it was recorded as 4.33 ± 1.1. The root gall index of the plants inoculated with five egg masses was recorded as 2.88 ± 1.3 , 2.83 ± 1.3, 2.66 ± 0.5, 2.66 ± 2.0 and 0.66 ± 1.1 at 2,4,6,8 and 10 ppm, treatment respectively. The same trend was observed in ten egg masses inoculated plants from 3.00 ± 1.0 (2 ppm) to 1.33 ± 0.6 (10 ppm). Thoden *et al.* (2009) proposed 1, 2 – Dehydropyrrolizidine alkaloids for the management of this nematode. Very recently Ntalli *et al.* (2010) proposed *Melia azedarach* (Meliaceae) for *M.incognita* management.From these observations, it has been augmented that the fruit extract of *C. colocynthis* has a telling effect on root gall index that is an indirect measure of nematode density. Therefore it is felt worthwhile to carryout further investigation for analyzing the reason for the toxic property of fruit extract of *C. colocynthis* in future.

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REFERENCES

Ahuja, S. 1982. Chemical control of root-knot nematode in nursery beds of tomato and egg plant and its effect on yield in the field. *Tropical Pest Management,* **28**(3) : 313-315.

Ali, S. S. 1986. Evaluation of nemacur against *Meloidogyne incognita* in cardamom nursery. *Indian Journal of Nematology,* **16** (1) : 48-50.

Baqri, Q. H. 2000. Diversity in plant and soil nematodes (Nematoda) of Rajasthan (India) Gaps in Research. In : Plant diseases. Ed. P. C. Trivedi pointer publishers, Jaipur (India). 286-304.

Das, N. and Sinha, A. K. 2005. Integrated management of root-knot nematode *Meloidogyne incognita* on okra (*Abelmoschus esculentus*). *Indian Journal of Nematology,* **35**(2): 175-182.

Jain, R. K. and Gupta, D. C. 1985. Control of root knot nematode (*Meloidogyne javanica*) through nursery bed treatment in tomato (cv. HS-100). *Indian Journal of Nematology*, **15** (2): 274.

Jayaraman, J. 1981. Laboratory manual in Biochemistry, Willey eastern Ltd., Madras 1-65.

Mishra, S. D. and Nageswari, S. 2000. Plant nematodes management in substainable and subsistence agriculture. **In :** *Plant diseases* Ed. P. C. Trivedi pointer publishers, Jaipur (India). 431-442.

Melakeberhan, H., Brooke, R. C., Webster, J. M. and Auria, J. M. D. 1985. The influence of *M. incognita* on the growth, physiology and nutrient content of *Phaseolus vulgaris*. *Physiological Plant Pathology,* **26**:259 – 268.

Ntail, N. G., Menkissoglu-Spiroudi, U. and Giannakou, I. 2010. Nematicidal activity of powder and extracts of *Melia azedarach* fruits against *Meloidogyne incognita*. *Annals of Applied Biology,* **156**(2): 309 - 317.

Nutman, P. S. 1958. The physiology of nodule formation **In:** Nutrition of the legumes, (Hallsworth, E. G. ed) Academic press. New york, 81-107.

Peach, K. and Tracey, M.V. 1956. Modern methods of plant analysis, Springer verlag, Berlin 33.

Prakash, A., Jagadiswari Rao and Nandagopal, V. 2008. Future of Botanical pesticides in rice, wheat, pulses and vegetables pest management. *Journal of Biopesticides*, **1**(2):154-169.

Ramarkrishnan, S., Gunasekaran, C. R. and Vadivelu, S. 1997. Efficacy of organics in the control of *M. incognita* on okra, *Indian Journal of Nematology*, **27**(1): 74-78.

Saka, V. W and Carter, C. C., 1987. Hosts and non-hosts of the root knot nematode, *Meloidogyne incognita*. North Carolina State University, Raleigh, N.C. 62.

Sikora, R. A. and Greco, N. 1993. Nematode parasites of food legumes. In: Luc, M., Sikora, R.A., Bridge, J. (eds.), plant parasitic nematodes in subtropical and tropical agriculture. (AB international, Institute of parasitology, Wallingford, U.K, 629).

Thoden, C., Halmann, J. and Boppre, M. 2009. Effect of Plants containing Pyrrolizidine alkaloids on the Northern root knot nematode *Meloidogyne hapla*. *European Journal of Plant Pathology,* **123**(1):27-36.

Vaitheeswaran, M., Ibrahim, S. M. and Senthilkumar, K. 2005. Carbohydrate metabolism in a host plant, *Hibiscus cannabinus* infected by *Meloidogyne incognita*. *Indian Journal of Nematology,* **35** (2): 205-206.

Wheeler, W. B., Thompson, M. P., Edelstein, R. L. and Krause, R. T. 1979. Ultrasonic extraction of carbofuran residues from radishes. *Bulletien of Environmental Contamination Toxicology* **21:**; 238-242.

Wellburn, A. R and Litchtenthaler, H. 1984. Formulae and programme to determine total carotenoids and chlorophyll a and b of leaf extracts in different solvents. In: Advances in Photosynthesis Research Sysberma, martinus Mishoff Junk, W. eds). The Hague. **2**: 9-12.

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