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EVALUATION OF NEMATICIDAL POTENCY OF BOTANICAL BIOPESTICIDES IN COMBINATION WITH TRIAZOPHOS AGAINST ROOT KNOT NEMATODE, *MELOIDOGYNE INCOGNITA* INFESTATION ON CHICKPEA, *CICER ARIETINUM* L

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ABSTRACT

Aqueous leaves extract of *Citrus aurantifolia* and chemical pesticide, Triazophos with different combinations have been tested against root knot nematode, *Meloidogyne incognita*. Experiments were conducted *in-vitro* and in micro-fields on host plant chickpea, *Cicer arietinum* L. Field application of mixtures was tested by seed soaking method, foliar spray method and direct soil treatment method. *In-vitro* experiments showed more than 95% juvenile (J_2) mortality of nematode parasite in 5: 5 combination of leaves extract and triazophos treatment. This combination was also found very effective by different treatment methods in micro-field experiments. A significant increase in shoot length and weight and root length and weight of host plant was observed. The greatest reduction in nematode population with nil root knot index (RKI) was noticed in 50:50 combination treatment of citrus leaves extract and triazophos when compared with infected control. This treatment also influences the number of bacterial nodules, percent germination of seeds and seedling vigour index (SVI) over normal control.

KEYWORDS: *Citrus aurantifolia*, Integrated Pest Management, *Meloidogyne incognita*, Nematicidal Efficacy, Root Knot Nematode, triazophos

INTRODUCTION

Pulses are an important ingredient of human diet because of their high nutritional value (iron and protein) at a low cost. They can be used for innovative and nutrient dense food products to combat malnutrition. In this era of Green Revolution with major focus on staple food like rice and wheat, pulses were relegated to the marginal lands with least of inputs. Pulses improve productivity of farming systems through reduced nitrogen requirements and improved soil health. After considering the importance of pulses for sustainable development FAO in 2013 declared the year 2016 as International Year of Pluses (IYOP-2016). During 2012-13, pulses production in the India was 18.34 million tones and is expected around 20 million tons in the 2013-14 with a record growth rate of more than 3% (Annual Report 2013-14, IIPR, India). Among the pulses, Chickpea (*Cicer arietinum L.*) is one of the most important pulse crops in India, ranking fourth among grain crops in acreage and production. It occupies about 6.67×10^6 hectare of agricultural land producing 5.3×10^6 tons annually in India (Singh 2012).

Plant parasitic nematodes are major pests of agricultural crops and are responsible for yield losses upto the 50% (Abbasi et al., 2008). Among these, root knot nematode, the genus *Meloidogyne* is distributed worldwide. They are known to have a wide range of host plants including monocotyledons, dicotyledons, herbaceous and woody plants (Sirca et al, 2004). Several nematode species are associated with chickpea causing damage to this crop. Root knot nematodes,

Meloidogyne arenaria, Meloidogyne incognita and Meloidogyne javanica are reported to cause 13.7- 60% losses in chickpea (Sharma and McDonald, 1990, Ali, 2009). Infection of four races of *M. incognita* (R1, R2, R3, and R4) and one race of *M. arenaria* (R2) was found to be prevalent in Uttar Pradesh (Mohiddin and Khan, 2014). In such situation, chickpea crop appears to suffer more seriously. Use of synthetic nematicides is one of the fast and most effective methods to control such parasites, but the injudicious and indiscriminate use of chemical pesticides resulted in contamination of food chain. In search of alternative methods to control the plant parasitic nematodes, the application of botanical biopesticides is a better option. Due to plant origin these botanicals are cheap, biodegradable and eco-friendly and are not harmful to non target fauna including human being. Till now many of the plants, plant parts and their active components have been evaluated for their nematicidal properties worldwide and positively these were found potent in reducing the nematode infection on plants (Saxena and Singh, 2002; Sarvanpriya and Sivakumar, 2005; Wiratno et.al., 2009; Rehman et. al., 2012; Wondimeneh et. al., 2013). Saxena and Singh (2001) also reported effectiveness of certain plant extracts for their nematicidal potentialities where they observed significant mortality of second stage juvenile of root-knot nematode. Botanical biopesticides release various active ingredients which have nematicidal properties (Trifonova and Atanasov, 2009; Du et al, 2011; Ojo and Umar, 2013).

Combinations of botanical biopesticides with some known chemical nematicides were also found to be effective against plant nematodes (Sayeeda and Ahmad, 2005; Mervata et at, 2012). The present work has been carried out to test the nematicidal potency of leaves extract of *Citrus aurantifolia* alone as well as in combination with synthetic chemical pesticide Triazophos against root-knot nematode *Meloidogyne incognita* infesting chickpea plants of susceptible variety, *Pusa 362*.

MATERIALS AND METHODS

In Vitro Experiments: Laboratory experiments (*in vitro*) were setup to evaluate the nematicidal effect of leaves extract alone and in combination with synthetic nematicide (Triazophos) against second stage juveniles (J_2) of *Meloidogyne incognita*. Dose combinations of leaves extract and Triazophos were prepared in different ratio *viz.* 9:1, likewise 8:2, 7:3, 6:4 and 5:5.

One ml. juvenile suspension containing approximately 35-48 juveniles in each vial mixed with same volume of each of the above concentration. Vials were covered by aluminum foil and kept in BOD incubator at 24+1^{0}C for the exposer of *3hrs, 6hrs, 24hrs, 48hrs* and *72hrs*. Each exposer was replicated thrice.

After the desired period of incubation, the mortal juveniles were counted with the aid of a stereoscopic binocular microscope. The percent juvenile mortality had been calculated according to Abbot's formula (Abbot, 1925) from the average of three replication and data were analyzed and converted to natural corrected % mortality.

$$Percent\ mortality\ of\ J2 = \frac{\text{Number of Mortal Larvae}}{Total\ Number\ of\ Larvae\ taken} \text{ x}100$$

Abbot's Formula

$$Correct\ \%\ mortality\ = \frac{\%\ \text{Test Mortality} - \%\ \text{Control Mortality}}{100 - \%\ Control\ Mortality}$$

In vivo Experiments

Micro-field experiments (*In vivo)* were processed by the following techniques-

Seed Soaking and Foliar Spray Methods

Field trials comprising of seven treatments were conducted with 390 ppm of lemon extract and Triazophos 40 EC as stock. Combinations of these two in 9:1, 8:2, 7:3, 6:4 and 5:5 ratio along with stock and control were taken for seed soaking method. Seeds were soaked for 24 hrs and then air dried later. The coated and dried seeds were then sown in the micro-fields of plot size 7' x 7' feet. Un-inoculated Control (NC) with seeds soaked in petroleum ether and a purely infested plant as Inoculated Control (IC) were also run parallel. After ten days of germination each plant was inoculated with 1000 freshly juveniles of *Meloidogyne incognita.* Each experiment was replicated thrice.

Foliar Spray Application of same treatment were given to the plant with the help of hand operated knap sprayer fitted with flat fan nozzles at the crop age of 40 days. The experiment was terminated after 60 days of sowing. Observations were made on plant growth characters and nematode population at the time of termination. Each treatment was replicated thrice. Un-inoculated and Inoculated Control experiments were run parallel for a check. Observations were recorded after 60 days inoculation on plant growth parameters and nematode population on termination of experiment for-

- Fresh shoot length (in cm)
- Fresh root length (in cm)
- Fresh shoot weight (in gm)
- Fresh root weight (in gm)
- Root-Knot Index (RKI)
- Number of Bacterial Nodules

Calculation Used in i*n Vivo* Experiment

Percent Germination of Seeds

$$\%\ Germination = \frac{\text{Total number of seeds germinated}}{\text{Total number of seeds sown}} \text{x } 100$$

Seedling Vigour Index (SVI)

$$SVI = (\text{Shoot length} + \text{Root length}) \text{ x } \%\ \text{germination of seeds}$$

Percent Increase/Decrease (I/D) in Length/Weight of Shoot/Root

$$Percent\ Increase\ or\ Decrease = \frac{\text{T} - \text{C}}{\text{C}} \text{x } 100$$

Where, T= Length or weight of experimental object and

C= Length or weight of control object

Root -Knot Index (RKI) was Calculated according to Reddy *et al* (1997)

$$RKI = \frac{\text{Number of total galls counted in each replicate}}{3}$$

Scale rating (0-5) was considered according to Taylor & Sasser (1978) *viz.*, 0= disease free, 1= very mild, 2 = mild, 3 = moderate, 4 = severe, 5 = very severe

RESULTS AND DISCUSSIONS

***In vitro* Experiment: Effect of Combinations of Leaves Extract of *Citrus aurantifolia* and Triazophos on Juvenile (J_2) Mortality**

Different combinations of *Citrus aurantifolia* with Triazophos were found effective against J_2 (s) of *M. incognita* as shown in Table 1. Treatment of 9:1ratio dose shows 54.16%, 69.16% and 86.67% mortality of J_2 after the exposer of 3 hrs, 6 hrs and 48 hrs respectively. 48 hrs of exposer gives 100% of juvenile mortality. In case of 8:2 concentration 3hrs and 6 hrs exposure shows 62% and 75.83% mortality which increased upto 94.16% and 100% after 24 and 48 hrs respectively. 7:3 and 6:4 both the concentrations were also found effective as these kill 100% population of J_2 (s) after 48 hrs of treatment. Out of all the concentrations, 5:5 were found to be the most effective as it recorded 75.87% after 3 hrs and 87.67% after 6 hrs, 97.66% and 100% after 24 & 48 hrs respectively. *Citrus aurantifolia* leaves extract alone was found to be equally good when compared to triazophos, against J_2 (s). Their population reduced to 31.66% after 3 hrs in comparison with triazophos which recorded 40.38% mortality after 3 hrs.

In the present work, combination of *C. aurantifolia* leaves extract and triazophos gave promising results as it reduced the population of J_2 (s) of *M. incognita.* The was in agreement with El-Nagdi and Youssef (2013), who carried out comparative studies between plant extract and a nematicide Cadusafos 10G (Rugby) to control J_2 (s) of *M. incognita.* They found that botanical extracts of garlic (*Allium sativum*) cloves and castor bean (*Ricinus communis*) seeds were more effective and significantly ($p \leq 0.05$) reduced nematode criteria including number of galls and egg masses on roots of tomato and number of juveniles in roots and soil. Here also *C. aurantifolia* leaves extract is as good as triazophos for the growth of chickpea. Previously Mojumdar and Mishra (1991) observed that soaking of chickpea seed in the aqueous extracts of leaves of bhang, kateli (*Argemone mexicana*) and neem reduced the penetration of *M. incognita* in chickpea seedlings. Kaushik, (2002) investigated the effect of factory effluents and same synthetic pesticide triazophos against *M. incognita* infecting chickpea where she recorded the 42.67% to 100% juvenile mortality after 6 hrs to 72 hrs exposure.

Table 1: Effect of *Citrus Aurantifolia* Leaves Extract and Triazophos Alone and in Combination against Juveniles of *M. Incognita* under *In Vitro* Condition

S. No.	Treatments	Percent Mortality After Hours				
		3	6	24	48	72
1.	9:1	54.16	69.19	86.67	100	-
2.	8:2	62	75.83	94.16	100	-
3.	7:3	69.33	84.16	95.83	100	-
4.	6:4	52.83	69.16	84.67	100	-
5.	5:5	75.87	87.67	97.66	100	-
6.	Lemon extract (390 ppm)	31.66	42.67	50.83	65.77	80.83
7.	Triazophos 40EC (390 ppm)	40.83	58.33	76.67	94.83	100

***In-Vivo* Experiments: Seed Soaking and Foliar Spray**

The results showed that seed soaking and foliar spray together improved the shoot-root length. All the treatments were found significantly superior to the control as presented in Table 2. *Citrus aurantifolia* and triazophos alone as well as in combination were proved to be effective against *M. incognita*, as mentioned above. All the treatments were effective but out of three combinations (5:5) was observed to be the best as it enhances the overall growth of the shoot length (31.0cms), root length (20.2cms), shoot weight (9.3cms) and root weight (6.6cms). The highest decrease in nematode

population, showing nil RKI (00.00) was noticed under 50-50 combination of *C. aurantifolia* and triazophos when compared with inoculated control of infected control (IC). Number of bacterial nodules were also increased (33.9) over un-inoculated control or normal control (NC) followed by the other combinations of *C. aurantifolia* leaves extract and triazophos 8:2>9:1>7:3>6:4.

Percent seed germination and Seedling Vigour Index (SVI) were also increased over un-inoculated control. Triazophos alone gave promising results against juveniles of *M. incognita* as it reported nil RKI (00.00) as shown in Table 2 and this was in agreement with earlier work of Bopaiah et al., (1976). In the present work, combination of *C. aurantifolia* leaves extract with triazophos as well as seed soaking and foliar spray method together reduced the nematode population but also there was increase in nodule formation as compared to inoculated control.

Table 2: Effect of Treatments Individually and in Combination of *C. aurantifolia* Leaves Extract & Triazophos by Seed Soaking & Foliar Spray Method

Treatments	Fresh shoot				Fresh root				No. of Nodules	% I/D	RKI	% I/D	Germination %(10 days)	Seedling Vigour index
	Length (cm)	% I/D	Weight (gm)	% I/D	Length (cm)	% I/D	Weight (gm)	% I/D						
Uninoculated Control(NC)	23.4	-	6.7	-	13.1	-	4.0	-	19.2	-	-	-	86.67	3163.455
C. aurantifolia +triazophos(9:1)	28.9	23.50	8.0	19.40	15.0	14.50	5.3	32.5	25.0	30.20	12.6	-93.54	73.33	3219.187
C. aurantifolia +triazophos(8:2)	29.1	24.35	8.3	23.88	17.6	34.35	5.6	40	28.4	47.91	9.8	-94.54	80	3736
C. aurantifolia +triazophos(7:3)	28.2	20.51	7.9	17.91	14.9	13.74	4.5	12.5	23.1	20.31	12.8	-92.88	73.33	3160.523
C. aurantifolia +triazophos(6:4)	26.9	14.95	8.7	29.85	14.4	9.92	4.3	7.5	21.6	12.5	15.8	-91.21	66.67	2753.471
C. aurantifolia +triazophos(5:5)	30.1	28.63	9.3	38.80	20.2	54.19	6.6	65	33.9	76.56	0	0	93.3	4692.99
C. aurantifolia	28	19.65	8.5	26.86	17.9	36.64	5.4	35	29.0	51.04	14.4	-91.99	80	3672
Triazophos	30	28.20	9.0	34.32	18.9	44.27	5.7	42.5	30	56.25	0	0	93.3	4562.37
Inoculated control(IC)	17.4	25.64	4.6	31.34	6.2	52.67	2.8	30	-	-	179.8	-	53.33	1258.588

Root knot nematodes are known to cause reduction in nodulation in leguminous plants, thus directly affecting the nitrogen fixation in leguminous plants. Coating on the seeds may create unfavourable environment around the growing points or emerging roots might have acquired some resistance tolerance the nematode. Recently Trifonova & Atanasov (2009) also studied the effect of some plant extracts against *Meloidogyne* and *Globodera* nematodes.

REFERENCES

1. Abbasi, WM., Ahmed, N., Zaki, N. and Shaukat, S.S (2008). Effect of *Barleria acanthoides* Vahl. on root-knot nematode infection and growth of infected okra and brinjal plants. *Pakistan Journal of Botany* **40 (5)**: 2193-2198.
2. Abbot, W.S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology* **17**: 167-265.
3. Ali, S.S. (2009). Estimation of unavoidable yield losses in certain rabi pulse crops due to the root-knot nematode, *Meloidogyne javanica. Trends in Biosciences.* 2 (2): 48-49

4. Bopaiah, B.M., Reddy, D.D.R. and Patil, R.D (1976). Effect of nematicides on nodulation and nitrogen fixation in mungbean. *Indian Journal Nematology* **6:** 156-160.

5. Du, S.S., Zhang H.M., Bai, C.Q., Wang, C.F., Liu, Q.Z., Liu, Z.L., Wang, Y.Y and Deng, Z.W. (2011). Nematocidal Flavone- C-Glycodise against the root knot nematode (*Meloidogyne incognita*) from *Arisaema erubescens* tubers. *Molecules* **16**, 5079-5086.

6. Kaushik, K. (2002). Comparative study of factory effluents and nematicides against root-knot nematode *Meloidogyne incognita* damaging pulse crops. *Thesis M.J.P. Rohilkhand University, Bareilly*. 55-56.

7. Mervata, A.A., Shawky S.M. and Shakera, G. S. (2012). Comparative efficacy of some bioagents, plant oil and plant aqueous extracts in controlling *Meloidogyne incognita* on growth and yield of grapevines. *Annals of Agricultural Sciences*. **57** (1), 7–18.

8. Mohiddin, F. A. and Khan, M. R. (2014). Root-knot nematode: Ignored soil borne plant pathogen causing root diseases of chickpea. *European Journal of Biotechnology and Bioscience. **2*** (1): 04-10.

9. Mojumdar, V. and Mishra, S.D. (1991). Nematicidal efficacy of some plant products and management of *Meloidogyne incognita* in pulse crops by soaking seeds in their aqueous extracts. *Current Nematology* **2**: 27-32

10. El-Nagdi, W.M.A. and Youssef, M.M.A. (2013). Comparative efficacy of garlic clove and castor seed aqueous extracts against the root-knot nematode, *Meloidogyne incognita* infecting tomato plants. *Journal of Plant Protection Research.* **53** (3), 285–288.

11. Ojo, G.T. and Umar, I (2013). Evaluation of some botanicals on root knot nematode (*Meloidogyne javanica*) in tomato (*Lycopersicon esculentum*, Mill) in Yola Adamwa State, Nigeria. *Biological forum*, **5** (2), 31-36.

12. Rehman, B., Ganai, M.A., Parihar, K., Siddiqui, M.A. and Usman, A. (2012). Management of Root Knot Nematode, *Meloidogyne incognita* affecting Chickpea, *Cicer arietinum* for sustainable production. *Bioscience International*, **1**(1):01-05.

13. Sarvanpriya, B and Sivakumar, M. (2005). Management of root knot nematode, *Meloidogyne incognita* on tomato with botanicals. *Natural Product radiance* **4** (3), 158-161.

14. Saxena, R. and Singh, R. (2001). Effectiveness of certain plant extracts for their nematicidal potebtialities. *J.Appl. Zool. Res.* **12** (1) 27-30.

15. Saxena, R. and Singh, R. (2002). Evaluation of some natural plant extracts against root knot nematode, *Meloidogyne incognita* infecting cucurbitaceous crops. *Thesis MJP Rohilkhand University, Bareilly Uttar Pradesh.* 112-113.

16. Sayeeda Fatema and Ahmad, M.U. (2005). Comparative efficacy of some organic amendments and a nematicide (Furadan-3G) against root-knot on two local varieties of groundnut. *Plant Pathology Journal*, **4**: 54-57.

17. Singh, N.P (2012). All India Coordinated Research Project on Pulses: *Indian Institute of Pulses Research, Kanpur, UP, India. Available at* http://www.iipr.res.in/aicrp_chickpea.htm

18. Sirca, S., Gregor, U & Gerrit, K (2004). The incidence of the root-knot nematode *Meloidogyne incognita* and *Meloidogyne hapla* in Slovenia. *Acta Agriculture Slovenia* **83 (1)**: 15-22.

19. Sharma, S.B and McDonald, D (1990). Global status of nematode problems of groundnut, pigeonpea, chickpea, sorghum and pearl millet and suggestions for future work. *Crop Protection* **9:** 453-458.

20. Trifonova, Z. and Atanasov, A (2009). Investigation on the nematicidal effects of some plant extracts on the mortality and hatching of *Meloidogyne incognita* and *Globodera rostochiensis*. *Plant Science.* **46,** 548-554

21. Wiratno, D., Taniwiryono, H., Van den Berg, J.A.G., Riksen, I.M.C.M., Rietjens, S.R., Djiwanti, J.E., Kammenga and Murk, A.J. 2009. Nematicidal activity of plant extracts against the root-knot nematode, Meloidogyne incognita. *The Open Natural Products Journal*. **2:** 77 – 85.

22. Wondimeneh Taye, Sakhuja P.K. and Tadele Tefera (2013). Root knot nematode (*Meloidogyne incognita*) management using botanicals in tomato (*Lycopersicon esculentum*). *Academic Journal Agricultural Research* **1** (1): 009-016