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PHYTO-NEMATICIDAL ACTIVITY OF *CASSIA TORA* L. ON ROOT-KNOT NEMATODE*MELOIDOGYNE INCOGNITA* RACE II ASSOCIATED WITH TOMATO, BRINJAL AND OKRA.

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Abstract: **Among the all nematodes plant parasitic nematodes are major harmful and destructive pest in agricultural plants so present investigation selected for study. The present study includes the phyto-nematicidal activity of *Cassia tora* L. on root-knot nematode *Meloidogyne incognita* race II associated with tomato, brinjal and okra. For the present study, the *Cassia tora* L. leaves extracts were diluted with 2% DMSO (Dimethyl Sulphoxide) to prepare concentration of (1mg/1ml). Observations were made for death time. Time of mortality was noted in 24 hours and 48 hour time intervals.**

Keywords: *Nematodes, Meloidogyne incognita race II*

I INTRODUCTION

Nematodes affect the quantity and quality of crop production in many perennial and annual crops. The annual crops losses due to plant parasitic nematode estimated in vegetables, fruits and nonedible field crops amounting over $80 billion annually [1]. Root-knot nematode (*Meloidogyne* spp.) were considered the most important key nematode pest but among them root knot nematode *Meloidogyne* species are more pathogenic in all cultivated crops especially vegetable crop [2].*M. arenaria, M. incognita and M. javanica* being the major ones responsible for yield losses vegetables crops [3].Plant parasitic nematodes are one of the economically important pests affecting crop growth and yield in all agricultural production sectors of the world [4,5].

The infected plants show *M. incognita race* II galls or knot on the roots main typical below ground symptoms. The above ground symptoms are chlorotic, stunted and necrotic at leaf margin and excessive wilting during periods of mild moisture stress. Mostly the young plants are more susceptible to disease. These symptoms impaired absorption of nutrients and water uptake of plants and severe infection less fruit set and finally death of plants occurs [6,7].

***Cassia tora, Sena tora* L.(Figure 1)**

Scientific classification

Kingdom : Plantae
Division : Magnoliophyta
Class : Magnoliopsida
Order : Fabales
Family : Fabaceae
Genus : *Cassia*
Species : *tora*

Parts used for present study: Leaves

Vernaculuar names

English: foetid cassia, Hindi: Chakvad, Marathi: Takala, Tamil: Tagarai, Kannada: Taragasi, Bengli: Chakunda, Telugu: Tatghambhu, Malyalama: Taghara, Punjabi: Pawasa.

Geographical Distribution

Cassia tora L. is annual shrub grows all over the tropical countries (India, Pakistan, Bangladesh and west China). It is widely distributed thought the India. They were grows well in coastal area, river banks, wasteland, and uncultivated fields, up to 1000-1400 meters.[8,9].

Botanical Description

It is annual shrub, 1-2 m height and leaves are green in colour with stipulate, paripinnate compound, up to 6-8 cm long, and leaflets are 3 pairs. Flowers with short peduncles, Flowers are pale yellow in colours, sessile pairs in the axil of leaves. Fruits are 10-20 cm long curved cylindrical in nature. Seeds are rhomboidal; Plants are produces flowers in rainy and fruits in winter seasons [8,9].

Phyto-chemical constituents

Chrysophanol is the major constituents in leaves, Anthraquinone glycosides, emodin, flavonoids, β-sitosterol, chrysophanol, aloe-emodin, myricyl alcohol, choline trigonelline. Rubrofusarin, Aurantio-obtusin, Norrubrofusarin, 1-desmethylchryso-obtusin, torlactone, torachrysone, triglucosides, naphthopyroneglucosides, chrysophanic acid, Physcion, Rubrofusarin, torlactone.[8,9].

Pharmacological uses

Antirheumatic activity, aperients, antiasthenic, diuretic, treatment of skin disorders like Ringworm, itch, skin ailments, for various rheumatic diseases and as laxative, curing intestinal disorders in children's, dysentery and in eye diseases. Seeds are also used in eye diseases, liver complaints and earache, leprosy etc [8,9].

II MATERIALS AND METHODS

Soil Samples:

Tomoato, brinjal and okra soil samples were used for collection of *M. incognita* race II

Sieves:

20, 60, 200 and 350 mesh sizes sieves were used for washing and filtration of soil sample and roots of rhizospheres.

Chemicals:

Alcohol grades, xylene, glycerine, chemical Nematicides, the micro plots Methanol, hexane, acetone, Formalin, 2% Dimethyl Sulphoxide (DMSO), D.W, Neem cake, Whatman filter paper No.1, Carbofuron, nailpolish, Silica gel 60-120 mesh, Silica gel column chromatography, etc are used for present study. All the research work was carried out in Department of Zoology, D. B. F. Dayanand College of Arts and Science Solapur (M.S.), India. A survey of soil and root system of vegetable crops Tomato, Brinjal and Okra plants for disease incidence and for the occurrence from farmers' fields around Solapur regions.

Methods

Collection of nematode from infected soil sample

Soil sample from infected fields of tomato, brinjal, and okra of 1 kg soil collected out of 200 cc soils was washed thoroughly and processed using Cobb's sieving and Decanting method [10]. Followed by modified Baermann's funnel methods. It was used to study seasonal incidence, prevalence of *M. incognita* race II.

Extraction of nematodes

Pure cultures of tomato, brinjal, okra are continuously grown in micro plots. Whenever nematode is required it is taken the soil from root zone region and processed by Cobb's Sieving and Decanting method [10].

Identification of root-knot nematode species

Meloidogyne incognita race II Species was identified on the basis of perennial pattern method described by [11]. The nematode *M. incognita* race II was identified by Kadam D. B. and Hole U. B. Plant Nematologist Nematology section Department of Agricultural Entomology, Mahatma Phule Krishi Vidypeeth Rahuri, Dist. Ahmednagar, (M.S.) India.

Preparation of crude extracts

The fresh collected plants parts were washed thoroughly with running tap water andthen rinsed with distilled water. Washed plant material was shade dried for 5 to 7 days. It cut into small pieces and pulverized in a mechanical blender. The dried plant materials were fine powdered with the help of electric grinder and preserved separately for further use.

Aqueous methanol extract preparation

Extracts were prepared in cold extraction methods described by (Gilani *et al.,* 2004). Powered plant material were soaked in methanol solution in the round bottom flask. 1 kg of dried powdered plant materials were soaked in 1500 ml of methanol solution. It was kept at room temperature for about 4 to 5 days after which the filter was collected in a large Petri dish with the help of Whatman filter paper No.1 the obtained filter was let for evaporation.

Fractions separated by Column chromatography

Extracts obtained were mixed with silica gel (60-120 mesh) and eluted by using Hexane and Acetone. By using column chromatography techniques different fractions A, B, C, D, E and F were collected[12]. Eluted bioactive fractions were further used for *in- vitro* antinematicidal activity.

***In-vitro* phyto-nematicidal testing method**

In vitro antinematicidal activity of the plant material method described [12,13]. The total 4 group prepared, in each petridish contains about 100 second stage juvenile larvae one group standard (Control), with distilled water, second group with standard (control), Carbofuran chemical nematicides, Third group control standard (control), plants bio pesticide Neem cake and fourth group with different extracts of medicinal plants. The extracts were diluted with 2% DMSO [14].Time of immobile phyto-nematodes was noted in 24 hours and 48 hour time intervals.

Statistical Analysis

Data were statistically analyzed by using Standard Statistical Methods.

III RESULTS

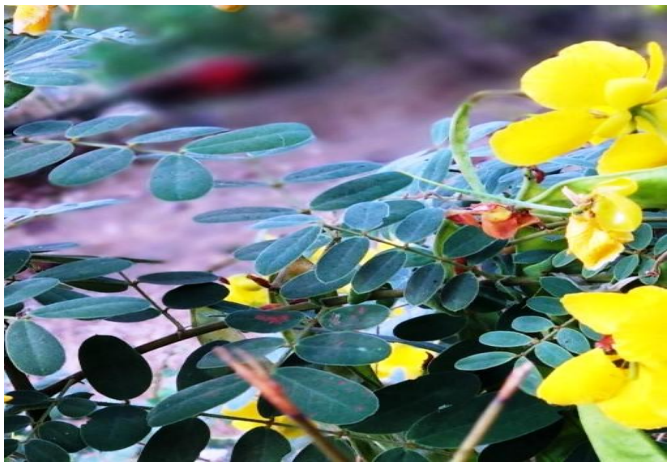

Figure 1. Medicinal plant*Cassia tora* L.**used for Phyto-nematicidal activity on *M. incognita* race II.**

The present investigations were made to study the phyto-nematicidal activityon *Meloidogyneincognita* race II from the roots of tomato, brinjal and okra plants. The results of the present research work shown inTable 1 to 4and Figure 1 to 3.*Meloidogyne incognita* reclassified on the basis of perineal pattern [14, 15].

Evaluation of phyto-nematicidal activity of medicinal plants.

In vitro studies were conducted to screen and evaluate the phyto-nematicidal activity of above mentioned plant extracts against the root-knot nematode *M. incognita* race II parasites of brinjal, tomato and okra.

The results evaluation of phtonematicidal activity of medicinal plants against the larva of root- knot nematode *M. incognita* race II parasites associated with brinjal, tomato and okraof are displayed in tables at the different concentrations which were kept constant for all the plant extracts that is (0.1, 0.2, 0.3, 0.4, and 0.5 mg /ml). The different grades in the tables indicate the different colored compound obtained by column chromatography using the different ratios of polar and non-polar solvents such as Hexane and Acetone.

The tested crude plant extracts shows mortality of juvenile larva *M. incognita* race II the overall test time recorded was 24 hr. to 48 hr. whereas the control time recorded was same as 24 hr. and 48 hr.In Table 1and 4 andFigure 2 and 3 summarize screening of this plant for observing the phyto-nematicidal activity on *M. incognita* race IIparasites.

Table 1.Shows the Mean ± SD values of the effect of *Cassia tora* L.plant extract,fraction B, C, D and Ewith control 2% DMSO, Neemcake and Carboafuranon mortality of *M. incognita* race II associated with brinjal, tomato and okra at 24 hrs.

Fractions and Standard Conc. mg/ml of 2% DMSO	Fraction B	Fraction C	Fraction D	Fraction E	2% DMSO	Neemcake	Carboafuran
	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD
0.1	25.6± 0.5	28.6± 0.5	34.2± 0.8	42.8± 1.3	0.0± 0.0	90.6± 0.5	100.0± 0.0
0.2	28.4± 0.9	35.4± 0.9	42.2± 0.8	46.8± 0.4	0.0± 0.0	93.6± 0.5	100.0± 0.0
0.3	32.4± 0.9	40.6± 0.5	44.2± 0.8	53.2± 0.8	0.0± 0.0	97.8± 0.4	100.0± 0.0
0.4	35.4± 0.9	45.6± 0.5	48.2± 0.8	57.4± 0.9	0.0± 0.0	100.0± 0.0	100.0± 0.0
0.5	38.2± 0.8	48.2± 0.8	54.6± 0.5	65.6± 0.5	0.0± 0.0	100.0± 0.0	100.0± 0.0

The methanolic leaves extract of *Cassia tora* L. shows phyto-nematicidal activity in concentration dependent manner. Among all the grades the minium laraval mortality of *M.incognita* race II in fraction B 38.2% mortality and highest in fraction E 65.6% mortality, neem cake and carbofuran shows 100% in 24 hr.

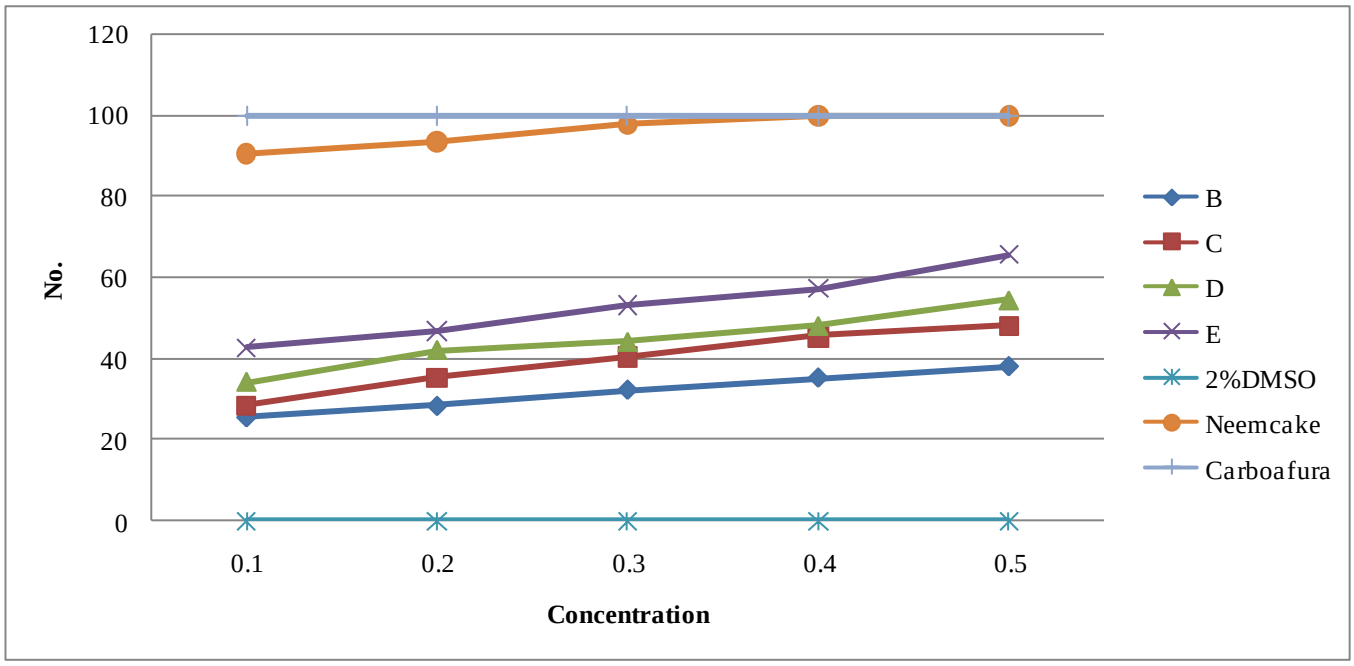

Figure2.Shows the immobilized *M. incogita* race II of *Cassia tora* L. at 24 hrs.

Table 2. Comparison of values of immobilized *M. incognita* race II, treated with *Cassia tora* L. fraction B, C, D, E, with Control 2% DMSO, Neemcake and Carboafuran at 24 hrs.

Fraction	Fraction and Standard Conc. mg/ml of 2% DMSO	2% DMSO		Neemcake		Carboafuran	
		t test	P value	t test	P value	t test	P Value
B	0.1	93.48	P<0.01	-167.83	P<0.01	-271.67	P<0.01
	0.2	63.50	P<0.01	-124.33	P<0.01	-160.10	P<0.01
	0.3	72.45	P<0.01	-130.80	P<0.01	-151.16	P<0.01
	0.4	79.16	P<0.01	-144.45	P<0.01	-144.45	P<0.01
	0.5	91.32	P<0.01	-147.73	P<0.01	-147.73	P<0.01
C	0.1	104.43	P<0.01	-160.08	P<0.01	-260.72	P<0.01
	0.2	79.16	P<0.01	-110.98	P<0.01	-144.45	P<0.01
	0.3	148.25	P<0.01	-161.79	P<0.01	-216.90	P<0.01
	0.4	166.51	P<0.01	-198.64	P<0.01	-198.64	P<0.01
	0.5	115.22	P<0.01	-123.83	P<0.01	-123.83	P<0.01
D	0.1	81.75	P<0.01	-112.80	P<0.01	-157.29	P<0.01
	0.2	100.88	P<0.01	-102.80	P<0.01	-138.17	P<0.01
	0.3	105.66	P<0.01	-113.00	P<0.01	-133.39	P<0.01
	0.4	115.22	P<0.01	-123.83	P<0.01	-123.83	P<0.01
	0.5	199.37	P<0.01	-165.78	P<0.01	-165.78	P<0.01

E	0.1	65.65	P<0.01	-67.60	P<0.01	-87.74	P<0.01
	0.2	209.30	P<0.01	-132.37	P<0.01	-237.92	P<0.01
	0.3	127.17	P<0.01	-94.03	P<0.01	-111.87	P<0.01
	0.4	128.35	P<0.01	-95.26	P<0.01	-95.26	P<0.01
	0.5	239.54	P<0.01	-125.61	P<0.01	-125.61	P<0.01

Statistically average values of immobilized *M. incognita* race IItreated with leaves of *Cassia tora*L. shows in Table 63, in B fraction, C fraction, D fraction and E fraction are significantly more than 2% DMSO positive control and significantly less than Neemcake and Carboafuran negative control at 0.1,0.2,0.3,0.4 and 0.5 concentrations (P<0.01) at 24 hrs.

Table 3. Shows the Mean ± SD values of the effect of *Cassia tora* L.plant extract,fraction B, C, D and Ewith control2% DMSO, Neemcake and Carboafuranon mortality of *M. incognita* race II associated with brinjal, tomato and okra at 48 hrs.

Fractions and Standard Conc. mg/ml of 2% DMSO	Fraction B	Fraction C	Fraction D	Fraction E	2% DMSO	Neemcake	Carboafuran
	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD
0.1	31.4± 0.9	37.4± 0.9	40.8± 0.8	47.4± 0.9	0.0± 0.0	94.8± 0.4	100.0± 0.0
0.2	35.2± 0.8	42.4± 0.9	46.6± 0.5	53.4± 0.9	0.0± 0.0	97.6± 0.5	100.0± 0.0
0.3	41.0± 0.7	46.6± 0.5	52.0± 0.7	62.0± 0.7	0.0± 0.0	100.0± 0.0	100.0± 0.0
0.4	51.6± 0.5	56.8± 0.4	63.4± 0.5	67.2± 0.8	0.0± 0.0	100.0± 0.0	100.0± 0.0
0.5	58.2± 0.8	66.0± 0.7	70.6± 0.5	74.0± 1.0	0.0± 0.0	100.0± 0.0	100.0± 0.0

The methanolic leaves extract of *Cassia tora* L. Shows phyto-nematicidal activity in concentration dependent manner. Among all the grades the minium larval mortality in*M.incognita* race II fraction B 58.2% mortality and highest in fraction E74.0% mortality, neem cake and carbofuran shows 100%mortality in 48 hrs.

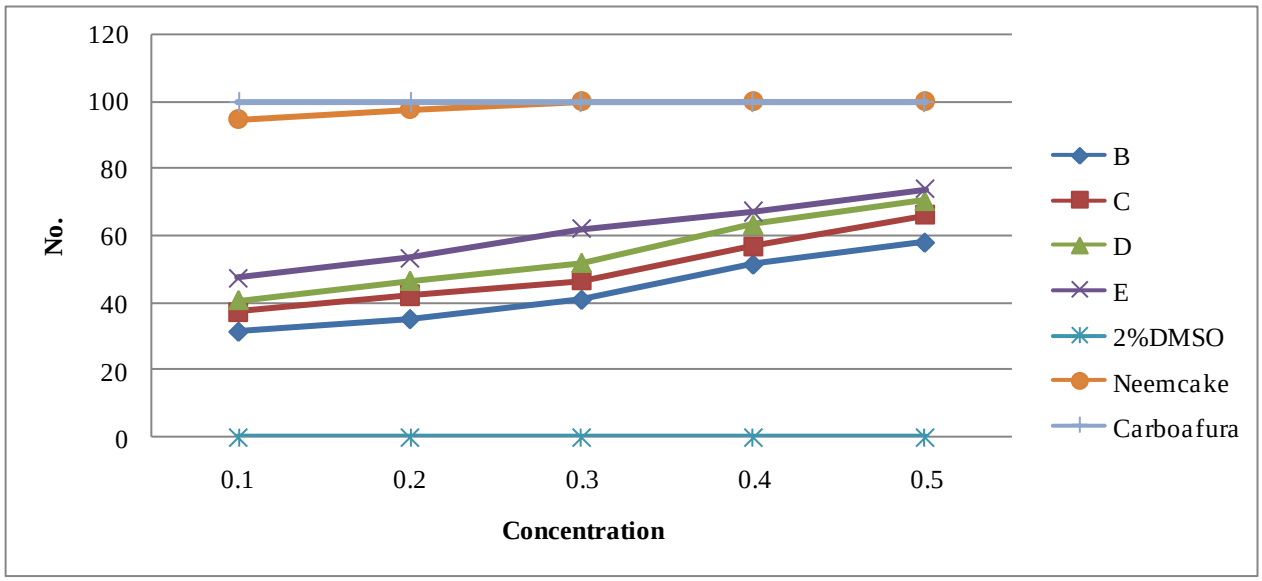

Figure3.Shows the immobilized *M. incogita* race II of *Cassia tora* L. at 48 hrs.

Table 4. Comparison of values of immobilized *M. incognita* race II, treated with *Cassia tora* L. fraction B, C, D, E, with Control 2% DMSO, Neemcake and at 48 hrs.

Fraction	Fraction and Standard Conc. mg/ml of 2% DMSO	2% DMSO		Neemcake		Carboafuran	
		t test	P value	t test	P value	t test	P Value
B	0.1	70.21	P<0.01	-126.80	P<0.01	-153.39	P<0.01
	0.2	84.14	P<0.01	-124.80	P<0.01	-154.90	P<0.01
	0.3	115.97	P<0.01	-166.88	P<0.01	-166.88	P<0.01
	0.4	188.42	P<0.01	-176.73	P<0.01	-176.73	P<0.01
	0.5	139.12	P<0.01	-99.92	P<0.01	-99.92	P<0.01
C	0.1	83.63	P<0.01	-114.80	P<0.01	-139.98	P<0.01
	0.2	94.81	P<0.01	-105.26	P<0.01	-128.80	P<0.01
	0.3	170.16	P<0.01	-194.99	P<0.01	-194.99	P<0.01
	0.4	254.02	P<0.01	-193.20	P<0.01	-193.20	P<0.01
	0.5	186.68	P<0.01	-96.17	P<0.01	-96.17	P<0.01
D	0.1	97.53	P<0.01	-113.84	P<0.01	-141.52	P<0.01
	0.2	170.16	P<0.01	-131.68	P<0.01	-194.99	P<0.01
	0.3	147.08	P<0.01	-135.76	P<0.01	-135.76	P<0.01
	0.4	231.50	P<0.01	-133.64	P<0.01	-133.64	P<0.01
	0.5	257.79	P<0.01	-107.35	P<0.01	-107.35	P<0.01

E	0.1	105.99	P<0.01	-94.80	P<0.01	-117.62	P<0.01
	0.2	119.41	P<0.01	-84.29	P<0.01	-104.20	P<0.01
	0.3	175.36	P<0.01	-107.48	P<0.01	-107.48	P<0.01
	0.4	160.64	P<0.01	-78.41	P<0.01	-78.41	P<0.01
	0.5	148.00	P<0.01	-52.00	P<0.01	-52.00	P<0.01

Statistically average values of immobilized *M.incognita* race IItreated with leaves of *Cassia tora*L. Shows in Table 65,in B fraction, C fraction, D fraction and E fraction are significantly more than 2% DMSO positive control and significantly less than Neemcake and Carboafuran negative control at 0.1,0.2,0.3,0.4 and 0.5 concentrations (P<0.01) at 48 hrs.

***Cassia tora* L.**

In the present study *Senna tora* L. or *Cassia tora* Leaf extracts shows 66% and 75% juvenile larval mortality in 24 hr. and 48 hr. respectively and the present research findings, *Cassia tora* L. fractions shows the very high phyto-nematicidal activity as compaired to findings of other researchers [16, 17].*In vitro* tested the efficacy on eggs and juveniles mortality were exposed at 24, 48 and 72 h in different concentrations (S, S /2, S /10, S /100, S is the standard concentration and S/2, S/10, S/100 is the dilution of Standard solution), showed mortality (48-52%) after 24 h of exposure period respectively [16]. Tanweer and Hisamuddin [17]. Pot Experiment was conducted to the leaf extract of *Cassia tora* against the *Meloidogyne incognita* it more effective reduce egg hatching in the nematode population. Plant extract may posse's phytoneaticidal propertires due to it contains nematicidal compoents contained alkaloids, flavonoids, saponins, benzamide and ketones singly and combination inhibited hatching of nematode eggs [18].

IV CONCLUSION

From this study it is concluded that tomato, brinjal and okra vegetable plants are highly infected by Plant pathogenic root-knot nematodes *M. incognita* race II.For present study observed the efficacy of different crude plant extracts on juvenile larval mortality of *M. incognita* race II nematode population. *Cassia tora* L. (leaves), shows the positive phyto-nematicidal activity.From the above results it indicates that the phytonematicidal efficacy of plants differ with respects to different groups of plant parasitic nematode. The data obtained from this study could be utilized for developing control programmers against *M. incognita* race II infection of vegetable in the study area and other geographical areas with similar environmental conditions.

REFERENCES

[1]. Agrios G. N. (2005). Plant Pathology. Elsevier. URL: http://doi.org/10.1016/B978-0-08-047378-9.50021-X.

[2]. Sikora R. Fernandez E. (2005). Nematode parasites of vegetables. In: Luc M, Sikora RA, Bridge J, eds. Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. Wallingford, UK: CAB International. Pp. 319–392.

[3]. Gautam S. K., Gamini S., Kumar V. B. and Aditi N. P. (2011). Status of root-knot nematode (*Meloidogyne* species) diseases in vegetable plants of some districts of central plain region of Chhattisgarch State, India. *Afr. J. Microbiol. Res.* 8(16): 1663-1667.

[4]. Ritter M. (1972). Role economique et importance des *Meloidogyne* en Europe et dans le bassin Mediterraneen. *OEPP/EPPO Bull*. 2: 17-22.

[5]. Sharma S. B. and Swarup G. (1984). Cyst forming nematodes of India. New Delhi. Ind. Cosmo Publ. 1: 150.

[6]. Wilcox D. A. and Luria R. (1986). Water relation's growth and yield in two snap beans cullivars infected with root-knot nematodes. *Meloidogyne hapla* (Chitwood). *J. Am. Soc. Hortic. Sci.* 111:34-38.

[7]. Chandel Y. S., Kumar S., Jain R. K. and Vashisth S. (2010). An analysis of nematode problems in green house cultivation in Himachal Pradesh and avoidable losses due to *Meloidogyne incognita* in tomato. *Ind. J. Nematol.* 40:198–203.

[8]. Kirtikar K. R. and Basu B. D. (1918). Indian Medicinal Plants. Sudhindra Nath Basu, M.B. Panini Office, Bhuwanéswari Asrama, Bahadurgan. pp. 72.

[9]. Kirtikar K. R. and Basu B. D. (1935). In Indian Medicinal Plants, 2nd edn. Lalith Mohan Publication, Calcutta. pp. 499.

[10]. Cobb N. A. (1918). Estimating the Nematode population of the soil. Bur. PI. Ind. *Agr. Tech.Clr.* 1: 1-48.

[11]. Eisenback J. D., Hirschmann H., Sasser J. N., and Triantaphyllou A. C. (1981). A guide to the four most

common species of root-knot nematodes (*Meloidogyne* spp.). With a pictorial key. Plant Pathology Departments of Plant Pathology and Genetics North Carolina State University and the United States Agency for International Development. Pp. 1-51.

[12].Dama L. B. (2002). Effect of naturally occurring napthoquinones on root- knot nematode *Meloidogyne spp*.*Ind. Phytopathol.* 55(1): 67-69.

[13]. Dama L. B., Poul B. N., Jadhav B. V. and Hafeez M. D. (1999). Effect of Herbal "Juglone" on Development of the plant parasitic nematode (*Meloidogyn*e Spp.) on *Arachis hypogaea* L. *J. Ecotoxicol. Environ. Monit*. 9:73-75.

[14]. Chitwood B. G. (1949). Root- knot nematodes, Part I. A revision of the genus *Meloidogyne* goeldi 1887. Helminth. *Soc. Wash. D. C. Proc.* 16: 90-104.

[15].Chitwood D. J. (2002). Phytochemical based strategies for nematode control. *Ann. Rev. Phytopathol.* 40: 221–249.

[16]. Ganai M.A., Rehman B. K., Parihar M. A. and Siddiqui M. A. (2013). Phytotherapeutic approach for the management of *Meloidogyne incognita* affecting *Abelmoschus esculentus* (L.) Moench. *Arch. Phytopathol. Plant Prot.* pp. 1-9.

[17].Tanweer A. and Hisamuddin (2012). Evaluation of *Cassia tora* for Control of Root-Knot Nematode (*Meloidogyne incognita*) in Soybean. *Afr. J. Basic Appl. Sci.* 4 (1): 21-24.

[18]. Goswami B. K. and Vijayalakshmi V. (1986). Nematicidal properties of some indigenous plant materials against root-knot nematode *Meloidogyne incognita* on tomato. *Ind. J. Nematol.* 16