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Changes in Antioxidative Enzymes in Resistant and Susceptible Genotypes of Tomato Infected with Root-Knot Nematode (*Meloidogyne incognita*)

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ABSTRACT: Changes in activities of antioxidative enzymes (Catalase, Peroxidase, Superoxide dismutase, Polyphenol oxidase, esterase, monodehydro ascorbate reductase, and dehydro ascorbate reductase) after nematode inoculation were investigated in four varieties of tomatoes, (two resistant i.e. Hisar Lalit, PNR- 7 and two susceptible i.e. Punjab Varkha Bahar-1 and Punjab Varkha Bahar-2. These four varieties were infected at the nursery stage with root-knot nematode *(Meloidogyne incognita).* The egg masses of *M. incognita* were taken from the infected brinjal plants. Tomato roots and leaves from un-inoculated and inoculated plants were evaluated for their antioxidative enzyme activities after 7 days and 20 days of infection. The activity of antioxidant enzymes increased after infection with root-knot nematode in the resistant varieties of tomato as compared to that of susceptible varieties. The activities of all enzymes increased after inoculation except the activity of CAT enzyme where a decrease was observed. The isozyme banding patterns of peroxidase and esterase showed that the intensity of bands was more in the resistant genotypes as compared to that of susceptible genotypes, which means that the activity of antioxidant enzymes were more after inoculation in the resistant genotypes as compared to that of susceptible genotypes. An additional band was obtained in the isozyme banding pattern of esterase in the resistant genotypes.

Keywords: Tomato, nematode, *Meloidogyne incognita,* antioxidant enzymes, isozyme

Tomato (*Lycopersicon esculentum* Mill.) a member of family Solanaceae, is universally known as a protective food. It is used directly as raw vegetable, in sandwiches, salads etc. and also as purees, chutneys and pickles in processed forms. Tomato fruit has undoubtedly assumed the status of a functional food considering the overwhelming epidemiological evidence for its anti-cancer activity. Root-knot nematode (*Meloidogyne incognita*) causes considerable damage to tomato plants (Kamalvanshi *et al.,* 2004) and results in extensive losses in yield and quality (Mahajan & Singh, 2001). Though the nematicides are effective against nematodes, yet they are hazardous for health, soil and environment. Plant resistance is one of the eco-friendly options for the management of nematode diseases. A series of biochemical and physical reactions occur in plants in response to root-knot nematode infection. Plants synthesize certain compounds that are toxic to root-knot nematode. In the family of solanaceae, resistance is usually associated with hypersensitive reaction (HR), a rapid and localized cell death in the infected plant in response to nematode attack.

Reactive oxygen species (ROS) play an important role in plant defense and during pathogen attack, levels of ROS detoxifying enzymes like peroxidase (POX) and catalase (CAT) are often suppressed in resistant plants (Klessing *et al.,* 2000). As a result, plants produce more ROS and accumulation of these components leads to HR in plant cells. For example, hydrogen peroxide plays a major role in triggering HR in incompatible interactions (Dangl & Jones 2001). Antioxidant enzymes such as SOD, POD and CAT are considered to be the main protective enzymes engaged in the removal of free radicals and activated oxygen species (Blokhina *et al.,* 2003, Devi *et al.,* 2000). Oxidative enzymes such as POX and pro are reported to be involved in the mechanism of disease resistance.

Peroxidases are heme containing enzymes that catalyse one electron oxidation of several substrates at the expense of hydrogen peroxide and are located in cell walls and vacuoles. The role of peroxidase in producing active oxygen species (AO) is controversial. In cell wall peroxidase oxidizes NAD(P)H to produce O_2- and H_2O_2 (Elstner and Heupel 1976). Peroxidase in its activity uses H_2O_2 as the main oxidant and reduces it into H_2O, thus acting as an active AO scavenger. Electrophoretic analysis of peroxidase revealed that both the resistant and susceptible plants responded to parasitic invasion by synthesizing new peroxidases isozymes. SOD, the family of metallo-enzymes, catalyses the disproportionation of superoxide O_2- to molecular oxygen and H_2O_2 (Scandalios 1993).

Catalase catalyses breakdown of H_2O_2 using one molecule of H_2O_2 as substrate donor and another molecule of H_2O_2 as oxidant or electron acceptor. Since catalase repression results in H_2O_2 accumulation, this molecule may be responsible for the production of defense signals that directly trigger HR. Two enzymes are involved in the regeneration of reduced ascorbate, namely mono-dehydro-acsorbate reductase (MDHAR) which uses NAD(P)H directly to recycle ascorbate and dehydro-ascorbate-reductase (DHAR). However, the situation is further complicated because mono-dehydro-ascorbate itself is an efficient electron acceptor (Foyer and Lelandais 1993, Miyake and Asada 1992). Mono-dehydro-ascorbate is reduced directly to ascorbate using electrons derived from the photosynthetic electron transport chain.

MATERIALS AND METHODS

The seeds of tomato genotypes viz: (Hisar lal, PNR-7, Punjab Varkha Bahar-l and Punjab Varkha Bahar-2) were procured from the Department of Vegetables Crops, Punjab Agricultural University, Ludhiana.

Egg masses of *Meloidogyne incognita* were collected from pure culture already maintained on brinjal plants. These egg masses were transferred to tissue paper kept on wire gauge suspended in water in petri plate, to allow the eggs to hatch. The second stage juveniles (J2s) were then inoculated to brinjal plants growing in 30 cm diameter earthen pots for further multiplication. One week old seedlings of tomato genotypes sown in pots were inoculated with freshly hatched larvae of root knot nematode (*M. incognita*) @ 1 J2s/cc of soil. Leaf and root samples were collected at 7 and 20 days after inoculation with root-knot nematode. Simultaneously, root and leaf samples were also collected from uninoculated plants and carried to the laboratory in an ice box and analyzed for anti oxidative enzymes.

Sample was homogenized in 5ml of cold (4^0 C) extraction buffer using pre chilled pestle and mortar. The mixture was then centrifuged at 20,000g for 10 minutes and supernatant was collected and was analyzed for enzyme activity.

Catalase- To chilled sodium phosphate buffer added enzyme extract. The reaction was started by adding H_2O_2 to the reaction mixture. The rate of decrease in absorbance at 240 nm was measured at 10 second intervals for 1 min (Chance and Mahley 1955).

Superoxide Dismutase- The reaction mixture contained methionine, NBT , EDTA, Na_2CO_3, phosphate buffer and distilled water. The enzyme extract was added at last and the reaction was started by adding 0.1ml riboflavin. The intensity of the color developed was read at 560nm (Xing *et al.,* 2008).

Polyphenol Oxidase- To enzyme extract, phosphate buffer was added. Reaction was started by adding 2% of catechol. PPO activity was assayed by measuring the linear increase in absorbance at 410nm (Augustin *et al.,* 1985).

Dehydroascorbate Reductase- The reaction mixture contained 0.1M phosphate buffer, ImM EDTA, 2.5mM glutathione reduced, 0.2mM DHA. To this enzyme extract was added. DHAR was assayed by measuring the reduction of dehydro-ascorbate at 265nm with an extinction coefficient of 14 (Foyer *et al.,* 1989).

Esterase- To enzyme extract, phosphate buffer was added. Reaction was started by adding 10^{-3} M indophenyl acetate. Esterase activity was assayed by change in absorbance at 625 nm (Sae *et al.,* 1971).

Table 1: Superoxide dismutase (SOD) activity (unit SOD g^{-1} FW) in tomato roots (R) and leaves (L) of resistant and susceptible genotypes against root-knot nematode

Genotype	Days after inoculation (Specific activity of SOD)			
	7		20	
	U	I	U	I
Resistant genotypes				
Hissar lalit (R)	0.301±0.007 (0.071±0.005)	0.311±0.012 (0.076±0.006)	0.316±0.013 (0.074±0.007)	0.623±0.015 (0.134±0.008)
PNR-7 (R)	0.416±0.014 (0.104±0.007)	0.42800.016 (0.113±0.006)	0.527±0.015 (0.132±0.007)	0.831±0.016 (0.205±0.005)
Hisar lalit (L)	0.361±0.014 (0.069±0.006)	0.372±0.013 (0.072±0.006)	0.431±0.017 (0.082±0.006)	0.798±0.018 (0.148±0.008)
PNR-7 (L)	0.346±0.016 (0.059±0.004)	0.35100.015 (0.061±0.005)	0.398±0.016 (0.068±0.006)	0.842±0.014 (0.134±0.008)
Susceptible genotypes				
Varkha Bahar 1 (R)	0.211±0.016 (0.032±0.002)	0.223±0.010 (0.034±0.002)	0.275±0.016 (0.042±0.003)	0.301±0.007 (0.051±0.004)
Varkha Bahar 2 (R)	0.239±0.013 (0.033±0.002)	0.251±0.012 (0.03500.003)	0.306±0.014 (0.043±0.003)	0.377±0.014 (0.054±0.004)
Varkha Bahar 1 (L)	0.224±0.015 (0.028±0.001)	0.235±0.014 (0.031±0.002)	0.268±0.015 (0.034±0.002)	0.303±0.016 (0.042±0.003)
Varkha Bahar 2 (L)	0.271±0.017 (0.031±0.002)	0.280±0.015 (0.035±0.002)	0.298±0.016 (0.045±0.003)	0.316±0.015 (0.052±0.004)

R= Roots; L= Leaves; U= Uninoculated plants; I= Inoculated plants

CD ($P \leq 0.05$)	R	L		R	L		R	L
A	0.792	0.907	C	0.792	0.907	ABC	0.224	0.256
B	0.112	0.128	AC	0.112	0.128			
AB	0.158	0.181	BC	0.158	0.181			

Values are mean ± S.E. of three determinations

Values in parenthesis indicate specific activity (µmoles per mg of the protein) of the enzyme

Peroxidase- To chilled guaiacol added enzyme extract. The reaction was started by adding H_2O_2 and the rate of decrease in absorbance at 470 nm was measured at 30 seconds intervals for 3 mins. The unit of enzyme is defined as a decrease in O.D by 1.0 under standard conditions (Shannon *et al.,* 1966).

Monohydroascorbate Reductase- The reaction mixture contained Hepes buffer (pH 7.6), 1mM NADPH, 2.5mM ascorbate and 50µl extract. The reaction was started by adding 0.4 units of ascorbate oxidase to the reaction mixture. MDHAR was assayed by decrease in absorbance monitored at 340 nm using 6.2 as extinction coefficient (Hossain & Asada, 1984).

Electrophoresis (Native-Page)- Electrophoresis was done by according to Schagger *et al* (1994) method. Dissolve the sample extract in equal volume of 2x sample buffer. Carefully pour the freshly prepared separating gel solution into the chamber without generating bubbles.

Table 2: Catalase activity (CAT) (μmoles of H_2O_2 decomposed min^{-1} g^{-1} FW) in tomato roots (R) and leaves (L) of resistant and susceptible genotypes against root-knot nematode

Genotype	Days after inoculation (Specific activity of Catalase)			
	7		20	
	U	I	U	I
Resistant genotypes				
Hisar lalit (R)	98.75±2.11 (23.22±1.89)	84.63±3.12 (20.84±1.37)	75.21±4.14 (17.69±1.09)	68.39±5.09 (15.06±1.19)
PNR-7 (R)	101.52±3.91 (25.57±1.19)	84.32±4.19 (22.30±1.37)	74.61±2.95 (18.79±1.34)	54.51±4.16 (10.88±0.57)
Hisar lalit (L)	144.16±:4.97 (27.72±1.84)	109.84±2.84 (21.53±1.14)	117.56±:3.79 (22.60±1.18)	98.42±5.14 (18.25±0.98)
PNR-7 (L)	151.77±:3.46 (25.89±1.65)	116.75±2.66 (20.59±1.54)	123.90±1.77 (21.14±1.67)	102.77±4.59 (16.46±1.02)
Susceptible genotypes				
Varkha Bahar l (R)	48.73±3.40 (7.47±0.21)	59.26±3.98 (9.21±0.22)	54.17±3.04 (830:±0.22)	66.30±3:11 (10.01±0.23)
Varkha Bahar 2 (R)	64.97±2.31 (9.16±0.21)	78.17±4.19 (11.16±0.24)	73.90±1.41 (10.43±0.23)	89.20±2.96 (11.79±0.25)
Varkha Bahar 1 (L)	103.55±2.58 (13.19±0.22)	121.85±2.47 (15.72±0.23)	142.89±2.75 (18.20±0.20)	153.25±4.10 (19.47±0.21)
Varkha Bahar 2 (L)	126,69±2.06 (15.89±0.23)	134.51±3.63 (20.44±1.69)	166.30±2.83 (20.86±1.77)	177.74±2.91 (24.82±1.84)

R= Roots; L= Leaves; U= Uninoculated plants; I= Inoculated plants

CD (P≤0.05)	R	L		R	L		R	L
A	NS	2.017	C	2.058	2.017	ABC	NS	5.705
B	2.910	2.852	AC	NS	2.852			
AB	4.116	4.034	BC	4.116	4.034			

Values are mean ± S.E. of three determinations

Values in parenthesis indicate specific activity (μmoles per mg of the protein) of the enzyme

Carefully overlayer the acrylamide solution with H_2O-saturated n-Butanol without mixing. Polymerize the gel for 1 h. Pour off the n-butanol from the polymerized separating gel, wash the gel top with water and fill the gap remaining in the chamber with stacking gel solution, insert the comb. When the stacking gel has polymerized, remove the comb without distorting the shapes of the well. Fill the apparatus reservoir buffer. Load the gel with 10-30 μl sample solution by pipette. Start the electrphoresis immediately by turning on power. After electrophoresis, remove the gel from between the glass plates. Stain the gel in the staining solution for 2-3 hours. Remove the dye that was not bound to protein in destaining solution. The bands of the isozymes appeared in the gel. Finally the photographs were taken to have the permanent record of the isozymes banding patterns.

RESULTS AND DISCUSSION

The SOD activity in the roots and leaves of both inoculated as well as uninoculated plants of resistant and susceptible genotypes increased after 20 days of infection

Table 3: Peroxidase activity (POX) (units min^{-1} mg^{-1} FW) in tomato roots (R) and leaves (L) of resistant and susceptible genotypes against root-knot nematode

Genotype	Days after inoculation (Specific activity of peroxidase)			
	7		20	
	U	I	U	I
Resistant genotypes				
Hisar lalit (R)	1.04±0.08 (0.244±0.021)	3.85±0.27 (0.948±0.026)	4.17±0.32 (0.981±0.022)	8.64±0.39 (1.903±0.025)
PNR-7 (R)	2.06±0.11 (0.518±0.028)	5.10±0.42 (1.349±0.029)	4.28±0.39 (1.078±0.024)	9.37±0.64 (1.870±0.31)
Hisar lalit (L)	4.55±0.38 (0.875±0.019)	5.60±0.44 (1.098±0.021)	7.45±0.58 (1.432±0.023)	10.10±0.68 (1.873±0.031)
PNR-7 (L)	5.09±0.44 (0.868±0.032)	7.15±0.61 (1.261±0.034)	7.07±0.68 (1.206±0.029)	8.84:±0.69 (1.416±0.033)
Susceptible genotypes				
Varkha Bahar 1 (R)	3.28±0.21 (0.503±0.031)	2.49±0.13 (0.387±0.026)	2.64±0.15 (0.404±0.035)	1.98±0.07 (0.299±0.025)
Varkha Bahar 2 (R)	4.78±0.35 (0.674±0.033)	3.58±0.27 (0.511±0.022)	4.00±0.32 (0.564±0.036)	3.09±0.25 (0.408±0.031)
Varkha Bahar 1 (L)	5.31±0.47 (0.676±0.032)	4.52±0.30 (0.583±:0.028)	4.37±0.29 (0.556±0.037)	3.95±0.23 (0.501±0.034)
Varkha Bahar 2 (L)	9.32±0.56 (1.416±0.026)	8.57±0.46 (1.075±0.023)	8.16±0.61 (1.240±0.035)	6.60±0.44 (0.921±0.024)

R= Roots; L= Leaves; U= Uninoculated plants; I= Inoculated plants

CD (P≤0.05)	R	L		R	L		R	L
A	0.342	0.346	C	0.342	0.346	ABC	NS	NS
B	0.484	0.489	AC	0.484	NS			
AB	0.685	0.692	BC	0.685	0.692			

Values are mean ± S.E. of three determinations

Values in parenthesis indicate specific activity (µmoles per mg of the protein) of the enzyme

with *M. incognita.* There was very less change in SOD activity after 7 days of inoculation as compared to uninoculated plants in both resistant as well as susceptible genotypes. There was increase in the SOD activity in inoculated plants as compared to uninoculated plants at 20 days of infection. The activity of SOD from inoculated as well as uninoculated plants was higher jn resistant genotypes as compared to that of susceptible genotypes at different intervals of time. Similar results were found by Vanderspool *et al* (1994), reported that SOD activity was found to be higher in resistant soybean roots attacked by *M. incognita* compared with uninfested roots.

The catalase activity in the roots and leaves of resistant genotypes decreased in inoculated plants as compared to uninoculated ones. However, the roots and leaves of susceptible genotypes were found to contain higher levels of catalase activity in inoculated plants as compared to uninoculated ones at all intervals of time. Chen *et al* (1993) observed that Salicylic acid has been

found to inhibit CAT activity in many plants and thus involved in plant systemic acquired resistance (SAR). CAT inhibition enhances the cellular level of H_2O_2, which is presently recognized in HR, as a trigger for hypersensitive cell death as well as a strong antimicrobial molecule (Levine *et al.,* 1994).

The peroxidase activity was higher in the inoculated roots and leaves of the resistant genotypes after inoculation. However, in the susceptible genotypes, the roots and leaves from uninoculated plants showed higher level of peroxidases as compared to inoculated plants at different stages of inoculation. Mahdy and Sally (2011) reported that there was increase in the activity of both peroxidase and polyphenol oxidase in strawberry grown in the soil infested with root knot nematode when compared with untreated plants.

The polyphenol oxidase activity was higher in the inoculated roots and leaves of the resistant genotypes after 7 and 20 days of inoculation. However, in the susceptible genotypes, the roots and leaves from uninoculated plants showed higher level of polyphenol oxidase activity as compared to inoculated plants at

Table 4 : Polyphenol oxidase activity (PPO) (units min^{-1} mg^{-1} FW) in tomato roots (R) and leaves (L) of resistant and susceptible genotypes against root-knot nematode

Genotype	Days after inoculation (Specific activity of PPO)			
	7		20	
	U	I	U	I
Resistant genotypes				
Hisar lalit (R)	1.20±0.06 (0.282±0.019)	1.98±0.12 (0.413±0.025)	2.42±0.18 (0.569±0.022)	3.24±0.26 (0.713±0.024)
PNR-7 (R)	1.04±0.05 (0.261±0.018)	1.65±0.07 (0.420±0.024)	2. 12±0.16 (0.534±0.026)	3.01±0.24 (0.601±0.027)
Hisar lalit (L)	0.88±0.03 (0.169±0.014)	1.21±0.09 (0.237±0.021)	1.39±0.06 (0.267±0.023)	2.98±0.18 (0.552±0.027)
PNR-7 (L)	1.68±0.07 (0.286±0.019)	2.29±0.17 (0.368±0.020)	2.23±0.19 (0.380±0.022)	3.11±0.22 (0.498±0.025)
Susceptible genotypes				
Varkha Bahar 1 (R)	2.18±0.17 (0.334±0.025)	1.28±0.04 (0. 199±0.024)	2.00±0.11 (0.306±0.021)	1.06±0.03 (0. 160±0.012)
Varkha Bahar 2 (R)	2.58±0.19 (0.363±0.025)	1.76±0.06 (0.251±0.018)	2.11±0.17 (0.297±0.018)	1.36±0.04 (0. 179±0.016)
Varkha Bahar 1 (L)	1.83±0.04 (0.233±0.017)	0.98±0.02 (0. 126±0.008)	1.01±0.03 (0. 128±0.008)	0.65±0.02 (0.093±0.004)
Varkha Bahar 2 (L)	1.96±0.06 (0.297±0.021)	1.29±0.04 (0.161±0.010)	1.16±0.04 (0. 176±0.010)	0.75±0.03 (0.108±0.007)

R= Roots; L= Leaves; U= Uninoculated plants; I= Inoculated plants

CD (P≤0.05)	R	L		R	L		R	L
A	NS	NS	C	0.233	NS	ABC	NS	NS
B	0.329	0.254	AC	NS	0.254			
AB	0.466	0.360	BC	0.466	0.360			

Values are mean ± S.E. of three determinations

Values in parenthesis indicate specific activity (µmoles per mg of the protein) of the enzyme

different stages of inoculation. Rani *et al* (2008) found similar results that there was enhancement of the activities of PPO enzymes in the resistant cultivars of tomato.

The MDHAR activity in the roots and leaves ofboth inoculated as well as uninoculated plants of resistant and susceptible genotypes increased after infection with *M. incognita* at different time intervals. However, there was very less change in MDHAR activity after inoculation in the roots of susceptible genotypes as compared to that in uninoculated plants. The resistant genotypes recorded higher content of MDHAR activity in both inoculated and uninoculated leaves as compared to the susceptible genotypes after inoculation with *M. incognita.* The DHAR activity in the roots and leaves of both inoculated as well as uninoculated plants of resistant and susceptible genotypes increased after infection with *M. incognita* at different time intervals. However, there was very less change in DHAR activity after inoculation in the roots of susceptible genotypes as compared to that in uninoculated plants. El Beltagi *et al* (2011) observed the similar results that the specific activity of these enzymes increased as

Table 5 : Monodehydroascorbate reductase activity (MDHAR) (µmoles min^{-1} g^{-1} FW) in tomato roots (R) and leaves (L) of resistant and susceptible genotypes against root-knot nematode

Genotype	Days after inoculation (Specific activity of MDHAR)			
	7		20	
	U	I	U	I
Resistant genotypes				
Hisar lalit (R)	0.198±0.014 (0.046±0.002)	0.304±0.017 (0.075±0.003)	0.216±0.016 (0.051±0.004)	0.469±0.018 (0.103±0.005)
PNR-7 (R)	0.324±0.015 (0.082±0.00S)	0.722±0.022 (0.191±0.014)	0.421±0.018 (0.106±0.009)	0.848±0.020 (0.169±0.011)
Hisar lalit (L)	0.237±0.017 (0.045±0.002)	0.428±0.021 (0.084±0.005)	0.307±0.017 (0.059±0.004)	0.903±0.022 (0.170±0.012)
PNR-7 (L)	0.261±0.015 (0.044±0.002)	0.504±0.016 (0.088±0.005)	0.318±0.014 (0.054±0.003)	0.835±0.018 (0.134±0.011)
Susceptible genotypes				
Varkha Bahar 1 (R)	0.209±0.013 (0.032±0.001)	0.241±0.021 (0.037±0.001)	0.243±0.017 (0.038±0.002)	0.270±0.018 (0.040±0.003)
VarkhaBahar 2 (R)	0.185±0.016 (0.026±0.001)	0.225±0.019 (0.032±0.002)	0.211±0.017 (0.029±0.001)	0.254±0.019 (0.034±0.002)
Varkha Bahar 1 (L)	0.167±0.015 (0.021±0.001)	0.206±0.019 (0.026±0.002)	0.198±0.013 (0.025±0.001)	0.240±0.017 (0.030±0.002)
Varkha Bahar 2 (L)	0.219±0.015 (0.033±0.001)	0.301±0.021 (0.037±0.002)	0.279±0.016 (0.042±0.003)	0.317±0.018 (0.044±0.003)

R= Roots; L= Leaves; U= Uninoculated plants; I= Inoculated plants

CD (P≤0.05)	R	L		R	L		R	L
A	0.103	0.996	C	0.103	0.996	ABC	0.292	0.281
B	0.146	0.140	AC	0.146	0.140			
AB	0.206	0.199	BC	0.206	0.199			

Values are mean ±S.E. of three determinations

Values in parenthesis indicate specific activity (µmoles per mg of the protein) of the enzyme

Table 6: Dehydroascorbate reductase activity (DHAR) (μmoles min^{-1} g^{-1} FW) in tomato roots (R) and leaves (L) of resistant and susceptible genotypes against root-knot nematode

Genotype	Days after inoculation (Specific activity of DHAR)			
	7		20	
	U	I	U	I
Resistant genotypes				
Hisar lalit (R)	2.00±0.13 (0.470±0.015)	3.49±0.16 (0.859±0.016)	2.48±0.19 (0.583±0.017)	4.24±0.21 (0.934±0.018)
PNR- 7 (R)	1.44±0.11 (0.363±0.015)	4.32±0.26 (1.142±0.021)	2.09±0.16 (0.526±0.017)	6.34±0.28 (1.265±0.022)
Hisar lalit (L)	1.47±0.12 (0.282±0.022)	3.28±0.28 (0.643±0.023)	1.97±0.13 (0.378±0.014)	4.28±0.25 (0.794±0.024)
PNR-7 (L)	2.71±0.22 (0.462±0.018)	3.82±0.23 (0.673±0.023)	3.01±0.27 (0.513±0.021)	4.40±0.29 (0.705±0.024)
Susceptible genotypes				
VarkhaBahar l (R)	2.74±0.18 (0.420±0.019)	2.81±0.19 (0.427±0.022)	3.11±0.27 (0.476±0.021)	3.23±0.28 (0.482±0.022)
Varkha Bahar 2 (R)	2.04±0.14 (0.287±0.021)	2.26±0.16 (0.292±0.025)	2.99±0.23 (0.421±0.016)	3.14±0.24 (0.428±0.019)
Varkha Bahar 1 (L)	4.16±0.25 (0.529±0.015)	4.32±0.26 (0.537±0.023)	5.03±0.26 (0.640±0.018)	5.19±0.27 (0.651±0.022)
Varkha Bahar 2 (L)	2.48±0.16 (0.376±0.024)	2.61±0.17 (0.383±0.025)	2.85±0.24 (0.433±0.021)	2.97±0.26 (0.444±0.023)

R= Roots; L= Leaves; U= Uninoculated plants; I= Inoculated plants

CD (P≤0.05)	R	L		R	L		R	L
A	0.145	0.151	C	0.145	0.151	ABC	0.410	NS
B	0.205	0.214	AC	0.205	NS			
AB	0.290	0.303	BC	0.290	NS			

Values are mean ± S.E. of three determinations

Values in parenthesis indicate specific activity (μmoles per mg of the protein) of the enzyme

strong antioxidant defense compounds against induced oxidative damage.

The data on the esterase activity in the roots and leaves of different tomato genotypes after inoculation with *M. incognita* have been presented. The esterase activity in the roots and leaves of both inoculated as well as uninoculated plants of resistant and susceptible genotypes increased after infection with *M. incognita* at different time intervals. However, there was very less change in esterase activity after inoculation in the roots and leaves of susceptible genotypes as compared to uninoculated plants. The resistant genotypes recorded higher content of esterase activity in both inoculated and uninoculated leaves and roots as compared to the susceptible genotypes after inoculation with *M. incognita.* In plants with resistance to nematodes, esterase activity increased after nematode infection (Zacheo and Bleve-Zacheo 1995, Lambert *et al.,* 1999 and Andres *et al.,* 2001).

The native- PAGE analysis of esterase showed that the roots and leaves of resistant genotypes of tomato plants have more intense and wider bands as compared to those of the susceptible genotypes after inoculation with root knot nematode. An extra band was observed in the roots and leaves of the resistant genotypes of tomato after inoculation but there was no extra band present in the roots and leaves of the resistant genotypes which showed that the activity of esterase enzymes was higher in both the leaves as well as roots of resistant genotypes of tomato after inoculation with root knot nematode. Similar results were showed by Montes *et al* (2004) that the esterase activity increased in the resistant genotypes after inoculation with root knot nematode.

Native- PAGE analysis of tomato plants of both resistant and susceptible genotypes showed that the isozyme bands of peroxidase were more intense and wider in both leaves and roots of resistant genotypes of tomato whereas in the susceptible genotypes the bands are less intense and wider in both roots and leaves of the tomato plants. Similar results were showed by Wu &

Table 7: Esterase activity (units min^{-1} g^{-1} FW) in tomato roots (R) and leaves (L) of resistant and susceptible genotypes against root-knot nematode

Genotype	Days after inoculation (Specific activity of DHAR)			
	7		20	
	U	I	U	I
Resistant genotypes				
Hisar lalit (R)	1.87±0.17 (0.440±0.018)	2.66±0.23 (0.655±0.024)	2.11±0.19 (0.496±0.018)	3.43±0.27 (0.756±0.028)
PNR-7 (R)	2.51±0.21 (0.632±0.021)	3.16±0.23 (0.835±0.026)	3.01±0.25 (0.758±0.022)	4.80±0.27 (0.958±0.025)
Hisar lalit (L)	3.23±0.24 (0.621±0.026)	4.81±0.26 (0.943±0.027)	3.92±0.24 (0.753±0.024)	5.68±0.28 (1.053±0.028)
PNR-7 (L)	2.14±0.15 (0.365±0.025)	3.78±0.27 (0.667±0.027)	2.71±0.25 (0.426±0.022)	4.93±0.27 (0.790±0.025)
Susceptible genotypes				
Varkha Bahar 1 (R)	1.09±0.07 (0.167±0.013)	1.13±0.09 (0.175±0.014)	1.69±0.06 (0.259±0.022)	1.72±0.08 (0.267±0.024)
Varkha Bahar 2 (R)	2.12±0.15 (0.297±0.018)	2.25±0.21 (0.308±0.023)	2.73±0.23 (0.385±0.022)	2.85±0.26 (0.390±0.025)
Varkha Bahar 1 (L)	3.19±0.25 (0.406±0.016)	3.29±0.27 (0.417±0.024)	3.92±0.32 (0.499±0.021)	4.08±0.33 (0.512±0.022)
Varkha Bahar 2 (L)	1.96±0.14 (0.245±0.024)	2.07±0.16 (0.269±0.025)	2.21±0.17 (0.326±0.024)	2.34±0.19 (0.335±0.025)

R= Roots; L= Leaves; U= Uninoculated plants; I= Inoculated plants

CD (P≤0.05)	R	L		R	L		R	L
A	0.148	0.145	C	0.148	0.145	ABC	0.420	NS
B	0.210	0.205	AC	0.210	NS			
AB	0.297	0.291	BC	0.297	0.291			

Values are mean ± S.E. of three determinations

Values in parenthesis indicate specific activity (µmoles per mg of the protein) of the enzyme

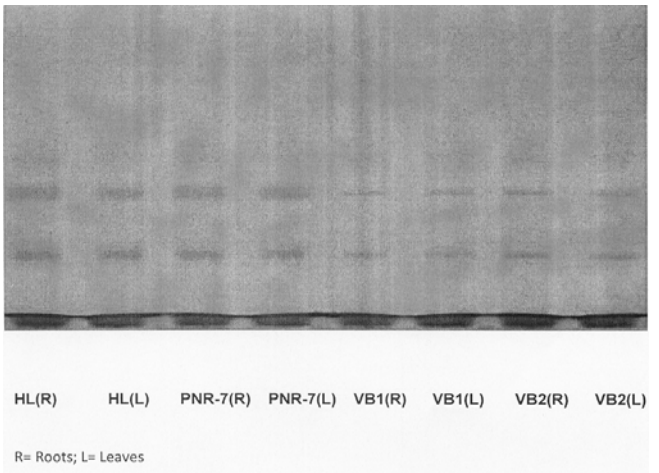

Fig. 1. Isozymes banding pattern of peroxidase after inoculation

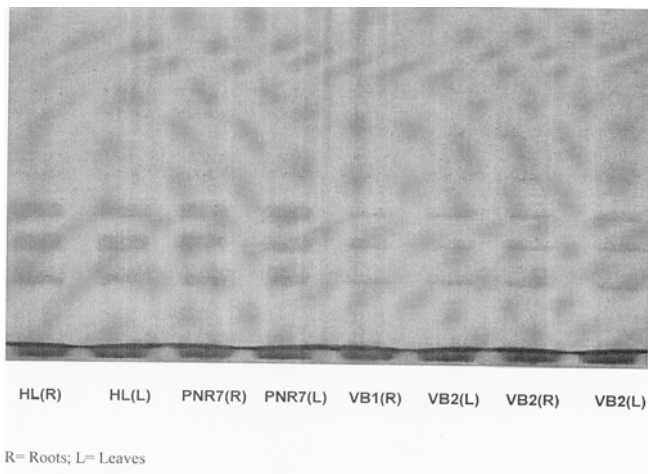

Fig. 2. Isozymes banding pattern of esterase after inoculation

Duan (2011) that the bands among the inoculated cultivars of resistant genotypes were wider than those of susceptible genotypes which showed that the peroxidase activity was higher in the resistant cultivars after inoculation.

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Effect of VA Mycorrhiza on Root Disease (*Pythium aphanidermatum* and *Meloidogyne incognita*) in Tobacco

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ABSTRACT: The interaction studies between five VAM fungi, i.e., *Glomus mosseae, G. fasciculatum, G. constrictum, Acaulospora laevis* and damping-off root rot pathogen (*Pythium aphanidermatum*) on tobacco (*Nicotiana tabacum* L.) seedbeds revealed that all the VAM fungi colonized the roots efficiently within 30 days of inoculation. However, the per cent colonization was higher in the seedlings inoculated with *G. fasciculatum.* The per cent disease severity in 30 day old seedlings was significantly less in treatments with VAM fungi compared to non-mycorrhizal treatment. The impact of VA-mycorrhizal fungi i.e., *Glomus mosseae, G. fasciculatum, G. constrictum, Acaulospora laevis* and root-knot nematodes on tobacco plants and the possibility of reducing the harmful effects of root-knot nematodes using VAM fungi, was carried out in pot experiments. The VAM fungus *G. fasciculatum* significantly increased VAM infection of tobacco plants infected or not infected with nematodes, whereas *Meloidogyne incognita* significantly reduced them, Mycorrhizal infection percentage and number or galls were affected by variation in mycorrhizal strain. The results indicated that the use of *G. fasciculatum* as a biocontrol agent is a promising technology for tobacco production.

Key words: *Glomus fasciculatum, Meloidogyne incognita,* root-knot nematodes and tobacco.

Damping-off is the most common and widespread nursery disease of tobacco *(Nicotiana tabaccum* L.) caused by soil-borne, hydrophilic fungus *Pythium aphanidermatum* (Edson) Fitzp. It is responsible for poor seedling stand. Conspicuous symptom of this disease is the sudden collapse of young seedlings in patches leading to uneven stand. Brown watery soft rot of young seedlings, girdling of hypocotyls and finally toppling and death of seedlings, leading to wet rot are the characteristic symptoms. The pathogen spreads quickly and affects the entire seed bed causing enormous loss of seedlings. The use of fungicides at present is unavoidable. However, chemical control of the disease is expensive and disturbs soil ecology (Alagarasamy & Jeyarajan, 1989). VAM fungi are known to impart resistance against soil borne plant pathogens, especially causing root rots and wilts (Moiler Kaare *et al.,* 2009). Apart from this, the beneficial effects of vesicular-arbuscular mycorrhizal (VAM) fungi on the growth of various crop plants have been explored and documented in literature (Hamel & Strullu, 2006). Hence, the present study was carried out to see the effect of VAM fungi on the severity of damping off disease in tobacco seed beds. Root- knot disease is one of the major problems effecting crop production throughout India. The disease is caused by root-knot nematodes, which belong to the genus *Meloidogyne.* Among the susceptible crops the most important are tobacco, tomato, egg plant, papaya and cotton. The losses due to nematode damage in tobacco, egg plant, cotton and coffee were 50% or more (Borah *et al.,* 2009). Indirect losses associated with root-knot disease are caused by secondary attack of other pathogens, inefficient utilization of fertilizers and water and high cost of chemical treatments. Vesicular-arbuscular mycorrhizal (VAM) fungi are beneficial soil fungi which form a symbiotic association with roots of many plants. Many research papers about the importance of VAM fungi in agriculture have been published (Shreenivasa *et al.*, 2007).

The subject of biological antagonism in the rhizosphere and root region is of long standing. The VAM fungi and

root-knot nematodes are members of the microbial population of the root region and they can compete with each other for the same site in the rhizosphere. Hence, the beneficial VAM fungi might be expected to reduce or even eliminate the harmful effects imposed by root-knot nematodes. In plants infected with both mycorrhizal fungi and nematodes, mycorrhizal inoculation substantially reduced adult nematode development (Kantharaju *et al.* , 2005). Therefore, the concept of using VAM fungi as a biocontrol agents is a promising perspective of these fungi. Objective of this work is to study the interaction of VAM fungi and root pathogens on tobacco.Since the data available on the potential of mycorrhiza in controlling soil borne diseases of tobacco is meagre, the present investigation to study the effects of VA mycorrhiza on host resistance is an attempt in this direction.

MATERIALS AND METHODS

Under the present interaction studies between VAM and *P. aphanidermatum* (damping -off causing pathogen), four VAM fungi namely *G. mosseae, G. fasciculatum, G. constrictum, Acaulospora laevis* and damping-off root rot pathogen *(P. aphanidermatum)* were used. The inoculum of these mycorrhizal endophytes was multiplied on *Zea mays* (maize) in pot culture under sterile conditions.

The experiment was conducted during 2007-8 at Central Tobacco Research Institute nursery site. The soil was analysed before starting the experiment for its chemical characteristics such as pH-7.6, electrical conductivity-1.76 , available N-o.25, available P-0.23 and available K-118.

Tobacco seeds were sown on m2 seed- beds along with the VA mycorrhizal inoculum. Around 50g soil including root bits containing 10-20 viable arbuscular mycorrhizal fungal propagules / g soil were used as inoculum and spread as a thin layer one cm below soil surface on each seed- bed. Three replications of each treatment were grown for a period of 60 days with every day watering up to field capacity.

All the treatments were inoculated with propagules (mycelium) of P. *aphanidermatum* raised on wheat grain medium @ 50g/m^2 nursery bed. The observations on disease severity, mycorrhizal root colonization were recorded after 30 days of pathogen inoculation.Four weeks old mycorrhizal tobacco seedlings were transplanted at the rate of one seedling per pot Mycorrhizal tobacco plants received *Meloidogyne incognita* inoculum at the rate of 2000 eggs/plant. The treatments were Nematode, Mycorrhiza, Nematode + Mycorrhiza and control.Each treatment was replicated three times. Clay pots (30 cm in diam.) were filled with 50 kg silty soil and sterilized in oven at 140°C for 2 h. Each plant received about 500 ml of tap water every two days. Pots were arranged in a randomized complete block design. Roots were thoroughly washed from surrounding soil and the following parameters were determined. The infection percentage was determined by root technique (Giovanetti & Mosse, 1980), (c) for assaying nematode infection the number of galls per plant were counted. Also, gall rating index (R.I.) from 0-5, where 0 = 0, 1= 1-10; 2 = 11-30, 3 = 31-100, 4 = 100-200 and 5 = > 200 galls per plant was determined .

RESULTS AND DISCUSSION

The perusal of data presented in Table 1 indicates that all the VA- mycorrhizal fungi colonized the root tissue efficiently in the rhizosphere soil of tobacco seedlings, and significantly reduced the severity of damping-off disease casued by P. *aphanidermatum.* However, percentage of disease severity and VAM colonization varied with VAM species. Amongst the various mycorrhizal endophytes tested, G. *fasciculatum* showed highest root colonization (69%) followed by *G. mosseae* and *Acaulospora laevis. G. constrictum* showed minimum colonization Higher colonization with, *G. fasciculatum* may be attributed to the adaptation of VAM fungi in native soil.

VAM isolate (*G. fasciculatum)* showed highest reduction in the damping- off severity compared to other VAM fungi. Tobacco seedlings inoculated with this VAM endophyte registered less disease severity compared to 100% in non- mycorrhizal seedlings (control).G. *fasciculatum* inoculated seedlings had healthy feeder roots except some rotting of the main root, where as in control plants, complete rotting of the feeder

Table 1: Mycorrhizal Root Infection and number of *Pythium* infected seedlings before and after inoculation of *Pythium* in the soil treated with 4 mycorrhizal cultures.

Treatment	Mycorrhizal root infection%	Damped off seedlings	
		B.I.P	A.I.P
G. fasciculatum	69	1.60 (1.5)	6.22 (37.6)
G. constrictum	36	3.19 (9.1)	9.52 (89.6)
G. mosseae	54	2.91 (7.4)	13.19 (172.9)
Aculospora	47	4.19 (16.5)	9.73 (93.6)
Control	0	7.96 (62.3)	15.79 (248.3)
CD (P=0.05)		0.45	0.34

Figures in parentheses are retransformed means

roots and coverage of the main root with the mycelium of the pathogen was observed. The VAM fungus *G. mosseae* was the next best in disease reduction followed by *Acaulospora laevis* and *G. constrictum.* Similar observation on the reduction of root infection due to VAM inoculation in chilli seedlings has been reported by Alejo-lturvide (2008).

The *G. fasciculatum* successfully infected tobacco roots forming typical VAM structures. Amongst the various mycorrhizal endophytes tested, *G. fasciculatum* showed highest root colonization (63%) followed by *G. mosseae*(50%) and *Acaulospora laevis* (49%). *G. constrictum* (38%)showed minimum colonization.(Table-2) Higher colonization with, *G. fasciculatum* may be attributed to the adaptation of VAM fungi in native soil.

Root diseases caused by nematodes are influenced by the mycorrhiza, less nematode galls were found in most of the mycorrrhizal plants tested (Table-2). The penetration of the larvae into the roots was not inhibited but their development within the mycorrhizal roots was restricted (Subhashini & Ramakrishnan, 2011). *M. incognita* formed conspicuous galls on roots of tobacco which were significantly greater in nonmycorrhizal than in mycorrhizal plants.(Table-3) The reduction in galls number caused by mycorrhizal inoculation was also observed by Jalaluddin *et al.,* (2008). The tolerance of mycorrhizal plants to nematode infection reported here agreed with that documented in many crops such as tomato (Shreenivasa *et al.,* 2007) and green gram (Sorah

Table 2: Root-Knot index of tobacco plants as influenced by inoculation with 4 different strains of mycorrhiza

Treatment	Mycorrhizal root infection (%)	Root-knot index
G. fasciculatum	63	3.75
G. constrictum	38	4.87
G. mosseae	50	2.50
Aculospora	49	3.75
Control	0	5.00
CD (P=0.05)		0.52

Table 3: Root-knot index of tobacco plants as influenced by mycorrhizal inoculation and root-knot nematode (Pot culture)

Treatment	Mycorrhizal root infection (%)	Root-knot index
Nematode	0	5.00
Mycorrhiza	60	2.12
Nematode + Mycorrhiza	49	2.37
Control	0	4.12
CD (P=0.05)		0.37

et al., 2009). The tolerance of mycorrhizal plants to nematode damage was attributed to changes in root physiology which in turn reduced nematode penetration and/or retarded adult nematode development (Grandison

& Cooper, 1986). These physiological changes were described by Heald *et al.,* (1989) as increased concentration of lignin, sugar, amino acids, phenol synthesis, and ethylene production.

To conclude with the present investigation clearly indicates the beneficial role of VA mycorrhiza in improving the resistance of host plant both interms of damping off, root-knot nematode and substantiates the view of Jalaluddin *et al.,* (2008) that these biological systems could be profitably exploited in the control of soil borne diseases.

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New Reports of Free-Living Marine Nematodes, *Chromadorina granulopigmentata* (Weeser, 1951) and *Neochromadora poecilosomoides* (Filipjev, 1918) (Chromadorida: Chromadoridae) from Indian Waters

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ABSTRACT: This paper describes new distributional records of two species of free-living nematodes belonging to the order Chromadorida and family Chromadoridae (*Chromadorina granulopigmentata* (Wieser, 1951) and *Neochromadora poecilosomoides* (Filipjev, 1918)) from India waters. It also provides a description for their identification based on the morphological features.

Key words: *Chromadorina granulopigmentata*, *Neochromadora poecilosomoides*, continental shelf, Indian waters

Free-living marine nematodes constitute as much as 60-90% of the benthic meiofauna (Sajan *et al.,* 2010). While the importance of parasitic nematodes has been recognized for many decades, this is not the case for free-living marine nematodes, especially those of aquatic environments (Heip *et al.,* 1985). An important feature of nematode population is the large number of species present in any habitat, often an order of magnitude higher than for any other taxon (Schratzberger *et al.,* 2006). Order Chromadorida is the largest order in class Adenophorea and family Draconematidae currently includes 15 genera and 33 species and family Epsilonematidae includes 8 genera and 11 species (Steyaert *et al.,* 2005). While few studies have been carried out on the qualitative and quantitative aspects of nematodes (Ansari *et al.,* 1980; Harkantra *et al.,* 1980; Ansari & Gauns, 1996; Nanajkar & Ingole, 2007; Sajan & Damodaran, 2007; Anila Kumary, 2008), only few studies have been carried on their taxonomy in the Indian waters (Timm, 1961, 1967; Sulthan Ali *et al.,* 1998; Chinnadurai & Fernando, 2006a, 2006b). In this backdrop the present study describes new distributional reports of two species of free-living nematodes namely *Chromadorina granulopigmentata* (Wieser, 1951) and *Neochromadora poecilosomoides* (Filipjev, 1918) for the first time in Indian waters.

MATERIALS AND METHODS

Totally 35 sediment samples were collected along 6 transects (off Karaikkal, Parangipettai, Cheyyur, Chennai, Tammenapatanam and Singarayakonda) at the depths of 30-50m, 51-75m, 76-100m, 101-150m, 151-175m and below 176m. In addition, due to the presence of an industrial cluster in Cuddalore (SIPCOT -State Industries Promotion Corporation of Tamil Nadu), sampling was also done here at depths of 30-50m and below 176m. Free-living marine nematode samples were collected onboard FORV (Fishery and Oceanographic Research Vessel) *"Sagar Sampada"* during December, 2008 (Cruise No. 260) along the southeast continental shelf of India.

Two sediment samples were collected using a Smith McIntyre grab (having a bite area of 0.2 m^2) from each depth. Immediately after the grab was hauled to the deck, sub-samples were taken from undisturbed grab samples using a glass corer (having an internal diameter of 2.5 cm and a length of 15 cm) from the middle of grab sample (Platt & Warwick, 1983). The samples were fixed in buffered formalin at a concentration of 4%. In the laboratory, samples were washed through a set of 0.5 mm and 0.053 mm sieves. The sediment retained in the

0.053 mm sieve was decanted to extract meiofauna following the method by Higgins & Thiel (1988). Sorting of meiofauna from sediment was done by flotation technique. The efficiency of this technique is around 95% (Armenteros *et al.,* 2008). The meiofaunal organisms were stained with Rose Bengal prior to extraction and were sorted and enumerated under a stereomicroscope (Meiji, Japan). All the nematodes were mounted onto glass slides, using the formalin-ethanol-glycerol following Vincx (1996). Identification of nematode species was carried out to the highest taxonomic level possible using the compound microscope following the standard pictorial keys of Platt & Warwick (1988) and the NeMys Database by Steyaert et al. (2005). Drawings and morphometric measurements were made using compound microscope (Olympus CX 41 under higher magnification of 1000x attached with ocular micrometer) with a Camera Lucida.

RESULTS

Totally 192 species were identified along the southeast continental shelf of India. Among these, two species (*Chromadorina granulopigmentata* (Wieser, 1951) and *Neochromadora poecilosomoides* (Filipjev, 1918)) belonging to the order Chromadorida and family Chromadoridae were found to be new distributional reports from Indian waters. Detailed systematic account, materials examined (number of specimens, place, depth and date of collection), brief description, feeding type, habitat and geographical distribution besides remarks of the above two species are given here.

1. *Chromadorina granulopigmentata* (Wieser, 1951) (Fig. 1 & Plate – 1 A-F)

Phylum: Nematoda Rudolphi, 1808

Class: Adenophorea von Linstew, 1905

Order: Chromadorida Filipjev, 1929

Family: Chromadoridae Filipjev, 1917

Genus: *Chromadorina* Filipjev, 1918

Species: *Chromadorina granulopigmentata* (Wieser,1951)

Material examined: 7 males and 3 females collected from Cheyyur 30-50m, 51-75m and 101-150m depths (18.12.2008).

De Man ratio:

	a	b	c
Male :	26±1.42 (24.07- 28.36)	4.83±0.24 (4.33-5.11)	8.01±0.18 (7.62-8.32)
Female :	25.70±1.58 (24.32- 27.42)	4.81±0.15 (4.68- 4.97)	7.99±0.07 (7.93- 8.07)

Description

Body long and slender with the length of 0.3 - 0.6 mm in male and 0.4 - 0.6 mm in female and anterior tapering and posterior conico-cylindrical. Body Maximum diameter 14 – 15 µm in male and 14 – 16 µm in female. Cuticle in two layers, inner one being thicker than the other one. The homogeneous cuticle with transverse rows of dots but without lateral differentiations. Lip region rounded and six small rounded papillae present in apex. Four small cephalic setae 3 - 4 µm. Silt-like amphids, 2 - 3 µm wide in male and 2 - 2.5 µm wide in female. Buccal cavity with a large solid dorsal tooth and two smaller equal subventral teeth. Granular pigment spots located in the anterior region with two pairs of setae. Oesophagus cylindrical with well developed posterior bulb (73 - 89 µm in male and 78 - 84 µm in female). Tail conico-cylindrical with tapering (3.2 - 4.6 anal body diameter in male and 3.9 - 4.2 anal body diameter in female). Spicules 16 - 23µm (1.9 anal body diameter) measured as a curve; arcuate with a ventral proximal swelling, rounded distal ends and a delicate ventral ala. Gubernaculum 10 - 12 µm. A pair of large and a pair of smaller precloacal setae present around the cloacal region. Ovaries paired, equal, opposed and reflexed. Vulva present at 56- 58% of total body length (Plate 1).

Feeding type: The specimens showed buccal cavity armed with small. According to the classification of buccal cavity by Wieser (1953), this species is an epigrowth feeder (2A).

Habitat: Sandy sediments.

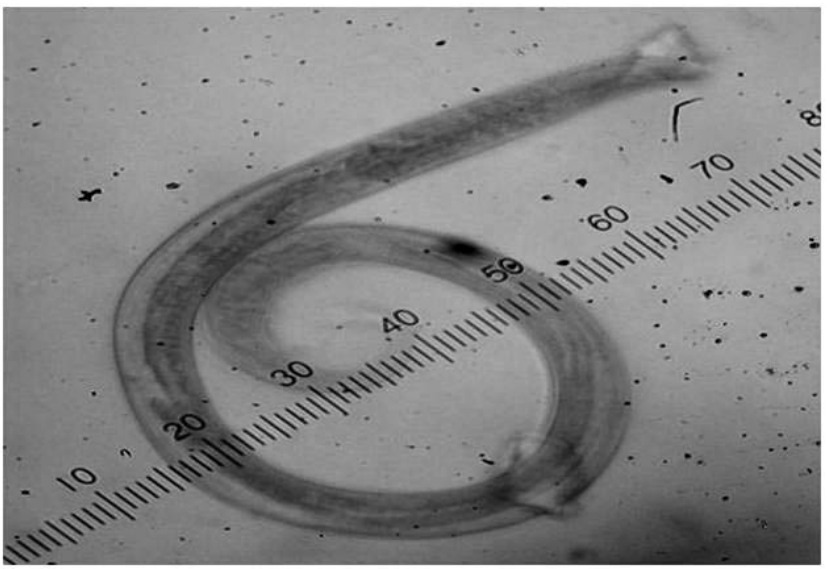

Fig. 1. Photomicrograph (40X) of *Chromadorina granulopigmentata* (Wieser, 1951)

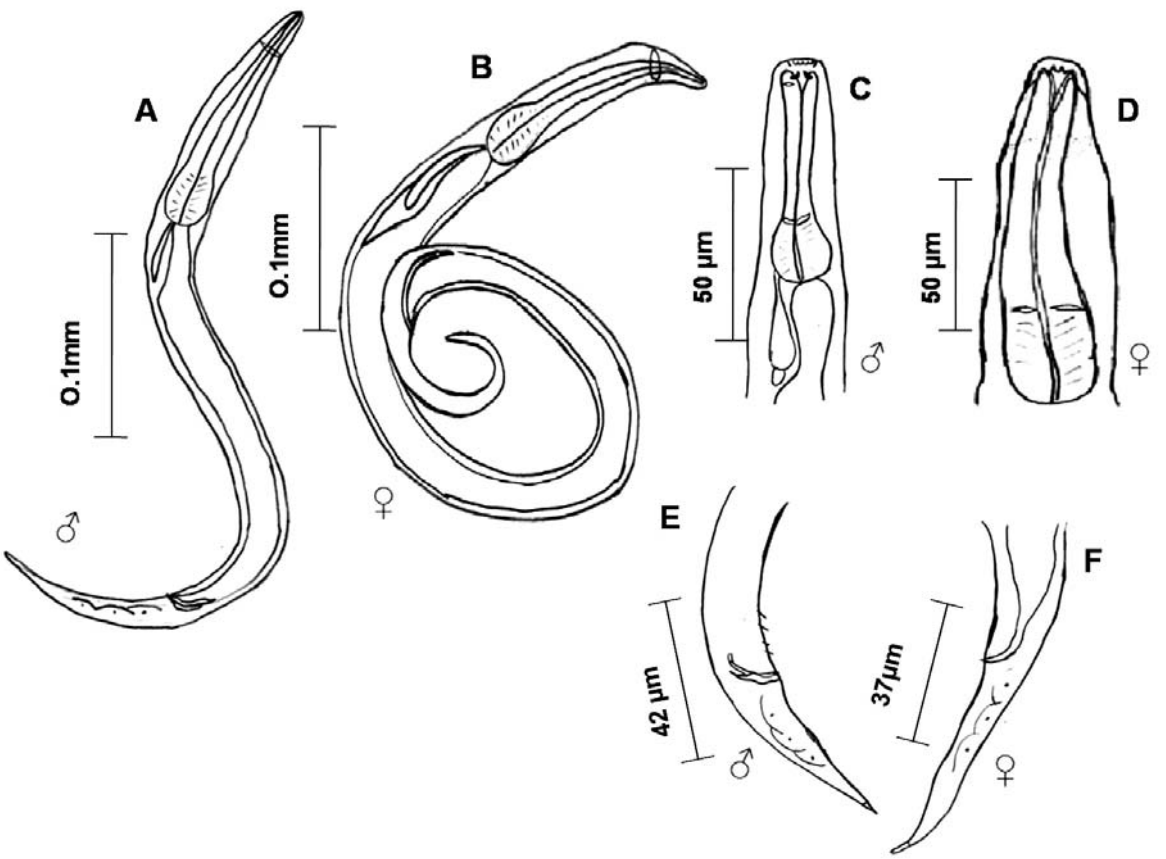

Plate 1. *Chromadorina granulopigmentata* (Wieser, 1951)
A) entire male B) entire female, C) male head, D) female head, E) male tail, F) female tail

Distribution

India: Cheyyur.

Elsewhere: Southwest England (Hansson, 1998) and Plymouth (Platt & Warwick, 1988).

Remarks

The specimens examined conformed well to the earlier description, except for the smaller body size. The total body length described by Platt & Warwick (1988) was 0.6 - 0.7 mm and tail length was 4 - 4.5 anal body diameter in male and in female these were not recorded. The body length of the specimen studied at present was found smaller being 0.3 - 0.6 mm and the tail length 3.2 - 4.6 anal body diameter in male and in female 0.4 - 0.6 mm body length and tail length 3.9 -4.2 anal body diameter This is the first record of the species from the Indian waters.

2. *Neochromadora poecilosomoides* (Filipjev, 1918) (Fig. 2 & Plate – 2 A-F)

Phylum: Nematoda Rudolphi, 1808

Class: Adenophorea von Linstew, 1905

Order: Chromadorida Filipjev, 1929

Family: Chromadoridae Filipjev, 1917

Genus: *Neochromadora* Micoletzky, 1924

Species: *Neochromadora poecilosomoides* (Filipjev, 1918)

Material examined: 5 males and 3 females collected from Cuddalore - SIPCOT (19.12.2008).

De Man ratio:

	a	b	c
Male :	44.26±1.59 (42.61-46.07)	7.10±0.31 (6.78- 7.55)	10.58±0.45 (9.98- 11.12)
Female :	43.14±0.56 (42.57-43.72)	6.89±0.11 (6.64- 6.99)	10.29±0.27 (9.96-10.46)

Description

Body long and slender with the length of 0.9 - 1.4 mm in male and 0.8 - 1.2 mm in female and body anteriorly narrow blended and posteriorly conical with tapering. Maximum body diameter 21 – 24 µm in male and 22 – 23 µm in female. Cuticle ornamentation complex; in anterior oesophageal region transverse rows of punctations with lateral differentiation of two longitudinal rows of dots beginning at base of oesophagus and continuing down length of body, terminating half way down tail. Rounded lip with surrounded by six short (6 – 7 µm) and four long (10 – 12 µm) cephalic setae. Somatic setae also present throughout the body. Silt-like amphids 1 – 4 µm wide in male and 2 – 4 µm wide in female. Buccal cavity small and narrow with small pointed dorsal tooth and two subventral denticles. Oesophagus cylindrical with slight oval posterior bulb (134 – 156 µm in male and 130 – 151 µm in female). Tail conical (4.8 -5.1 anal body diameter in male 4.9 – 5 anal body diameter in female). Spicules 13 - 16µm (0.8 - 0.9 anal body diameter), arcuate. Gubernaculum curved with length of 9 – 12 µm. Eight small squarish precloacal supplements. Ovaries paired, equal, opposed and reflexed. Vulva present at 52 – 53 % of body length (Plate 2).

Feeding type: The specimens showed buccal cavity armed with small. According to the classification of buccal cavity by Wieser (1953), this species is an epigrowth feeder (2A).

Habitat: Sandy sediments.

Distribution

India: Cuddalore – SIPCOT.

Elsewhere: England (Platt & Warwick, 1988); European waters and Mediterranean (Hansson, 1998).

Remarks

The specimens examined conformed well to the earlier description, except for the larger body size. The total body length described by Platt & Warwick (1988) was 1 - 1.1 mm and tail length was 5 - 5.7 anal body diameter in male and in female these were not recorded.

Fig. 2. Photomicrograph (40X) of *Neochromadora poecilosomoides* (Filipjev, 1918)

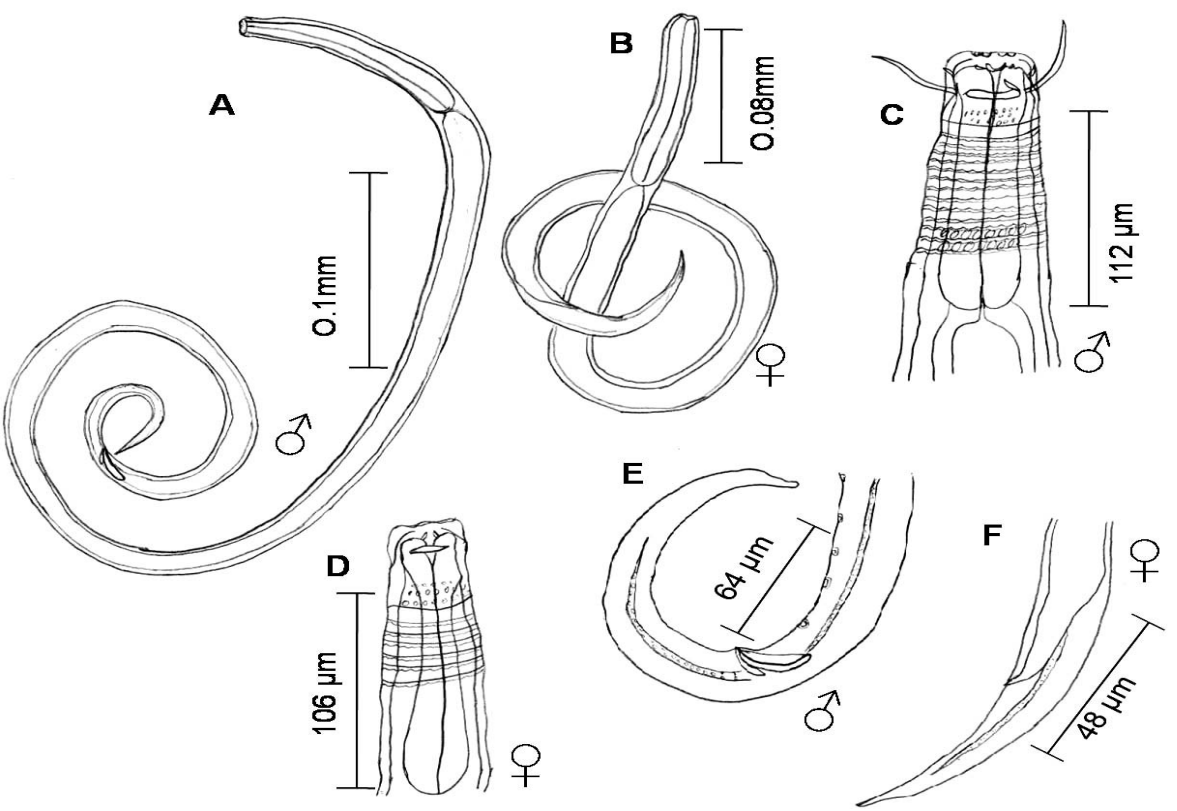

Plate 2. *Neochromadora poecilosomoides* (Filipjev, 1918)
A) entire male B) entire female, C) male head, D) female head, E) male tail, F) female tail

The body length of the specimen studied at present was found larger being 0.9 - 1.4 mm and the tail length 4.8 - 5.1 anal body diameter in male and in female 0.8 - 1.2 mm body length and tail length 4.9 -5 anal body diameter. This is the first record of the species from the Indian waters.

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Chemo-Therapeutic and Nematoxic Effect of Composts on the Survival of *Meloidogyne hapla* Juveniles

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ABSTRACT: Aqueous extract from five different composts (Terra Ecosystem, wyvern waste, garden waste, farm yard manure and green waste) were assessed for their effects on juvenile survival of *Meloidogyne hapla in vitro*. It was found that graded extracts (5, 10, 15 and 20% w/v) of the composts effectively killed *M. hapla* juveniles. At the 8th day exposure time,*M. hapla* juveniles in Terra Ecosystem, wyvern waste, garden waste and green waste were dead, whereas few *M. hapla* were alive in the farm yard manure aqueous extract till day 10. The *in vitro* assessment shows that Terra Ecosystem, wyvern waste, garden waste and green waste were significantly toxic to *M. hapla.*Phyto-chemical analysis of the compost revealed that Terra Ecosystem contained sterols and flavonoids; wyvern waste contained sterols, glycosides and flavonoids; garden waste contained only flavonoids; farm yard manure contained saponins, sterols, glycosides and flavonoids; and green waste contained saponins, sterols and flavonoids.

Key words: Compost, nematode,*Meloidogynehapla*, Chemo-therapeutic, nematoxic

The ten most important nematode genera include *Meloidogyne, Pratylenchus, Heterodera, Ditylenchus, Globodera, Tylenchulus, Xiphinema, Radopholus, Rotylenchulus*and*Helicotylenchus* (Sasser & Freckman, 1987). Root knot nematodes, *Meloidogyne* species, are economically important pathogens worldwide. They are obligate parasites infecting plant species, including major food crops, vegetables, fruits and ornamental crops. They reduce the yield and quality of susceptible crops (Jonathan & Hedwig, 1991; Yousef & S'Jacob, 1994; Olabiyi, 2008).

There are various and recent European Legislations which restricted and revised the use of chemical pesticides on agricultural crops (Reg. CE 396/2005; 1095/2007; 33 and 299/2008 and 1107/2009). Agricultural crop consumers are poisoned and in most cases, the prices of synthetic nematicides are very high and not always available for the users at the time of need (Thomas, 1996). All these spurred researchers/ scientists globally, to find alternative strategies that are environmentally friendly and economically convenient. Therefore, research on measures that could have no or low environmental impact, alternatives to synthetic pesticides, had received very strong impulse and considered a wide range options. One of the options is the application of compost in agricultural crop production. Application of compost, as soil amendment or aqueous plant extract, had caused significant reduction in population of root knot nematode, *Meloidogyne* spp. and reduced the nematode damage which had resulted to improve plant growth and yields (Verma, *et al*., 1997; Ribeiro, *et al*., 1998; D'Addabbo, *et al*., 2000).

Recently, the safety and health of food have become major concern, due to indiscriminate use of pesticides for food production and its resultant negative impacts on human health, livestock, soil and environment (Primentel, 2005). For this reason, farmers in the environmental conscious world have started replacing synthetic fertilizers with compost. Composts have been reported to augment soil nutrient and suppress soil inhabiting nematodes (Olabiyi,*et al.* 2007).

Decomposed plant materials have been reported to be toxic to nematodes and have been effectively used as nematode pest control measure. These composted plant materials include wild sunflower, neem cake, neemleaf, maize stover and cassava peel (Egunjobi and Onayemi, 1981; Riga and Lazarovits, 2001; Abbasi, et al. 2005; Olabiyi,*et al*. 2007). The objective of this study is therefore to assess the potential of different organic composts on the juvenile survival of root knot nematode,*Meloidogynehapla* and also to determine the phyto-chemical constituents of the compost.

MATERIALS AND METHODS

Preparation of compost solution: Terra ecosystem, Wyvern waste, Green waste, Farm yard manure and Garden waste were ground (2mm) using attrition mill in the Environmental Science Laboratory of the Coventry University, UK. Ten (10) ml each of the air-dried compost was transferred to plastic poly-bottles to which were added 25ml of deionised water. The bottles were capped and then shaken for 15 minutes on a reciprocating shaker (200 – 250 hub/min) and the resulting suspension was filtered through Whatman filter paper number 1.

Preparation of the test organism (root knot nematode): Tomato (*Lycopersicumesculentus* L. Mill), was grown in pot in the greenhouse and inoculated with stock culture of *Meloidogynehapla*. Ten weeks after planting (10 WAP), eggs of *Meloidogynehapla* were extracted, using sodium hypochlorite (Hussey and Baker, 1973) from tomato galled roots. The eggs were incubated at temperature of 10 - 15°C for 24 hours. The freshly hatched second stage nematode juveniles were collected. Juveniles of *M. hapla* in the distil water suspension was concentrated and standardized so that each 1ml suspension contained approximately 100 juveniles.

Effect of different compost on *M. hapla* juveniles (nematode bio-assay): Different compost solutions were prepared into 1, 5, 10,15% and 20% w/v concentrations in the Environmental Science Laboratory, Coventry University, UK. Aliquot of 20 ml of each compost solution was dispensed into Petri-dish and thereafter, 1 ml each of nematode suspension containing approximately 100 *M. hapla* population was added. The Petri dish containing only 1ml juvenile suspension containing approximately 100 *M. hapla* juveniles in 20 ml distil water only, served as the control. Each treatment, including the control, was replicated five times. The experimental design was complete randomized design. A count of live nematodes was made and cumulative numbers of dead nematodes were put on record on daily basis for a period of 10 consecutive days.

Phyto-chemical analysis

Two-hundred and fifty gram (250gm) of each compost (Terra ecosystem, Wyvern waste, Green waste, Farm yard manure and Garden waste) was ground using Glen Creston Rotary hammer mill, to pass a 1mm mesh. The samples were then stored in re-sealable polythene bags. An hundred gram (100g) of the compost was weighed separately with sensitive electric weighing balance (four digital) into 500 ml ethanol (100%) and soaked for 3 days in a closely air-tight one (1) litre sized measuring cylinder. The content was filtered using Whatman filter paper number 1. The filtrate (ethanol extract) was used for the chemotherapeutic analysis.

Flavonoids

(i) Aliquot of 4 ml of aqueous NaOH was added to 2ml of each of ethanol extract. If a yellow precipitate was observed, it indicates the presence of flavonoids in the extracts. But if otherwise, it indicates the absence of flavonoids

(ii) Shinoda test: A little amount of magnesium powder and 3 drops of concentrated HCl were added to 4 ml of each of the ethanol extract. If a red colour was observed, it indicates the presence of flavonoids. If otherwise, it shows the absence of flavonoids

Sterols

Aliquot of 2ml concentrated H_2SO_4 was added to 1 ml of each ethanol extract. If a brownish red colour was observed, it shows the presence of sterols. But, if otherwise, it shows the absence of sterols.

Glycosides

Aliquot of 10 ml of 50% H_2SO_4 was added to 1 ml of each ethanol extract. The mixture was heated on water bath for 15 min. Then, 10ml of fehling solution (5cm^3 each of fehling solutions A and B) was added and boiled. If a red precipitate was observed it shows the presence of glycoside. But if otherwise, it shows the absence of glycosides.

Tannins

To 2ml of each of the ethanol extract was added 5 drops of Fe_2Cl_3 solution. If a dirty green precipitate was observed, it indicates the presence of tannins. But if otherwise, it indicates the absence of tannins.

Test for Saponins

Frothing test: Aliquot of 2ml of each ethanol extract was shaken vigorously in the test tube for 2 minutes, frothing shows the presence of saponins. But if otherwise, it indicates absence of saponins.

Emulsion test: Aliquot of 5 drops of olive oil was added to 3 ml of the weed extract in test tube and mixture was vigorously shaken. If a stable emulsion is formed, it indicates the absence of saponins. But if froth is formed, it indicates presence of saponins.

RESULTS AND DISCUSSION

Table 1 shows the survival potential of *Meloidogynehapla* in different compost concentrations

Table 1: Survival of *M. hapla* in different compost concentrations

Different compost		Number of live nematodes at day									
Concentrations (% w/v)		1	2	3	4	5	6	7	8	9	10
Terra ecosystem	5	100	52^{e}	38^{b}	22^{b}	7^{a}	4^{a}	3^{a}	0^{a}0^{a}0^{a}		
	10	100	47^{d}	31^{b}	20^{b}	9^{a}	4^{a}	1^{a}	0^{a}0^{a}0^{a}		
	15	100	40^{d}	25^{b}	11^{a}	4^{a}	2^{a}	1^{a}	0^{a}0^{a}0^{a}		
	20	100	26^{bc}	18^{a}	8^{a}	3^{a}	1^{a}	0^{a}0^{a}0^{a}0^{a}			
Wyvern waste	5	100	32^{c}	14^{a}	10^{a}	7^{a}	1^{a}	0^{a}0^{a}0^{a}0^{a}			
	10	100	28^{c}	13^{a}	11^{a}	9^{a}	0^{a}0^{a}0^{a}0^{a}0^{a}				
	15	100	20^{b}	10^{a}	8^{a}	7^{a}	1^{a}	0^{a}0^{a}0^{a}0^{a}			
	20	100	15^{a}	12^{a}	10^{a}	8^{a}	1^{a}	0^{a}0^{a}0^{a}0^{a}			
Garden waste	5	100	24^{b}	20^{a}	13^{a}	12^{a}	4^{a}	1^{a}	0^{a}0^{a}0^{a}		
	10	100	24^{b}	17^{a}	7^{a}	5^{a}	2^{a}	1^{a}	0^{a}0^{a}0^{a}		
	15	100	20^{a}	14^{a}	7^{a}	4^{a}	1^{a}	0^{a}0^{a}0^{a}0^{a}			
	20	100	21^{b}	12^{a}	6^{a}	5^{a}	0^{a}0^{a}0^{a}0^{a}0^{a}				
Farm yard manure	5	100	85^{g}	72^{c}	38^{c}	28^{b}	26^{b}	24^{b}	24^{b}	22^{b}	20^{b}
	10	100	80^{g}	66^{c}	34^{c}	22^{b}	21^{b}	20^{b}	20^{b}	19^{b}	17^{b}
	15	100	88^{g}	68^{c}	33^{c}	25^{b}	22^{b}	20^{b}	20^{b}	18^{b}	17^{b}
	20	100	76^{f}	61^{c}	35^{c}	28^{b}	25^{b}	21^{b}	20^{b}	20^{b}	18^{b}
Green waste	5	100	21^{b}	14^{a}	10^{a}	9^{a}	3^{a}	0^{a}0^{a}0^{a}0^{a}			
	10	100	18^{a}	16^{a}	11^{a}	7^{a}	3^{a}	1^{a}	0^{a}0^{a}0^{a}		
	15	100	16^{a}	14^{a}	13^{a}	13^{a}	4^{a}	0^{a}0^{a}0^{a}0^{a}			
	20	100	13^{a}	13^{a}	12^{a}	11^{a}	1^{a}	0^{a}0^{a}0^{a}0^{a}			
Control (ddH_2O)	NS	100	100^{h}	94^{d}	91^{d}	90^{c}	85^{c}	81^{c}	80^{c}	71^{c}	65^{c}

NS = Not statistically significant

Means followed by the same letter along the same column are not statistically different at 5% probability

Table 2: Phyto-chemical analysis of different composts

	Saponins	Tannins	Sterols	Glycosides	Flavonoids
Terra ecosystem	A	A	P	A	P
Wyvern waste	A	A	P	P	P
Green waste	P	A	P	A	P
Farm yard manure	P	A	P	P	P
Garden waste	A	A	A	A	P

Key: P = Present A = Absent

within 10 days. It was observed that compost have lethal effect on *M. hapla* and the toxic effects are days to exposure and level of concentration dependent. However, toxic potential of compost differ from one type of compost to the other. By day 8 of exposure, all nematodes in Terra ecosystem, Wyvern waste, Garden waste and Green waste were dead, whereas few were still found living in the Farm yard manure, even till after day 10. Though there were reduction in the number of live nematodes in the control (deionised distilled water), but live nematodes recorded, showing that deionised distilled water does not have toxic effect on *M. hapla*.

Table 2 shows the chemotherapeutic potentials of the different composts. Presence of saponins, tannins, sterols, glycosides and flavonoids were examined on each of the compost. The results elicit the presence of saponins in green waste and farm yard manure; none of the compost had tannins; terra ecosystem, wyvern waste, green waste and farm yard manure showed the presence of sterols and flavonoids; while only wyvern waste and farm yard manure had glycosides. Garden waste did not contain any of the tested chemical compounds.

Aqueous extract from Terra Ecosystem, wyvern waste, garden waste, farm yard manure and green waste compost were assessed for their effects on juvenile survival of *Meloidogyne hapla in vitro*. The tested composts significantly reduced the juvenile survival rate of *M. hapla* compared to the control.The result corroborates the earlier findings of Sasanelli and Addabbo (1993), Pandey *et al.* (1997) and Olabiyi *et al.* (2007) who reported that organic manure significantly reduced the population of *Meloidogyne incognita*. Aqueous extracts (100% concentrations) of *Lactua sativa, Amnimajus, Artemisia pallens* and *Artemisia annua* resulted in 100% *Meloidogyne incognita* mortality within 24 hours of exposure in vitro (Pandey, 1990).

The result of phyto-chemical analysis revealed that the compost contained saponins, tannins, sterols, glycosides and flavonoids. Several plants and compost are known for their toxicants and metabolites. Natural plant products have the ability to produce environmentally friendly bio-toxicants that are bio-degradable and efficacious against pathogenic organisms, including soil inhabiting plant parasitic nematodes (Schmutterer, 1990; Jackai, *et al*., 1992).Brimstone, *Morindalucida*, contained saponins and flavonoids which were reported to have bio-nematicidal properties (Olabiyi,*et al*., 2008).

CONCLUSION

The current research indicates that compost contained certain bio-chemical which was proved to be toxic to *Meloidogyne hapla* in the laboratory. However, there is need to do further investigation on the field.

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Efficacy of Wild Sunflower Compost on Root Lesion Nematode, Pest of Maize

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ABSTRACT: Field trials were conducted during 2010 and 2011 planting seasons to assess the bio-nematicidal potential of wild sunflower (*Tithonia diversifolia*) compost in the control of *Pratylenchus brachyurus* infection on maize (*Zea mays*) cv ACR 97. Field trials were arranged in randomized complete block design, with 5 treatments, each replicated 4 times. Experimental plots where no compost were applied served as the control; while other treatments were 1.0, 1.5, 2.0 and 2.5 tons/ha. The result obtained shows that higher application rates of 2.0 and 2.5 tons/ha. gave significant ($p<0.05$) control measure on *P. brachyurus* with resultant higher growth and yield of maize. The soil nematode population where 2.0 and 2.5 tons/ha were applied had significant ($p<0.05$) lower number of *P. brachyurus*. The result of the phytochemical screening revealed the presence of tannins, saponins, alkaloids and flavonoids, which were hypothesized to be the nematoxic principles in wild sunflower compost.

Key words: Compost, *Pratylenchus brachyurus, Zea mays,* sunflower

Maize (*Zea mays* L.) belongs to the family Graminae. Maize is widely cultivated throughout the world and it is one of the most important cereal grains in the world, providing nutrients for humans and animals. It also serves as a basic raw material for the production of starch, oil, protein, alcoholic beverages foods, and fuel. Maize has very high average yield per hectare when grown under optimum condition. Despite the wide spread use of maize, its production and cultivation has been greatly reduced by insect pests, diseases and soil inhabiting pathogens that affect the growth and yield of maize on the field.

Root lesion nematodes, *Pratylenchus brachyurus* Filipjev, are the major species of nematodes found on maize and are mainly root parasites. The below ground symptoms on the roots are the presence of lesions/ blisters on the root, excessive branching of root, and shortening of the root. The above ground symptoms manifested by affected crops are dwarfing, patchy growth and low yield. Poor germination and death of young seedlings may be observed in cases of heavy infestation (Egunjobi and Ekundare, 1981).

The use of chemicals has been found to be the most effective method of controlling pests on Agricultural crops. However, the prohibitive costs as well as hazards associated with their application have greatly reduced their use by many farmers (Olabiyi, 2004). Today the world is becoming more and more aware of the environmental dangers posed by chemical pesticides. Even in the advanced countries where these chemicals are produced and farmers have sufficient skill for their application, synthetic chemicals are now being replaced with other environmentally friendly methods of pest and disease control for various reasons including water and atmospheric pollution which continue to render the world agricultural foods unsafe for mankind (Egunjobi and Afolabi, 1976; Thomas, 1996; Olabiyi, 2005).

Organic manure when incorporated in large amount into the soil reduces the population of the soil inhabiting

pest and pathogen. Wild sunflower used as soil amendment is very promising for nematode control (Olabiyi *et al.* 2007). The use of compost in crop protection when fully developed or integrated will enhance safe food crop production, reduce environmental pollution and also make our agricultural produce to gain international recognition. The objective of this paper is therefore to assess the effect of wild sunflower (*Tithonia diversifolia*) compost on root lesion nematode (*Pratylenchus brachyurus*) pest of maize (*Zea mays*).

MATERIALS AND METHODS

Preparation of compost materials

A windrow method was used for the preparation of compost at the Teaching and Research farm, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. Freshly harvested wild sunflower (100 kg) was chopped (5-10 cm sizes), wrapped with plastic and properly covered up for a period of 5 months with regular turning (monthly interval) with garden spade. The fully decomposed materials (compost) were spread and air dried. The compost, in air-dried form, was ground into powder form using attrition mill.

Field experiment

The experimental field of 350m^2 size was ploughed twice and partitioned into plots. Five ridges of 4m^2 were constructed within plots. The field experiment was carried out at the Organic Garden section, Teaching and Research Farm, Ladoke Akintola University of Technology, Ogbomoso, Nigeria in 2009 and 2010. The experimental design was randomized complete block design, with 5 treatments replicated 4 times. Three seeds were planted per stand which was later thinned down to one healthy maize plant per stand with an average distance of 25cm intra-row on the ridges. Each of the maize stands was inoculated two weeks after planting with 100g *P. brachyurus* infected maize root. The compost, in power form, was applied as soil amendments in ring form, 2-3cm away from maize plant and at the rates of 1.0, 1.5, 2.0 and 2.5tons/ha. The experimental plots where no compost was applied served as the control.

Phyto-Chemical analysis: The presence of tannins, saponins, flavonoids, alkaloids, glycosides and sterols in wild sunflower compost was assessed at the University Central Research Laboratory, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.

Test for Saponin (frothing test): 2 ml of crude extract of wild sunflower compost in a test tube was vigorously shaken for 2 min. Frothing in the extract indicates the presence of saponins.

Test for Saponin (emulsion test): Five drops of olive oil were added to 3 ml of the extract of wild sunflower compost in a test tube and the mixture were vigorously shaken. A stable emulsion formed in the extract indicates the presence of saponins.

Test for Flavonoids (Shinoda test): 1 ml of 10% NaOH was added to 4 ml of extracts of wild sunflower compost. A yellow colour observed indicates the presence of flavonoids.

Test for tannins: Two drops of 5% ferric chloride were added to 1 ml of crude extract of wild sunflower compost. An observed dirty green precipitate in the extract indicates the presence of tannins.

Test for sterols: 1 ml of concentrated sulphuric acid (H_2SO_4) was added to 3 ml of crude extract of wild sunflower compost. A colourless solution observed indicates the absence of sterols.

Test for glycosides: 10 ml of 50% concentrated sulphuric acid (H_2SO_4) was added to 1 ml of crude extract of wild sunflower compost in a test tube. The mixtures were heated in boiling water for 15 min. Fehling's solution (5 ml each of Fehling's solution A and B) were added and the mixture was boiled. A colourless solution observed indicates the absence of glycosides.

Test for alkaloids: 1 ml of 1% hydrochloric (HCl) acid was added to 3 ml of crude extract of wild sunflower compost in the test tube. The mixture was boiled for 20 min and filtered. Portions of the filtrate were treated with two drops each of Mayer's and Wagner's reagent separately. An observed creamy white (Mayer's reagent)

and reddish brown (Wagner's reagent) precipitates indicates the presence of alkaloids.

Data collection

Data were collected on plant height, number of leaf per plant, plant girth, number of cob per plant, number of grain per cob, fresh cob weight, population of root lesion nematode in 10g root sample at both years were pooled together, and thereafter subjected to analysis of variance (ANOVA) and means were separated using Duncan Multiple Range Test (DMRT) at 5% probability level. Data were also collected on the phyto-chemical analysis of wild sunflower compost.

RESULTS AND DISCUSSION

Two years field trials were carried out during 2010 and 2011 planting seasons in order to assess the bio-nematicidal potentials of wild sunflower compost in the control of maize root lesion nematode, *Pratylenchus brachyurus*. The effects of wild sunflower compost on the growth of maize infected with *P. brachyurus* were presented on Table 1. It was evident that maize treated with wild sunflower compost significantly have higher plant height, number of leaves per plant and plant girth than the control. Maize plants that were treated with wild sunflower compost of between 1.5 and 2.5 tonnes per hectare performed better than those treated with 1.0 tonne per hectare and control.

Table 1: Effects of wild sunflower compost on the growth of maize infected with *P. brachyurus*

Treatments	Mean number of leaf/plant	Mean plant height (cm)	Plant girth (cm)
Control	9.3[b]	65.4[d]	2.0[b]
1.0 tonne/ ha.	9.8[b]	73.3[c]	2.8[a]
1.5 tonne/ ha.	10.7[a]	119.6[b]	3.0[a]
2.0 tonnes/ ha.	13.0[a]	152.1[a]	4.0[a]
2.5 tonnes/ ha.	13.1[a]	151.5[a]	4.0[a]

Means followed by the same letter(s) along the same column are not significantly different at 5% probability level according to Duncan multiple range tests (DMRT).

The result presented on Table 2 elicits the effects of application rates of wild sunflower compost on the yield of maize. While there was no significant difference between the treated maize and control with respect to number of cob per plant, there were significant differences on the number of grain per cob and fresh cob weight in view of the maize plants that were treated and those that were not treated (control). Maize plants that were treated with wild sunflower compost have higher number of grains per cob and fresh cob weight than the untreated maize (control). Maize plants treated with wild sunflower compost of between 1.5 and 2.5 tonnes per hectare performed significantly better than those maize plants treated with 1.0 tonne per hectare wild sunflower compost and control.

Table 2: Effects of wild sunflower compost on the yield of maize infected with *P. brachyurus*

Treatments	Mean number of cob/ plant	Mean number of grain/ cob	Fresh cob weight (kg)
Control	1	105.4[d]	0.06[c]
1.0 tonne/ ha.	1	311.3[c]	0.09[b]
1.5 tonne/ ha.	1	412.6[b]	0.14[a]
2.0 tonnes/ ha.	1	582.1[a]	0.15[a]
2.5 tonnes/ ha.	1	581.5[a]	0.16[a]

Means followed by the same letter(s) along the same column are not significantly different at 5% probability level according to Duncan multiple range tests (DMRT).

Table 3 shows the effect of different application rates of wild sunflower compost on the population dynamics of *P. brachyurus* in the root of maize. Maize plants that were treated with wild sunflower compost significantly have lower *P. brachyurus* population in the root (10g) of maize.

Phyto-chemical components of wild sunflower compost were presented on Table 4. It was evident that wild sunflower compost contains tannins, saponins, flavonoids and alkaloids while it lacks sterols and glycosides. The presence of flavonoids in the sunflower compost might be the reason why it gave effective control measure on maize.

Table 3: Effects of wild sunflower compost on the root lesion nematode population

Treatments	Mean root lesion nematode in 10gm root sample
Control	110.5[b]
1.0 tonne/ ha.	32.5[a]
1.5 tonne/ ha.	31.7[a]
2.0 tonnes/ ha.	31.5[a]
2.5 tonnes/ ha.	30.5[a]

Means followed by the same letter(s) along the same column are not significantly different at 5% probability level according to Duncan multiple range tests (DMRT).

The results of this experiment showed that wild sunflower (*Tithonia diversifolia*) compost significantly reduced the infection potential of *Pratylenchus brachyurus* on maize. The bio-nematicidal effects of composted materials as soil amendments with plant- and animal-based materials have been reported previously (D'Addabbo, 1995; Akhtar and Malik, 2000; Olabiyi, 2005). Application of composts as soil amendments are found to improve soil quality and soil microbial population dynamics, and also suppress the activity of plant soil-inhabiting pathogens and pests, including plant parasitic nematodes (Bailey and Lazarovitz, 2003; Chang *et al.* 2007). Composts prepared from fresh olive pomace combined with a low percentage of chicken manure; cow manure and lettuce crop residues; municipal green wastes combined with sewage sludge; municipal green wastes combined with soil; and mycelium waste from penicillin production were reported to significantly reduce the soil population soil inhabiting nematodes of barley (Renco *et al*. 2010). Moreover, Olabiyi (2007) reported reduction in number of galls and egg masses as well as increase in plant growth as a result of treating nematode infected cowpea with composted wild sunflower.

The phytochemical analysis revealed the presence of flavonoids in the wild sunflower compost. This investigation supported earlier reports of the nematicidal properties of flavonoids by Verma et al (1978), Zavaleta-Mojia et al (1993) and Submaniyan and Vadivelu (1990) who reported that *Crotolaria spectabilis* leaves and roots posses certain phyto-chemicals that killed

Table 4: Phyto-chemical analysis of wild sunflower compost

Chemical constituents	Inference
Tannins	Present
Saponins	Present
Flavonoids	Present
Alkaloids	Present
Gycosides	Absent
Sterols	Absent

Pratylenchus brachyurus juveniles within 6 days exposure at concentration of 1.5 *in vitro*.

In conclusion, the results of this study unveil the bio-nematicidal potentials of wild sunflower compost and show that it could be used in the control of *Pratylenchus brachyurus* infection on maize.

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Evaluation of Heterocystous and Non Heterocystous Cyanobacterial Species for Nematicidal Activity

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ABSTRACT: Nematicidal potential of ten terrestrial heterocystous and non heterocystous cyanobacteria were compared against root-knot nematode, *Meloidogyne incognita* J2s. The extracts of all the ten unsonicated cyanobacteria caused an average immobility of 4.8-22.9% and mortality of 1.5-5.6% which increased to 88.2-96.5% and 4.2-29.3% on sonication, respectively. The highest per cent mean mortality was observed in *S. nidulans* (29.3) followed by *P. tenue* (26.8), *M. tenera* (25.8), *T. nodosa* (22.6), *M. vaginatus* (21.3), *S. bohmeri* (17.5), *N. punctiforme* (6.5), *A. variabilis* (5.6), *C. brevissima* (5.1) and least in *H. luteolus* (4.2); with an increase in exposure time from 24 to 72h. The immobility and mortality were significantly higher in sonicated extracts compared to unsonicated extracts.

Key words: cyanobacteria, extracts, heterocystous, non heterocystous, immobility, mortality. *Meloidogyne incognita*

Cyanobacteria are photosynthetic prokaryotic organisms that are cosmopolitan in habitat and constitute a substantial biomass in the soil. Heterocystous cyanobacteria have been valued for their ability to fix atmospheric nitrogen, bind and enrich soil and are employed as biofertilizers (Venkataraman, 1972; Das, 1991). Both heterocystous and non heterocystous forms are reported to produce a large number of metabolites which have been identified as toxins (microcystins, nodularins, neurotoxins), antibiotics, and protease inhibitors. Majority of the known toxins are peptides, alkaloids or non ribosomally synthesized molecules (Namikoshi & Rinehart, 1996); some have been chemically characterized as neurotoxins causing inhibition of acetylcholine esterase and/or functions like acetylcholine (Carmichael, 1994). Few investigations have been carried out on cyanobacteria to evaluate their bionematicidal potential (Dhanam *et al*., 1994; Gaur, 1995; Khan *et al*., 1997; Sharma *et al*., 2007; Sharma & Gaur, 2008). A large number of cyanobacterial species remain to be examined for their nematicidal effects. In the present study, ten species of terrestrial heterocystous and non heterocystous cyanobacteria were evaluated for their bionematicidal potential against root-knot nematode, *Meloidogyne incognita.*

MATERIALS AND METHODS

Culture of cyanobacteria (Blue green algae)

The heterocystous cyanobacteria viz; *Hapalosiphon luteolus, Anabaena variabilis*, *Calothrix brevissima*, *Nostoc punctiforme*, *Tolypothrix nodosa*, *Scytonema bohmeri* and non heterocystous cyanobacteria viz; *Synechococcus nidulans*, *Phormidium tenue*, *Microchaete tenera* were obtained from Centre for Conservation and Utilisation of Blue Green Algae (CCUBGA), I.A.R.I., New Delhi. The non heterocystous cyanobacterium, *Microcoleus vaginatus* was obtained from Department of Botany, University of Allahabad, Allahabad. The cyanobacteria were grown axenically in 1000 ml of in BG-11 (N^-) medium for heterocystous species and BG-11(N+) medium (Stanier *et al*., 1971) for non heterocystous species in 3 l Haffkin flasks using cotton plugs to allow aeration. The medium was sterilised at 121æ%C for 20 min at 103421.25 Pa pressure followed

by inoculation with 10 ml of fresh cyanobacterial culture and incubated in a culture room at 4000 lux with a 16 h:8 h light/dark period at 28 ± 2æ%C for 14 days. The flasks were manually shaken each day to aerate the culture.

Preparation of extracts of sonicated cyanobacteria

The sonication of microbial cells in suspension with high frequency sound waves results in their inactivation and disruption. One gram fresh weight of cyanobacterial biomass obtained from 14-day-old culture flasks was concentrated by centrifugation (1008 *g*, 10 min) and was subjected to sonication in 2 ml Eppendorf tubes, using 1 ml sterile distilled water. Sonication was carried out intermittently (0.5 sec) using labsonic (B. Braun Biotech International, Melsungen, Germany) at amplitude of 65 Hz, four times for 7 min, at an interval of 7 min. The tubes were maintained at low temperature by placing them in compressed ice to avoid degeneration of the toxin during sonication. Sonicated cells were centrifuged again at 1008 *g* for 10 min, the supernatants were collected and the volume was made to 10 ml with sterile distilled water. The sonicated extract so obtained was used for nematicidal assays.

Preparation of extracts of unsonicated cyanobacteria

The cyanobacterial cultures were centrifuged at 1008 *g* for 10 min. The supernatant was filtered through Whatman No. 1 filter paper. The filtrate was used as a unsonicated extract for nematicidal assays

***In vitro* nematicidal activity**

The cultures of all 10 cyanobacterial species were harvested at 14 days and tested for their nematicidal efficacy with and without sonication against *M. incognita* J2s. Freshly hatched J2s of *M. incognita*, was obtained from eggmasses extracted from tomato plant pot cultures maintained in the glasshouse of the Division of Nematology, I.A.R.I., New Delhi. At least 100 nematodes in 0.5 ml sterile distilled water were poured into each well of the multiwell plate containing 1 ml cyanobacterial extract, distilled water (control) and medium (control). Three replications were maintained for each cyanobacterial species and for each treatment. The immobility of nematodes was checked by probing the tail tip with a nematode pick and the immobile nematodes were counted after 24, 48 and 72 h. Further, the extracts of each exposure period were replaced with distilled water, rinsing five times. After the final rinse, nematodes were kept in distilled water for 24 h to observe if nematode activity resumed. The numbers of active and inactive nematodes were counted to calculate the percent mortality.The total chlorophyll and total soluble proteins of 10 cyanobacterial species were also estimated in 14-day-old cultures (Mckinney, 1941; Lowry *et al*., 1951; Herbert *et al*., 1971)

Statistical analysis

The data on immobility and mortality were analysed by two factorial analysis of variance (ANOVA) and the chlorophyll and total soluble protein by a single factor ANOVA (Gomez & Gomez, 1984). The data on percent immobility and mortality were arc sine transformed before analysis. The critical difference (CD) values at 0.05 P were used to determine the significance of treatment mean differences.

RESULTS AND DISCUSSION

The unsonicated extracts of all the ten cyanobacteria caused an average immobility in the range of 1.3-39.1% within 72h. *M. vaginatus*, *S. bohmeri* and *P. tenue* caused significantly higher percent immobility of 22.9, 21.5 and 19.4, respectively, compared to *M. tenera* (17.4) and *T. nodosa* (12.5). The percent immobility due to *S. nidulans* (11.5) was at par with *H. luteolus* (9.7) and *A. variabilis* (9.5) but significantly higher than that in *N. punctiforme* (5.3) or *C. brevissima* (4.8) compared to 1.1 in the medium and 0.9 in the distilled water (Table 1). The average per cent mortality in the unsonicated extracts ranged from 1.5–5.6; the minimum for *C. brevissima* and maximum for *S. bohmeri* (Table 2). The differences in per cent mortality were non-significant between all the treatments except for *S. bohmeri* which showed significantly higher mortality than other extracts without sonication.

Sonication significantly increased the toxic effect of cyanobacteria on the nematodes. The sonicated extracts of all the ten cyanobacteria caused an average immobility

Table 1. Effect of extracts from unsonicated heterocystous and non heterocystous cyanobacteria and exposure time on per cent immobility of *Meloidogyne incognita* (J2)

Treatments	% immobility of *M. incognita* (J2) after			Mean
	24h	48h	72h	
Heterocystous cyanobactera				
Anabaena variabilis	3.0(9.9)	8.3(16.5)	17.3(24.6)	**9.5(17.0)**
Calothrix brevissima	2.3(8.7)	3.2(10.1)	8.9(16.5)	**4.8(11.8)**
Hapalosiphon luteolus	3.7(11.1)	8.7(17.0)	16.7(24.0)	**9.7(17.4)**
Nostoc punctiforme	1.5(6.7)	7.0(15.2)	7.5(15.8)	**5.3(12.6)**
Scytonema bohmeri	20.5(26.7)	21.7(27.6)	22.4(28.1)	**21.5(27.5)**
Tolypothrix nodosa	5.5(13.3)	6.1(14.0)	25.8(30.2)	**12.5(19.2)**
Non heterocystous cyanobacteria				
Microchaete tenera	1.3(6.3)	11.8(19.7)	39.1(38.6)	**17.4(21.6)**
Microcoleus vaginatus	11.2(19.4)	27.9(31.7)	29.7(32.9)	**22.9(28.0)**
Phormidium tenue	16.6(23.5)	18.0(25.1)	23.5(29.0)	**19.4(25.9)**
Synechococcus nidulans	4.0(11.4)	11.5(19.7)	18.9(25.4)	**11.5(18.8)**
Control				
Medium	0.3(1.7)	1.4(5.5)	1.6(7.3)	**1.1(4.9)**
Water	1.1(4.9)	0.9(4.4)	0.7(3.9)	**0.9(4.4)**
Mean	**5.9(12.0)**	**10.6(17.2)**	**17.7(23.0)**	
CD (0.05P)				
(treatment)	**(3.6)**			
(time)	**(1.8)**			
(treatment x time)	**(6.2)**			

*Figures in parentheses are arc sine transformed values. Average of 3 replications.

Table 2. Effect of extracts from unsonicated heterocystous and non heterocystous cyanobacteria and exposure time on per cent mortality of *Meloidogyne incognita* J2

Treatments	% mortality of *M. incognita* (J2) after			Mean
	24h	48h	72h	
Heterocystous cyanobactera				
Anabaena variabilis	1.6(5.8)	2.8(9.4)	3.9(11.4)	**2.8(8.9)**
Calothrix brevissima	1.0(4.6)	1.4(5.4)	2.3(8.5)	**1.5(6.2)**
Hapalosiphon luteolus	0.5(2.4)	1.5(5.6)	2.7(9.1)	**1.6(5.7)**
Nostoc punctiforme	1.4(5.1)	1.4(6.8)	5.4(10.6)	**2.7(7.6)**
Scytonema bohmeri	3.7(11.1)	4.4(11.4)	8.5(16.9)	**5.6(13.1)**
Tolypothrix nodosa	1.9(6.4)	3.3(8.0)	3.9(10.6)	**3.0(8.3)**
Non heterocystous cyanobacteria				
Microchaete tenera	2.1(8.0)	3.4(10.2)	5.9(13.9)	**3.8(10.7)**
Microcoleus vaginatus	2.0(6.7)	2.4(8.9)	3.8(10.0)	**2.7(8.5)**
Phormidium tenue	2.7(9.3)	3.9(11.1)	7.0(15.2)	**4.5(11.9)**
Synechococcus nidulans	1.8(7.5)	2.7(9.3)	4.8(12.5)	**3.1(9.8)**
Control				
Medium	1.0(4.6)	0.0(0.0)	0.7(3.9)	**0.6(2.9)**
Water	0.0(0.0)	0.0(0.0)	0.4(2.1)	**0.1(0.7)**
Mean	**1.6(6.0)**	**2.3(7.2)**	**4.1(10.4)**	
CD (0.05P)				
(treatment)	**(3.9)**			
(time)	**(1.9)**			
(treatment x time)	**N.S**			

*Figures in parentheses are arc sine transformed values. Average of 3 replications.

in the range of 81.3-96.6% and occurred within 24 as exposed J2 were immobile within this time. After 72 h, immobility increased in the range of 91.5-98.3% in sonicated extracts compared to 1.6% in medium and 0.7% in water (Table 3). It appears from the observations that the cyanobacteria like *M. vaginatus*, *S. bohmeri* and *P. tenue* which caused significant immobility in J2s of *M. incognita* even without sonication, released their toxic metabolites in the medium. Other cyanobacteria like, *S. nidulans* do not appear to release sufficient amount of their toxic metabolites in the medium as their unsonicated extracts caused significantly lesser immobility compared to *M. vaginatus*, *S. bohmeri* and *P. tenue*. It is only after the breakdown of the cells that the nematicidal components are released in *S. nidulans* extract, as sonication significantly enhanced the per cent mortality of *M. incognita* juveniles (Dutta *et al*., 2007; Holajjer *et al*., 2012).

Significant differences in mortality were also observed among the cyanobacterial species. The highest mean mortality (29.3%) was observed in *S. nidulans* followed by *P. tenue* (27.8%), *M. tenera* (25.8%), *T. nodosa* (22.6%), *M. vaginatus* (21.3%) and *S. bohmeri* (17.5%). However, in some cyanobacteria, the per cent immobility was reversible or temporary. This was observed in *A. variabilis*, *H. luteolus, C. brevissima* and *N. punctiforme* where the per cent immobility was 96.5, 95.7, 92.8 and 95.7 but the mortality values were 5.6, 4.2, 5.1 and 6.5%, respectively.

Table 3. Effect of extracts from sonicated heterocystous and non heterocystous cyanobacteria and exposure time on per cent immobility of *Meloidogyne incognita* (J2)

Treatments	% immobility of *M. incognita* (J2) after			Mean
	24h	48h	72h	
Heterocystous cyanobactera				
Anabaena variabilis	96.6(80.0)	94.6(76.6)	98.3(82.9)	**96.5(79.8)**
Calothrix brevissima	90.8(72.6)	92.9(76.0)	94.6(77.7)	**92.8(75.4)**
Hapalosiphon luteolus	93.9(76.2)	95.2(77.6)	98.0(82.2)	**95.7(78.7)**
Nostoc punctiforme	94.8(77.1)	96.1(79.0)	96.1(78.8)	**95.7(78.3)**
Scytonema bohmeri	90.9(72.4)	91.4(73.4)	93.4(75.1)	**91.9(73.6)**
Tolypothrix nodosa	86.6(69.4)	87.9(69.8)	91.5(73.6)	**88.7(70.9)**
Non heterocystous cyanobacteria				
Microchaete tenera	86.3(68.4)	91.8(73.7)	92.5(74.1)	**90.2(72.1)**
Microcoleus vaginatus	83.5(66.8)	86.3(69.9)	94.9(77.5)	**88.2(71.4)**
Phormidium tenue	81.3(64.9)	89.5(71.2)	94.6(76.9)	**88.5(71.0)**
Synechococcus nidulans	94.9(77.6)	93.1(76.1)	94.6(77.5)	**94.2(77.0)**
Control				
Medium	0.3(1.7)	1.4(5.5)	1.6(7.3)	**1.1(4.9)**
Water	1.1(5.0)	0.9(4.4)	0.7(3.9)	**0.9(4.4)**
Mean	**75.1(61.0)**	**76.8(62.8)**	**79.2(65.6)**	
CD (0.05P)				
(treatment)	**(4.8)**			
(time)	**(2.4)**			
(treatment x time)	**N.S.**			

*Figures in parentheses are arc sine transformed values. Average of 3 replications.

Table 4. Effect of extracts from sonicated heterocystous and non heterocystous cyanobacteria and exposure time on per cent mortality of *Meloidogyne incognita* (J2)

Treatments	% mortality of *M. incognita* (J2) after			Mean
	24h	48h	72h	
Heterocystous cyanobactera				
Anabaena variabilis	3.3(10.3)	6.3(14.5)	7.1(15.4)	**5.6(13.4)**
Calothrix brevissima	3.6(10.8)	4.0(11.4)	7.7(15.6)	**5.1(12.6)**
Hapalosiphon luteolus	1.6(6.0)	2.0(8.1)	9.0(17.4)	**4.2(10.1)**
Nostoc punctiforme	4.9(12.5)	5.5(13.6)	9.1(17.0)	**6.5(14.3)**
Scytonema bohmeri	16.2(23.7)	17.0(24.3)	19.1(25.7)	**17.5(24.5)**
Tolypothrix nodosa	20.5(26.8)	21.6(27.7)	24.7(29.6)	**22.6(28.0)**
Non heterocystous cyanobacteria				
Microchaete tenera	22.0(27.8)	26.4(30.9)	29.1(32.6)	**25.8(30.4)**
Microcoleus vaginatus	13.0(21.0)	17.8(24.8)	33.3(35.1)	**21.3(27.0)**
Phormidium tenue	24.1(29.2)	26.3(30.7)	30.0(33.1)	**26.8(31.0)**
Synechococcus nidulans	13.3(21.1)	32.2(34.5)	42.4(40.6)	**29.3(32.0)**
Control				
Medium	1.0(4.6)	0.0(0.0)	0.7(3.9)	**0.6(2.8)**
Water	0.0(0.0)	0.0(0.0)	0.4(2.1)	**0.1(0.7)**
Mean	**10.6(16.4)**	**13.3(18.4)**	**17.7(22.3)**	
CD (0.05P)				
(treatment)	**(3.1)**			
(time)	**(1.6)**			
(treatment x time)	**(5.4)**			

*Figures in parentheses are arc sine transformed values. Average of 3 replications.

An attempt was made to compare the mortality of juveniles with the protein and chlorophyll content of the cyanobacteria. The data on total chlorophyll (µg/g fresh weight) and total soluble protein (mg/g fresh weight) are given in Table 5. The highest chlorophyll content was observed in *H. luteolus* (273.6 µg/g), which was at par with that in *S. nidulans* (268.8 µg/g) at CD(0.05P)=21.5. The highest protein content (48.8 mg/g) was observed in *N. punctiforme,* which was at par with that in *H. luteolus* (47.2 mg/g), which were significantly higher than that in *S. nidulans* (18.4mg/g). Thus, both chlorophyll and protein content could not be related to the toxic effects of the cyanobacteria. .

The nematicidal culture filtrates or cell extracts from cyanobacteria can be formulated using suitable carriers for seed or soil treatment as protectants against plant parasitic nematodes. The nematicidal effect of *S.*

Table 5. Chlorophyll and total soluble protein content of heterocystous and non heterocystous cyanobacteria

Treatment	Chlorophyll (µg/g fresh weight)	Total soluble protein(mg/g fresh weight)
Heterocystous cyanobacteria		
Anabaena variabilis	214.2	41.2
Calothrix brevissima	171.7	16.1
Hapalosiphon luteolus	273.6	47.2
Nostoc punctiforme	202.5	48.8
Scytonema bohmeri	128.7	38.2
Tolypothrix nodosa	231.8	7.2
Non heterocystous cyanobacteria		
Microchaete tenera	31.6	20.1
Microcoleus vaginatus	199.5	30.8
Phormidium tenue	69.7	18.5
Synechococcus nidulans	268.8	18.4
CD (0.05P)	**21.5**	**5.2**

nidulans using formulations has been investigated in microplot experiments against *M. incognita,* infecting brinjal, wherein significant reduction in nematode multiplication and root galling was observed (Holajjer *et al*., 2010).

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In vitro Production of *Steinernema carpocapsae* in Different Artificial Media

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ABSTRACT: The successful implementation of entomopathogenic nematodes (EPN) in the families *Steinernematidae* and *Heterorhabditidae* against soil inhabiting insects is due to their ease of culture. Mass multiplication of *S. carpocapsae* was studied (*in vitro*) on different artificial media *i.e.*, Wout's, Modified egg yolk and Egg yolk media. Out of these highest (25.88) lakhs IJs were obtained from Wout's media followed by modified egg yolk (20.78) lakhs and Egg yolk (0.91) lakhs media. It was observed that *S. carpocapsae* multiplied on both animals as well as plant protein based media.

Key words: Entomopathogenic nematodes, *Steinernema carpocapsae*, *In vitro*, artificial media, mass production.

Rhabditid nematodes of the families *Heterorhabditidae* and *Steinernematidae* are potential biocontrol agents of various insect pests, (Poinar, 1979; Klein, 1990; Kaya *et al.*, 1993). They provide some of the most promising new alternatives to chemical insecticides and are non-polluting, non toxic and environmentally friendly with comparative safety to non-target and beneficial organisms. Although these nematodes are easily produced *in vitro* on various complex semi-solid organic media. The successful implementation of entomopathogenic nematodes (EPN) in the families *Steinernematidae* and *Heterorhabditidae* against soil inhabiting insects is due to their ease of culture (Georgis, 1990). The associated bacteria convert a wide range of substrates into media suitable for nematode development and the nematode-bacterium complex is culturable on a variety of non-restrictable artificial media (Bedding, 1981; Friedman, 1990). Mass production at low cost is a pre requisite for the use of EPN. *In vitro* methods using artificial media come handy for mass production of IJs in large numbers. Protein and fatty acids play a vital role in the progeny production of EPN.

MATERIALS AND METHODS

The ingredients were mixed together according to different media composition with polyether polyurethane sponges (1.5 cm^3). The flasks were filled with foam chips medium mixture (1.5 g of foam chips: 8-9 g of medium, w/w) and plugged tightly with cotton. The flasks were autoclaved for 20 min. at 121°C and allowed cooling at room temperature before inoculation with infective juveniles (IJs) fresh IJs extracted from the infected insect cadavers were used.

The nematodes were inoculated aseptically @ 1000 IJs/flask. Care was taken not to shake the flasks after the inoculation of nematodes. The sealed flasks were incubated at 28°C for 30 days. Colonies of the nematodes were observed on the walls of the flasks after 20 days post inoculation. The harvesting of the nematodes was done after 30 days. The nematode yield from each treatment medium harvested was expressed in terms of number of IJs/flask.

Wout's medium: Nutrient-broth 0.88 g; Yeast-extract 0.32 g; Groundnut-oil 10.40 g; Soy flour 14.40 g; Distilled-water 60 ml.

Egg yolk medium: Solid egg yolk 7.00 g; Yeast-extract 2.00 g; Sodium chloride 0.80 g; Groundnut oil 15.00 g; Distilled-water 60 ml.

Modified egg yolk medium: Egg yolk 7.00 g; Soy flour 20.00 g; Yeast-extract 2.00 g; Sodium chloride 0.80 g; Groundnut oil 15.00 g; Distilled-water 60 ml. The observation was taken after 30 days of inoculation.

RESULTS AND DISCUSSION

The mass multiplication (*in vitro*) of *S. carpocapsae* was done on different artificial media. The infective juveniles of *S. carpocapsae* were inoculated @ 1000 IJs/ 250ml flask. It was observed that IJs multiplied both on plant and animal protein media. Out of these media Wout's media was found better as compared to other media. The maximum number (in lakh) of *S. carpocapsae* obtained in Wout's media (25.88) lakhs followed by Modified egg yolk (20.83) lakhs and Egg yolk media (0.91) lakhs. Similar studies in this regard were made by Hussaini *et al.* (2000) who reported maximum yield (30.58×10^5) of *S. carpocapsae* in Wout's media as compared to other media. Yadav (2006) also studied the mass multiplication of *S. carpocapsae* on artificial media, and recorded maximum (9.37×10^5) yield from Wout's media. However, Vyas *et al.* (2001) tested 21 animals and plant protein based media for the mass production of *S. carpocapsae* and recorded poor yield of *Steinernema* sp. in plant protein as compared to animal protein based media. Hussaini (2007) tested Wout's medium for *in vitro* mass multiplication of indigenous isolate of *S. carpocapsae* was obtained in medium supplemented with better Soya and Sunflower maximum 64.6×10^5 and 48.9×10^5 IJs/250 ml flask. Similar studies were made on *in vitro* mass multiplication and estimation of cost production of *Steinernema seemae* Ali *et al.* (2008) which cost Rs. 2.20 to get 60×10^5 IJs. Vyas *et al* (2010) worked out *in vitro* production cost of *S. glaseri* for 1 million IJs of *S. glaseri* for Rs. 0.25 in egg yolk and Rs. 0.72 in dog food biscuit media. As for as economics of mass production of infective juveniles is concerned out of these artificial media were taken, out of which Wout's media was found more economical than modified egg yolk and egg yolk media in respect to cost involved and production of IJs/250 ml flask. The Wout's media was synthesized using locally available ingredients such as nutrient broth 0.88 g, yeast extract 0.32 g, groudnut oil 10.40 g, soyflour 14.40 g and distilled water 60 ml which has costed Rs. 12.20 to get 25.88 lakh IJs of *S. carpocapsae* as against modified egg yolk and egg yolk media costed 16.20 and Rs. 18.50 to obtain 20.78 and 0.91 lakh IJs respectively.

Economics of *in vitro* mass production of *S. carpocapsae* on different artificial media

Media	Composition production cost/ 250 ml flask(Rupees)
Wout's	Rs. 12.20 (25.88 lakh IJs)
Egg yolk	Rs. 16.80 (20.78 lakh IJs)
Modified egg yolk	Rs. 14.20 (0.91 lakh IJs)

Table 1: Yield of *Steinernema carpocapsae* obtained on different artificial media (30 DAI)

Treatments	Yield of *S. carpocapsae* (in lakhs)		
	2010-11	2011-12	Pooled Mean
T_1 = Wout's medium (1000 IJs/250 ml flask)	25.75	26.00	25.88
T_2 = Modified egg yolk medium (1000 IJs/250 ml flask)	21.55	20.80	20.78
T_3 = Egg yolk medium (1000 IJs/250 ml flask)	0.81	1.00	0.91
SEm±	0.49	0.50	0.35
CD at 5%	1.56	1.60	1.04

(Treatments of 4 replications)

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Effect of Methanol Neem Extracts on Mortality of *Meloidogyne incognita* Juvenile

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ABSTRACT: Root-knot nematode *Meloidogyne incognita* is regarded as one of the obstacles in the agriculturel productivity. Natural products are used as safer alternative to control root knot nematode. An experiment was carried out in vitro to test the neem plant parts methanol extract. Different concentration of shade-dried leaves, bark and seeds of neem were tested for mortality of *Meloidogyne incognita* juveniles. Increase in concentration and exposure period resulted in increased mortality rate of Juveniles. Methanol neem seed (5 ml concentration) proved to be the most effective among used plant parts viz. bark, leaves, and seed. Neem bark was the least effective among the three.

Key words: *M. incognita*, Methanol, Plant extract, Mortality

The root-knot nematode, *Meloidogyne incognita* is a serious problem to the cultivation of agricultural crop through out the world. It causes severe damage and yield loss to a wide group of vegetable crop in all parts of India (Khan & Khan, 1996). Almost all the chemical nematicides are expensive and create environmental problems. Modern agriculture moves to adopt more environment friendly practices. There is increasing interest in the use of biopesticides that are pest specific and non toxic to human and beneficial organisms. Extract of plant product contain nematicidal compound. Thousand of plants possessing insecticidal properties are known today (Banerji et al. 1985). Use of neem and neem product has been advocated for the management of root-knot nematode by many workers (Dekha & Rehman, 1998). Therefore the present investigation was known about the nematicidal effect of neem plant parts as methanol extract on mortality of *M. incognita* juveniles.

MATERIALS AND METHODS

Preparation of Extracts: The Neem (*Azadirachta indica)* plant parts viz. neem bark, neem leaves, neem seeds were shade dried and then oven dry at 60°C for overnight, powder of plant parts were prepared by using a mixer or blender. Five gm powder of neem bark, neem leaves and neem seeds were soaked in 50 ml methanol for 7-9 days for evapouratation after that it was washed with distilled water and absolute organic solvents. Extract were filtered through four ply muslin cloth and then passed through Whatman filter paper no.1 filtered extract were make up 50 ml with required amount of distilled water and then centrifuged at 4000 rpm for 10 minutes then again filtered through Whatman filter paper no.1. The extract so obtained was put in conical flasks and steam sterilized in an autoclave. The plant part extract viz. neem bark, neem leaves and neem seed designated as stock solution for preparing different dilution. The sterilized distilled water served as control.

Effect of methanol extracts on juvenile mortality of *M. incognita*: Seven dilutions viz. (5 ml, 2.5ml, 2 ml, 1.5 ml, 1 ml, 0.50 ml, 0.25 ml) were prepared from stock solution. Five ml of each plant parts methanol extracts were prepared by adding the required amount of distilled water were taken in sterile beaker of 50 ml capacity. Two hundred freshly hatched second stage juveniles J2s of *M. incognita* were transferred in beaker. Equal

number of J2s was also transferred to separate beaker containing sterilized water to serve as control. Three replicated were taken completely randomized design (CRD). Observations of both live and dead nematodes were made at 24, 48, 72, 96 and 120 h after inoculation.

RESULTS AND DISCUSSION

Result present in table (1, 2 & 3) revealed the maximum mortality was recorded in 5ml concentration which increased with increase in methanol extract con. more number or percentage of juveniles mortality with increase in exposure period too. Minimum mortality per cent was observed in neem bark methanol extract (Table 1) there was 81 percent mortality at its highest 5ml con. and 24 h exposure period. Only 24 percent mortality recorded at its lowest concentration (0.25ml) after 120 hrs of exposure period. Highest concentration of neem leaf extract (5ml) revealed that cent percent mortality after 48 h of exposure period and 41.66 percent mortality recorded at it lowest concentration (0.25ml) after 120 h of exposure period. Neem seed methanol extract on the other hand was found to be the most effective in juvenile's mortality (Table 3), the highest concentration (5ml) were found to be most effective caused cent percent mortality from 24 h exposure and its lowest concentration had only 54% mortality after 120 h of exposure period.

Cumulative percent mortality of *M. incognita* was maximum in neem seed extract and minimum in neem bark extract which could be due to the fact that Neem bark does not possess sufficient active ingredient which are nematicidal. Neem leaves and Neem seed having more concentrated nematicidal/nematostatic properties. The present investigation in adjustable conformity with the finding of Vijayalaxmi *et al*. (1979) found leaf extract of *A. indica* caused increased Mortality of *M. incognita* juvenile. These results also agree with the Joymatidevi (2010) on effect of ten different medicinal plants methanol extract against second stage juveniles of *M. incognita*. Haseeb *et al*. (2007) reported that *A. indica* seeds powder were found effective against *M. incognita*. Joymatidevi (2008) tested methanol extracts in laboratory condition and found *A. indica* and *Melia azaderach* show highest larval mortality. Neem producte (Neem Kernel, Neem leaf, Neem bark extract) are known to posses nematicidal activity against nematode population (Zaki & Bhatti, 1989, Derkar *et al*., 1990). The present study also revealed that the mortality rate was increased with increased expose period and concentration as reported by Siddiqui & Alam (1988). Singh & Sitaramaiah

Table 1. Effect of Neem Bark Extract (Methanol) on juveniles Mortality of *Meloidogyne incognita* at different time intervals

(Observations are mean of three replicates)

Extract Con.	% Mortality at different exposure period				
(ml)	24 h	48 h	72 h	96 h	120 h
0.25	4.000	12.333	18.000	22.000	24.000
0.50	5.667	17.333	21.000	28.000	35.667
1.0	13.667	19.667	39.667	48.667	55.333
1.5	21.000	36.000	53.000	61.333	69.333
2.0	32.333	41.667	60.333	64.667	75.333
2.5	27.000	55.333	66.333	78.000	81.667
5.0	81.000	87.333	91.000	93.000	94.667
Control	0.000	0.000	0.000	0.000	0.000
SEM ±	5.923	1.258	2.309	1.080	1.354
C.D. (P=0.05)	13.659	2.902	5.325	2.491	3.122

Table 2. Effect of Neem Leaf Extract (Methanol) on juveniles Mortality of *Meloidogyne incognita* at different time intervals (Observations are mean of three replicates)

Extract Con.	% Mortality at different exposure period				
(ml)	24 h	48 h	72 h	96 h	120 h
0.25	3.000	15.000	24.667	32.000	41.667
0.50	8.000	21.667	30.667	39.000	52.000
1.0	14.333	31.667	43.333	52.667	64.000
1.5	27.000	44.667	55.667	63.333	73.333
2.0	49.333	57.000	73.000	84.000	90.667
2.5	75.333	85.667	92.667	99.000	100.000
5.0	98.333	96.667	100.000	100.000	100.000
Control	0.000	0.000	0.000	0.000	0.000
SEM ±	1.236	2.055	1.724	1.280	1.633
C.D. (P=0.05)	2.850	4.738	3.976	2.952	3.766

Table 3. Effect of Neem Seed Extract (Methanol) on juveniles Mortality of *Meloidogyne incognita* at different time intervals (Observations are mean of three replicates)

Extract Con.	% Mortality at different exposure period				
(ml)	24 h	48 h	72 h	96 h	120 h
0.25	9.000	20.333	38.000	46.667	54.000
0.50	18.000	30.667	47.333	52.000	69.667
1.0	30.333	42.333	55.000	66.000	78.333
1.5	43.000	58.667	72.000	78.333	87.667
2.0	74.000	81.667	86.333	92.667	100.000
2.5	83.333	97.333	100.000	100.000	100.000
5.0	100.000	100.000	100.000	100.000	100.000
Control	0.000	0.000	0.000	0.000	0.000
SEM ±	1.143	1.528	1.247	1.354	1.190
C.D. (P=0.05)	2.635	3.522	2.876	3.122	2.745

(1973) and Khan et.al. (1974) reported that the nematostatic and nematicidal properties of Neem products might be due to azadirachtin as in active principles and toxic chemicals present in them, which could have nematicidal/nematostatic effect. It was responsible for mortality of *M. incognita* juveniles. It is therefore concluded that the incorporation of plant product like neem could provide a safer, suitable and cheaper alternative to root-knot nematode *M. incognita*.

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A Check-List of Globally Known Species of *Tylenchorhynchus* Cobb, 1913 along with Compendium of the Indian Species

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ABSTRACT: Stunt nematodes of the genus *Tylenchorhynchus* are widely distributed in association with almost all the field and horticultural crops, and about 158 species are known globally, of which 60 species have been described from India. This paper provides a checklist of all the known species of *Tylenchorhynchus*, along with compendium of the nominal Indian species and other recorded species of genus from India.

Keywords: Checklist, compendium, crop host, locality, morphometrics, Species, Stunt nematodes, synonyms, taxonomy, India.

The soil dwelling stunt nematodes, earlier characterized under one genus *Tylenchorhynchus* are now split into eight more genera namely, *Bitylenchus, Dolichorhynchus, Quinisulcius, Merlinius, Amplimerlinius, Triversus* and *Trilineelus*. Most of the information, however, on their association with diseased conditions of plants is under the former complex genus, *Tylenchorhynchus.* Members of these genera possess similar anatomy and may be easily mistaken for one another.

The cosmopolitan genus *Tylenchorhynchus* was established by Cobb (1913) for *T. cylindricus* found in southern California. Allen (1955) enhanced taxonomic criteria for *Tylenchorhynchus*. Tarjan (1973) gave a synopsis, key, and diagnostic data of the genera and species in the Subfamily Tylenchorhynchinae. The history of the genus was discussed by Hooper (1978), Golden *et al.*, (1987), and Anderson and Potter (1991). Siddiqi (1986 & 2000) recognized 71 and 104 total species, respectively as being in *Tylenchorhynchus*. Fortuner and Luc (1987), in their reappraisal of Tylenchina, included *Tylenchorhynchus* under the family Telotylenchidae, subfamily Telotylenchinae, and recognized 129 valid species, defining *Tylenchorhynchus* as having 2 to 5 lines in the lateral field, which is sometimes areolated. Mahajan (1988) gave a diagnostic compendium to species of *Tylenchorhynchus* and included 89 valid species in the genus. Esser (1991) listed 257 nominal species in his checklist of the genus. Brzeski and Dolinski (1998) compiled a compendium containing 177 species with 2 to 5 lines in the lateral field. The taxonomy of stunt nematodes has been advanced through scanning electron microscopy (Fortuner and Luc, 1987; Powers, 1983; Powers *et al.,* 1983; Siddiqi, 1986). Handoo (2000) prepared a key and a compendium containing morphometric and related details to facilitate easy identification of 111 valid species of *Tylenchorhynchus*. As a result of the inevitable taxonomic changes, development of a dichotomous key to *Tylenchorhynchus* spp. has become increasingly difficult. Many species previously included in *Tylenchorhynchus* have been placed in newer genera, and numerous species within related genera have been either shifted to *Tylenchorhynchus* or synonymized with other species. The most important character used in distinguishing these genera is the number of lateral lines or incisures, which can range from three to six.

In the present study, we compiled a check list of 158 globally known nominal species of *Tylenchorhynchus*, (Fig. 1), listed 47 species which are either transferred to

other genera or synonymized with other genera/ species (Siddiqi, 2000) and other species reported from India (Fig.1 & Table 1). We also present a check-list and compendium of 60 nominal Indian species of *Tylenchorhynchus* containing morphometric and related details to facilitate easy identification (Table 2 & 3).

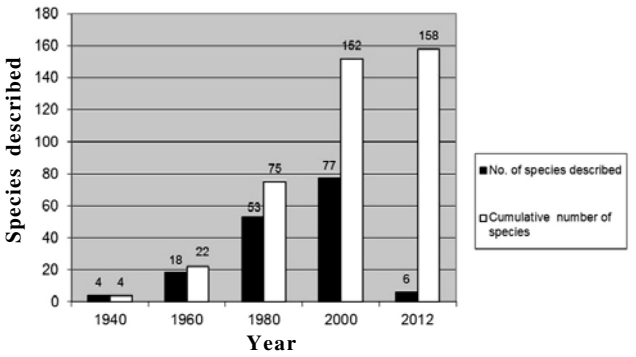

Fig. 1. *Tylenchorhynchus* species described in different decades along with the cumulative number of species

Subfamily Telotylenchinae Siddiqi, 1960

Diagnosis: Lateral fields each with three to five incisors. Amphidial aperture pore-like, labial, indistinct. Derids absent. Stylet small to moderately long (about 11-40µm), with conus about as long as shaft and distinct basal knobs. Median oesophageal bulb well developed; Oesophageal glands enclosed in a basal bulb or dorsal gland enlarged, formimg a long lobe extending over intestine. Vulva slit like, lacking epiptygma. Vagina not sclerotized. Ovaries paired except *Trophurus.* Female tail Conoid, subcylindroid or subclevate, rarely spicate, between two and six anal body widths long. Male tail conical, about as long as that of female. Bursa simple, well developed, enveloping tail. Hypoptygma absent. Spicules arcuate, with large distal flanges and pointed or indented tip. Gubernaculum rod-like, usually protrisible, distally boat shaped in lateral view, with or without crust. Type genus: *Telotylenchus* Siddiqi, 1960

Other genera: *Bitylenchus* Filipjev, 1934

Histotylenchus Siddiqi, 1971

Neodolichorhynchus Jairajpuri & Hunt, 1984

Paratrophurus Arias, 1970

Quinisulcius Siddiqi, 1971

Sauertylenchus Sher, 1974

Telotylenchoides Siddiqi, 1971

Trichotylenchus Whitehead, 1960

Trophurus Loof, 1956

Tylenchorhynchus Cobb, 1913

Uliginotylenchus Siddiqi, 1971

Genus *Tylenchorhynchus* Cobb, 1913

Diagnosis: Telotylenchinae. About 1 mm or less long. Cuticle prominently annulated may be marked by longitudinal striae, longitudinal ridges or lamellae outside lateral fields absent; not abnormally thickened on tail. Lateral field with three or four incisures, generally not areolated behind oesophageal region. Cephalic region offset from body (type genus) or continuous, annulated, or rarely smooth, without longitudinal indentations of annules; labial disc indistinct, in SEM *en face* squarish, flattened, fused with first lip annule; framework light to moderately sclerotized. Stylet well developed, generally 15-25 µm long, knobs prominent. Median bulb round or oval, with distinct refractive thickenings, usually marked off from pre-corpus and isthmus. Basal bulb offset from intestine, or base with slightly extending over intestine. Cardia prominent. Vulva median; Ovaries paired, outstretched. Female tail usually conoid with blunt tip as in type species, but may be subcylindroid, cylindroid or subclavate; terminus smooth, rarely striated or narrowing to a point. Males generally present. Tail enveloped by a large simple bursa. Spicules distally flanged. Gubernaculum well developed, about half as long as spicule, generally rod-like and with a velum, protrusible.

Table 1. A check-list of globally known species of *Tylenchorhynchus* Cobb, 1913.

S.No	Species	Author	Year	Remarks
1.	*aduncus*	de Guiran	1967	
2.	*aerolatus*	Tobar Jime´nez	1970	
3.	*agri*	Ferris	1963	
4.	*alami*	Shaw & Khan	1996	
5.	*allii*	Khurma & Mahajan	1987	(syn. of *Bitylenchus quaidi)*
6.	*amgi*	Kumar	1981	
7.	*ancorastyletus*	Ivanova	1983	
8.	*annulatus*[a]	(Cassidy, 1930) Golden	1971	
9.	*antarcticus*	Wouts & Sher	1981	
10.	*ascicaudatus*	Chang	1991	
11.	*aspericutis*	Knobloch	1975	
12.	*badliensis*	Saha & Khan	1982	
13.	*bambusi*	Singh, Lal, Rathour & Ganguly	2010	
14.	*berberidis*	Sethi & Swarup	1968	(syn. with *Nagelus hexagramus*)
15.	*bicaudatus*	Khakimov	1973	
16.	*bohrrensis*	Gupta & Uma	1980	
17.	*brassicae*	Siddiqi	1961	
18.	*brevilineatus*[a]	Williams	1960	(Transferred to *Bitylenchus*)
19.	*bryobius*	Sturhan	1966	(Transferred to *Bitylenchus*)
20.	*cacti*	Chawla, Bhamburkar, Khan & Prasad	1968	(Transferred to *Quinisulcius*)
21.	*canalis*	Thorne & Malek	1968	(Transferred to *Bitylenchus*)
22.	*chirchikensis*	Mavlyanov	1978	
23.	*chonai*	Sethi & Swarup	1968	
24.	*cicerus*	Kakat, Khan & Siddiqi	1995	
25.	*clarus*[a]	Allen	1955	
26.	*clathrocutis*	(Lewis & Golden) Fortuner & Luc	1987	
27.	*clavicaudatus*	Seinhorst	1963	(Transferred to *Bitylenchus*)
28.	*clavus*	Khan	1990	
29.	*claytoni*	Steiner	1937	
30.	*coffeae*	Siddiqi & Basir	1959	
31.	*contractus*	Loof	1964	
32.	*crassicaudatus*	Williams	1960	
33.	*cristatus*	Ivanova	1983	(Transferred to *Neodolichorhynchus*)
34.	*crotoni*	Pathak & Siddiqi	1997	
35.	*cuticaudatus*	Ray & Das	1983	(Transferred to *Bitylenchus*
36.	*cylindricus*	Cobb	1913	
37.	*cynodoni*	Kumar	1981	
38.	*dactylurus*	Das	1960	
39.	*delhiensis*	Chawla, Bhamburkar, Khan & Prasad	1968	

S.No	Species	Author	Year	Remarks
40.	*depressus*	Jairajpuri	1982	(Transferred to *Bitylenchus*
41.	*dewaeli*	Kleynhans	1992	(Transferred to *Telotylenchus*)
42.	*digittatus*	Das	1960	
43.	*dispersus*	Siddiqi & Sharma	1995	
44.	*divittatus*	Siddiqi	1961	
45.	*dubius*[a]	(Butschli, 1873) Filipjev	1936	(Transferred to *Bitylenchus*)
46.	*ebriensis*	Seinhorst	1963	
47.	*elamini*	Elbadri *et al.,*	2010	
48.	*elegans*	Siddiqi	1961	
49.	*eremicolus*	Allen	1955	
50.	*erevanicus*	Karapetjan	1979	
51.	*eroshenkoi*	Siddiqi	1986	
52.	*estherae*	Kleynhans	1992	(Transferred to *Neodolichorhynchus*)
53.	*ewingi*[a]	Hooper	1959	
54.	*fugianensis*	Chang	1990	
55.	*georgiensis*	Eliashvili	1971	
56.	*goffarti*[a]	Sturhan	1966	(Transferred to *Bitylenchus*)
57.	*goldeni*	Rashid & Singh	1982	(syn. of *T. elegans*
58.	*gossypii*	Nasira & Maqbool	1996	
59.	*graciliformis*	Siddiqi & Siddiqui	1983	
60.	*haki*	Fotedar & Mahajan	1971	
61.	*handooi*	Khan, A	2004	
62.	*hexincisus*	Jairajpuri and Baqri	1968	(Transferred to *Scutylenchus*)
63.	*hordei*	Khan	1972	
64.	*huesingi*	Paetzold	1958	
65.	*iarius*	Saha, Gaur & Lal	1998	
66.	*ibericus*	Mahajan & Nombela	1986	(Transferred to *Quinisulcius*)
67.	*impar*	Ray & Das	1983	
68.	*indicus*	(Siddiqi) Fortuner & Luc,1987	1960	(Transferred to *Quinisulcius*)
69.	*iphilus*	Minagawa	1995	(Transferred to *Bitylenchus*)
70.	*irregularis*	Wu	1969	
71.	*ismaili*	Azmi & Ahmad	1991	
72.	*kamlae*	Shaw & Khan	1996	
73.	*kangwoensis*	Geraert, Choi & Choi	1990	
74.	*karnalensis*	Saha, Singh, Lal & Kaushal	2002	
75.	*kashmirensis*	Mahajan	1974	
76.	*kegasawai*	Minagawa	1995	
77.	*kegenicus*	Litvinova	1946	
78.	*kidwaii*	Rashid & Heyns	1990	(Transferred to *Bitylenchus*)
79.	*labiatus*	(Jairajpuri, 1984) Siddiqi	1986	

S.No	Species	Author	Year	Remarks
80.	*lamilliferus*	(de Man, 1880)	1936	(Transferred to *Neodolichorhynchus*)
81.	*latus*[a]	Allen	1955	
82.	*leucaenus*	Azmi	1991	
83.	*leviterminalis*	Siddiqi, Mukherji & Dasgupta	1982	
84.	*madrasensis*	Gupta & Uma	1981	
85.	*malinus*	Lin	1992	
86.	*mangiferae*	Laqman & Khan	1986	
87.	*manubriatus*	Litvinova	1946	
88.	*marudharensis*	Lal, Mathur & Rajan	1989	(Transferred to *Telotylenchus*
89.	*mashhoodi*	Siddiqi & Basir	1959	
90.	*maximus*	Allen	1955	(Transferred to *Sauertylenchus*)
91.	*mexicanus*	Knobloch & Laughlin	1973	
92.	*microcephalus*	Siddiqi & Patel	1990	
93.	*microconus*	Siddiqi, Mukherjee & Dasgupta	1982	
94.	*minutus*	Karpetjan	1979	
95.	*musae*	Kumar	1981	
96.	*namibiensis*	Rashid & Heyns	1990	(Transferred to *Telotylenchus*)
97.	*natalensis*	Kleynhans	1984	(Transferred to *Bitylenchus*)
98.	*neoclavicaudatus*	Mathur, Sanwal & Lal	1979	
99.	*nilgiriensis*	Seshadri, Muthukrishnan & Shanmugam	1967	(syn. *Quinisulcius capitatus)*
100.	*nordiensis*	Khan & Nanjappa	1974	
101.	*novenas*	Nobbs	1989	(Transferred to *Neodolichorhynchus*)
102.	*nudus*[a]	Allen	1955	
103.	*obscurisulcatus*	Andrassy	1959	
104.	*oleraceae*	Gupta & Uma	1981	
105.	*oryzae*	Kaul & Waliullah	1995	
106.	*pachys*	Thorne & Malek,	1968	
107.	*paracanalis*	Khan	1991	
108.	*paranudus*	Phukan & Sanwal	1982	(syn. of *T. leviterminalis)*
109.	*paratriversus*	Brzeski	1991	
110.	*parvus*	Allen	1955	(Transferred to *Bitylenchus*)
111.	*paulettae*	Bloemers & Wanless,	1998	
112.	*penniseti*	Gupta & Uma	1980	(syn. of *T. elegans)*
113.	*persicus*	Sultan, Singh & Sakhuja	1991	
114.	*phallocercus*	Chang	1991	
115.	*phaseoli*	Sethi & Swarup	1968	(Transferred to *Neodolichorhynchus)*
116.	*projectus*	Khan	1990	
117.	*pruni*	Gupta & Uma	1981	
118.	*punensis*	Khan & Darekar	1979	(syn. of *T. elegans)*

S.No	Species	Author	Year	Remarks
119.	*punici*	Gupta and Uma	1980	(syn. *Quinisulcius*)
120.	*qasimi*	Ramzan, Handoo & Fayaz	2008	
121.	*quaidi*	Golden, Maqbool & Handoo	1987	(Transferred to *Bitylenchus*)
122.	*queirozi*	Monteiro & Lordello	1976	(Transferred to *Bitylenchus*)
123.	*robustus*	Thorne & Malek	1968	
124.	*rosei*	Zarina & Maqbool	1991	(syn. of *T. varicaudatus)*
125.	*sabourensis*	Shaw & Khan	1997	
126.	*sacchari*	Sivakumar & Muthukrishnan	1983	(syn. of *T. elegans)*
127.	*sanwali*	Kumar	1982	
128.	*sculptus*	Seinhorst	1963	
129.	*shimizui*	Talavera, Watanabe & Mizukubo	2001	
130.	*shivanandi*	Shaw & Khan	1992	
131.	*siccus*	Nobbs	1990	
132.	*silvaticus*	Ferris	1963	
133.	*solani*	Gupta & Uma	1982	(Transferred to *Neodolichorhynchus*
134.	*spinaceai*	Singh	1976	
135.	*striatus*	Allen	1955	
136.	*sudanensis*	(Decker, Yasin & El-Amin, 1975) Castillo, Siddiqi & Gomez-Barcina	1989	
137.	*swarupi*	Singh & Khera	1981	(Transferred to *Bitylenchus*)
138.	*tarjani*	Andrassy	1969	
139.	*tenuicaudatus*	Wouts & Sher	1981	
140.	*teeni*	Hashim	1981	(Transferred to *Bitylenchus*)
141.	*teres*	(Khan & Darekar, 1979) Siddiqi	1986	
142.	*thermophilus*	Golden, Baldwin & Mundo-Ocampo	1994	
143.	*tobari*	Sauer & Annells	1981	(Transferred to *Bitylenchus*)
144.	*triglyphus*	Seinhorst	1963	
145.	*trilineatus*	Timm	1963	
146.	*tritici*	Golden, Maqbool & Handoo	1987	
147.	*tuberosus*	Zarina & Maqbool	1994	
148.	*usmanensis*	Khurma & Mahajan	1987	(Transferred to *Bitylenchus*)
149.	*varainnus*	Mavlyanov	1978	
150.	*varicaudatus*	Singh	1971	
151.	*velatus*	Sauer & Annells	1981	(Transferred to *Sauertylenchus*)
152.	*ventrosignatus*	Tobar Jime´	1969	(Transferred to *Bitylenchus*)
153.	*vishwanathensis*	Pathak & Siddiqi	1997	
154.	*vulgaris*	Upadhyay, Swarup & Sethi	1972	(Transferred to *Bitylenchus*)
155.	*wilskii*	Kornobis	1980	(syn. of *sauertylenchus maximus*)
156.	*yugaensis*	Geraert, Choi & Choi	1990	
157.	*zambiensis*	Venditti & Noel	1995	(Transferred to *Bitylenchus*)
158.	*zeae*	Sethi & Swarup	1968	

([a] = Reported from India).

Table 2. Check list of *Tylenchorhynchus* species described from India, along with their type host, locality, Reference and Accession Number in National Nematode Collection of India (NNCI).

S.No.	Species	Author	year	Type host	Type Locality	Reference	Type accession no. in NNCI	Remarks
1	*alami*	Shaw & Khan	1996	Musa sp.	Ranchi, Bihar	*Journal of Research Birsa Agricultural University* **8 :** 1-8	-	-
2	*allii*	Khurma & Mahajan	1987	*Allium cepa*	Jalandhar , Panjab	*Indian Journal of Nematology* **17**: 202-207	-	(syn. of *Bitylenchus quaidi)*
3	*amgi*	Kumar	1981	*Coffea arabica*	Chickmaglur	*Journal of Coffee Research* **11**: 88-99	-	-
4	*badliensis*	Saha & Khan	1982	*Lycopersicon esculentum*	Rohtak	*Indian Journal of Nematology* **11**: 205-211	1192-1202	-
5	*berberidis*	Sethi and Swarup	1968	*Berberis aristata*	North -Western India	*Nematologica* **5:** 73-77	27-30	(syn. with *Nagelus hexagramus*)
6	*bambusi*	Singh, Lal, Rathour & Ganguly	2010	Bamboo	Assam	*Indian Journal of Nematology.***40** : 216-220	2291-2298	-
7	*bohrrensis*	Gupta & Uma	1980	Sugarcane	Bohrr	*Rev.Iber .Parasitology,* **40:** 423-427	-	-
8	*brassicae*	Siddiqi	1961	Cauliflower and cabbage	Aligarh	*Zeitschrift Parasitenkunde*, **21:** 46-64	-	-
9	*cacti*	Chawala, Bhamburkar, Khan & Prasad	1968	*Cactus* spp.	-	*Labdev Journal of Science and Technology.***10:** 205-210	-	(Transferred to *Quinisulcius*)
10	*chonai*	Sethi & Swarup	1968	*Bougainvillea* spp.	Western India	*Nematologica* **14**:77-88	31-34	-
11	*cicerus*	Kakar, Khan & Siddiqi	1995	Pigeon pea	Assam	*Annals of Plant Protection Science.* **3**:149-154	-	-
12	*dactylurus*	Das	1960	*Capsicum annum*	Hyderabad	*Zeitschrift fur parasitenkunde* **19**: 553-605	-	-
13	*digitatus*	Das	1960	*Lactuca sativa*	Hyderabad	*Zeitschrift fur Parasitenkunde* **19**: 553-605	-	-
14	*coffeae*	Siddiqi & Basir	1959	Coffee Plants	Mysore	*Proceedings of 46th Indian Science Congress* P.15	-	-

S.No.	Species	Author	year	Type host	Type Locality	Reference	Type accession no. in NNCI	Remarks
15	*crotoni*	Pathak & Siddiqi	1997	*Croton* sp.	Udaipur	*Indian journal Nematology* **27:** 99-103	-	-
16	*cuticaudatus*	Ray & Das	1983	Potato	Cuttack	*Indian Journal of Nematology* **13:** 16-25	-	(Transferred to *Bitylenchus*)
17	*cynodoni*	Kumar	1981	Coffee soils *Cynodon dactylon*	South India	*Journal of Coffee Research* **11:** 88-99	-	-
18	*delhiensis*	Chawla, Bhamburkar, Khan & Prasad	1968	*Anana squamosa*	Karol Bagh	*Labdev Journal of Science and Technology* **68:** 86-100	-	-
19	*elegans*	Siddiqi	1961	Grass	Aligarh	*Z. Parasitenkunde* **21:** 46-64	-	-
20	*divittatus*	Siddiqi	1961	-	Aligarh	*Z. Parasitenkunde* **21:** 46-64	-	-
21	*goldeni*	Rashid & Singh	1982	sugarcane	Lucknow	*Indian Journal of Nematology* **12:** 193-195	1572-74	(syn. *T. elegans*)
22	*haki*	Fotedar & Mahajan	1971	*Brassica oleracea*	Srinagar	*Kashmir Science*: 120	-	-
23	*hexincisus*	Jairajpuri & Baqri	1968	Citrus plants	Gujrata	*Nematologica* **14:** 217-222	-	(Transferred to *Scutylenchus*)
24	*indicus*	(Siddiqi) Fortuner & Luc,1987	1960	Grass	Aligarh	*Nematologica* **5:** 73-77	-	(Transferred to *Quinisulcius*)
25	*iarius*	Saha,Gaur & Lal	1998	Rice	IARI Farm	*Annals of Plant protection Science* **6:** 63-65	1833-1844	-
26	*impar*	Ray & Das	1983	Broad Bean	OUAT Orchard, Orissa	*Indian Journal of Nematology* **13:** 16-25	-	-
27	*ismaili*	Ajmi & Ahmad	1989	Albizta	Jhansi	*Indian Journal of Nematology* **19(2):** 279-282	-	-
28	*kamlae*	Shaw & Khan	1996	*Kenddiopspy-rosexsculpta*	Jamalpur ,Bihar	*Journal of Research.Birsa. Agriculture. University* **8:** 1-8	-	-
29	*karnalensis*	Saha, Singh, Lal & Kaushal	2002	Rice	Karnal	*Annanls of Plant Protection Science,***10(2):** 355-359	2124-2129	-
30	*Kashmirensis*	Mahajan	1974	*Brassica oleracea*	Srinagar	Proceedings of *Helminth-ological Society of Washington* **41:** 13-16	834-835	-

S.No.	Species	Author	year	Type host	Type Locality	Reference	Type accession no. in NNCI	Remarks
31	*leucaenus*	Azmi	1991	*Leucaena leucocephala*	Jhansi	*Current Nematology.***2:** 81-82	-	-
32	*madrasensis*	Gupta & Uma	1981	Nanja Herb Plant	Madras	*Helminthologica* **18:** 53-59	-	-
33	*mangiferae*	Luqman & Khan	1985	*Mangifera indica*	Meerut	*Indian Journal of Nematology* **15:** 202-208	1588-1589	-
35	*marudharensis*	Lal, Mathur & Rajan	1989	Datepalm *Phoenix dactylifera*	Bikaner ,Rajasthan	*Indian Journal of Nematology* **9:** 51-54	-	(Transferred to *Telotylenchus*)
34	*mashhoodi*	Siddiqi & Basir	1959	Sugarcane	Coimbatore	*Proc.46th Indian Science Congress.* P.15	-	-
36	*musae*	Kumar	1981	*Musa* spp	Chickmaglur	*Journal of Coffee Research* **11:** 88-89	-	-
37	*nilgiriensis*	Seshadri, Mutukrishnan & Shanmugam	1967	*Brassica oleracea*	Madras	*Current Science* **36:** 551-553	-	(syn. Q*uinisulcius capitatus)*
38	*neoclavi caudatus*	Mathur, Sanwal & Lal	1979	Imported Potato tubers	USA	*Indian Journal of Nematology* **8:** 148-150	1291-1292	-
39	*nordiensis*	Khan & Nanjappa nomen novum for *T. areolatus* Khan & Nanjappa, 1971	1974	Citrus	Panipat	*Indian Journal of Nematology* **2:** 216	-	-
40	*oleraceae*	Gupta & Uma	1981	*Brassica oleracea*	Panipat	*Revista Ibero Parasitologia* **42:**289-292	-	-
41	*oryzae*	Kaul & Walliullah	1981	*Prunus persica*	Srinagar	*Annals of Plant Protection Science* **3:** 155-157	-	-
42	*pruni*	Gupta & Uma	1995	*Prunus persica*	Srinagar	*Helminthologica* **18:** 53-59	-	-
43	*punici*	(Gupta & Uma, 1980) Fortuner & Luc	1987	*Punica granatum*	Srinagar	*Indian Academy of Science Animal. Science* **89:** 415-418		(syn. *Quinisulcius*)
44	*paranudus*	Phukan & Sanwal	1982	Areca catechu	Karim ganj assam	*Indian Journal of Nematology* **12:** 383-385	1371-1372	(syn. of *T. leviterminalis)*
45	*penniseti*	Gupta & Uma	1980	*Pennisetum typhoides*	Naurangabad , Haryana	*Indian Journal of Parasitology* **4:** 157-159	-	(syn. *T. elegans*)

S.No.	Species	Author	year	Type host	Type Locality	Reference	Type accession no. in NNCI	Remarks
46	*persicus*	Sultan,Singh & Sakhuja	1989	*Prunus persica*	Ludhiana, Panjab	*Indian Journal of Nematology* **19:** 215-222	-	-
47	*phaseoli*	Sethi & Swarup	1968	*Phaseolus aconotifolius*	Panjab	*Nematologica* **14:**77-78	35	(Transferred to *Neodoli-chorhynchus)*
48	*punensis*	Khan & Darekar	1978	*Solanum melongena*	Nasik, Maharashtra	*Indian Journal of Nematology* **8:** 43-48	942-946	(syn. of *T. elegans)*
49	*sacchari*	Sivakumar & Muthukrishnan	1983	Sugarcane	Quddalore, Tamilnadu	*Indian Journal of Nematology***12:** 393-395	1443	(syn. of *T. elegans)*
50	*sanwali*	Kumar	1982	*Brassica oleracea*	Lucknow	*Kanpur University Research* **1:** 185-192	-	-
51	*savourensis*	Shaw & Khan	1997	Blackgram	Bihar	-	-	-
52	*shivanandi*	Shaw & Khan	1992	Sugarcane	Nagaland . India	*Bulletin of Entomology* **33:** 713	-	-
53	*solani*	Gupta & Uma	1982	-	-	*Indian Journal of Parasitology* **5:** 37-38	-	(Transferred to *Neodoli-chorhynchus)*
54	*Spinaceai*	Singh	1974	*Spinacea oleracea*	Lucknow	*Indian Journal of Zootomy* **15**: 187-192	-	-
55	*Swarupi*	Singh & Khera	1981	Cauliflower	Hoogly, West Bengal	*Bulletin of Zoological Survey of India* **1:** 25-28	-	(Transferred to *Bitylenchus*)
56	*usmanensis*	Khurma & Mahajan	1987	*Cucunis melo*	Panjab	*Indian Journal of Nematology* **17:** 202-207	-	(Transferred to *Bitylenchus*)
57	*variacaudatus*	Singh	1971	Fern	Hyderabad	*Journal of Helminthology* **45:** 353-369	-	-
58	*vishwanathensis*	Pathak & Siddiqi	1996	Chandni (*Jasmium grandiflorum*	Udaipur	*Indian Journal 0f Nematology* **26(2):** 274-277	-	-
59	*vulgaris*	Upadhyay, Swarup & Sethi	1972	Zea mays	IARI,FARM	*Indian Journal of Nematology* **2:** 129-138	622-639	(Transferred to *Bitylenchus*)
60	*zeae*	Sethi & Swarup	1968	Zea mays	Sangrur, Panjab	*Nematologica* **14:** 77-88	36-37	-

Table 3. Diagnostic characters of nominal species of *Tylenchorhynchus* Cobb,1913 described from India.

Species	L (mm)	a ratio	b	c	c'	V %	Lip region	Lip annules	Frame-work Sclerotization	Stylet (µm)	Lateral incisure	Tail annules	Tail shape	Tail terminus	Spicule (µm)	Guberna-culum (µm)
aerolatus	0.55-0.68	27-34.4	5-6.3	14-17	2.4-2.7	52.7-56.6	CNT	4	LSC	11-13	4	14-19	BLROU	SMO	20	10
alami	0.61-0.70	26.3-33.3	4.75-5.64	12.5-16	3-4	54.5-57.1	CNT	3	WSC	19-21	4	25	CON	SMO	17-18	9-10
*allii**	0.54-0.68	27.1-33.9	5.3-5.8	11-16	2.7-3.6	51.9-55	OFF	6-7	MSC	15-17	4	34-47	SCYL	SMO	23-24.6	10-12.3
amgi	0.67-0.75	33-38	4.3-4.7	16-17	3.7	56-59	CNT	0	LSC	19-20	4	16-20	SCYL	SMO	-	-
badliensis	0.62-0.71	28-35	5.0-5.1	15-19	3-4	53-61	OFF	3	LSC	17-19	4	17-22	CON	SMO	20-22	10-11
*berberidis**	.72-.95	26-31	4.7-6.0	13-16	2.0-3.2	53-57	CNT	2-3	LSC	28-36	5-6	40	Bluntly ROU	SMO	23-27	12-14
bambusi	0.66-0.77	27.2-38.0	4.8-5.7	13.3-18.7	2.7-4.5	43.7-56.2	CNT	2-3	LSC	17-19	4	17-25	CLA	SMO	21-24	8-13
bohrrensis	0.61-0.75	24-30	4.7-6.0	13-18	2-2.3	53-57	CNT	2-3	LSC	15-17	4	17-21	SCYL	SMO	23-27	12-14
brassicae	0.58-0.72	26-35	5.0-6.0	14-27	1.9-3.0	52-58	OFF	4	LSC	16-17	4	18-33	CON	SMO	18-21	9-11
*cacti**	0.6-0.7	25-33	4-6	14-19	-	54-59	OFF	6	-	15-19	5	23	CON	SMO	-	-
chonai	0.56-0.66	28-32	4.3-5.8	15-17	2.5	57-62	CNT	3	-	25-28	3	14	CON	SMO	21	14
cicerus	0.5-0.6	28.7-34.0	5.1-5.9	12.5-15	-	53-58	SEM OFF	3	MSC	13-16	3	-	-	-	20-23	9-11
coffeae	0.57-0.63			15-17	3-3.3	-	-	2	-	17-20	4	19	SCYL	SMO	-	-
crotoni	0.53-0.58	26.5-31.9	4.7-5.7	14.318.1	-	55.6-57	OFF	4-5	MSC	13-18	4	Fine	CON	ANN	-	-
*cuticaudatus**	0.50-0.62	31-37	4.2-4.8	12.3-15	2.3-2.4	53-60	OFF	5-6	LSC	14-16	4	33-45	CYL	SMO	23-26	9-14
cynodoni	0.63-0.68	31-34	4.7-5.5	13-15	4.8	52-61	CNT	0-1	LSC	13-15	4	20-26	CLA	SMO	28	11
dactylurus	0.64-0.71	30.8-32.5	4.6-5.0	14.8-16	-	52-54	CNT	4	LSC	20-21	4	25	BLP-ROU	SMO	22	13
delhiensis	0.6-0.7	24-33	5-6	13-18	4	47-58	ROU	2	INC	14-16	4	29	SCYL	SMO	-	-
digitatus	0.67-0.72	24-27	4.7-5.2	14.0-16.5	-	52-73.7	CNT		INC	20	4	-	CYL	SMO	23	13
divittatus	0.55-0.77	32-38	5.4-6.4	16-19	2.8	53-55	OFF	5	INC	16-17	3	21	SCLA	SMO	17	8
elegans	0.56-0.70	25-29	4.4-5.2	14.5-16	3.0	54-55	CNT	3-4	LSC	15-17	4	20-26	SCYL	SMO	22-26	10-13
*goldeni**	0.57-0.82	21-40	4.5-5.6	13-15	-	55-59	CNT	2-3	-	16-19	4	19-36	SCYL	SMO	22-26	10-13
haki	0.55-0.63	26-33	5.0-5.9	20-27	2.5-4.0	56-59	CNT	3	LSC	16-18	4	14-20	CON	SMO	18-22	10-15
*hexincisus**	0.85-1.08	32-39	6.0-8.2	18-23	-	52-55	OFF	6	LSC	17-19	6	-	SCYL	ANN	28-31	8-9
*indicus**	0.5-0.67	24-33	4.3-5.7	11.7-16.2	-	53-57	OFF	7	-	14-16.5	4	-	-	-	22-24	11-12.5

Species	L (mm)	a ratio	b	c	c'	V %	Lip region	Lip annules	Frame-work Sclerotization	Stylet (µm)	Lateral incisure	Tail annules	Tail shape	Tail terminus	Spicule (µm)	Guberna-culum (µm)
impar	0.57-0.68	24-29	4.5-5.3	16-20	1.9-2.2	55-60	OFF	4	LSC	22-24	3	11-14	CYL	SMO	23-26	13-15
Ismaili	0.46-0.66	27-33	3.8-5.5	18-26	2.5-2.6	54-61	OFF	4-5	LSC	17-20	4	14-17	SCYL	ANN	-	-
kamlae	0.55-0.63	27.4-32.1	4.4-5.7	11.7-16.2	3-4.7	53-59	OFF	4	MSC	19-21	4	21	SCYL	SMO	22	11.7
karnalensis	0.59-0.74	24-29	4.5-5.5	12.5-15.5	2.7 – 3.2	50-55	CNT	3	LSC	16-17	4	22-28	CYL	SMO	-	-
kashmirensis	0.60-0.74	24-34	4.0-5.5	24-37	-	62-64	OFF	3	MSC	17-21	4	13-17	CON	ANN	19-21	-
leucaenus	0.55-0.56	36-37	4.6-4.7	16-22	-	55-56	OFF	4-5	LSC	17-18	4	18-21	SCYL	ANN	13.5	5
madrasensis	0.53-0.66	23-25	4.4-4.7	15	1.9-2.2	56-59	CNT	1-2	LSC	19-22	3	10-13	SHM	SMO	22-24	12
mangiferae	0.54-0.67	32-39	5.0-5.7	14-16	-	51-56	OFF	5-6	-	12-13	4	35-38	SCLA	SMO	22	7-9
mashhoodi	0.61-0.76	26-30	4.9-5.5	16-19	2.5-4.0	55-59	-	3-4	LSC	17-18	4	14-16	CYL	SMO	22-24	12-13
*marudharensis**	0.64-0.81	36-42	6.0-6.6	14-18.5	3.0-3.7	52-55	OFF	7-8	LSC	14.5-16.0	4	35-42	CON	SMO	23.5-25	11-13
musae	0.58-0.65	28.1-32.8	4.9-6.0	14.8-16.4	-	54.9-56.9-	OFF	1-2	LSC	18-19	4	16-20	SCYL	SMO	24-26	10-12
*nilgiriensis**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
neoclavic-audatus	0.59-0.72	23-31	4.0-5.8	12-15	27-36	53-56	CNT	2-3	LSC	20-23	4	32-50	CLA	SMO	23-25	11-15
nordiensis	0.55-0.68	27.4-34.4	5.0-6.3	14-17	-	52.7-56.6	CNT	4	LSC	11-13	4	14-19	ROU	SMO	20	10
oleraceae	0.48-0.68	-	-	12-16	2.2-3.2	-	OFF	4-5	-	12-15	4	38	CON	SMO	-	-
oryzae	0.43-0.61	24-29	3.6-5.1	12.8-18.7	-	53-62	OFF		LSC	20-23	4	>20	SCYL	SMO	20-24	11-12.5
pruni	0.58-068	25-30	5.0-5.9	16-22	1.7-2.6	55-62	OFF	4-5	LSC	14-17	3	17	CON	SMO	24	9
*punici**	0.65-0.76	-	-	15-18	2.1-3.2	-	OFF	6-7	-	16-17	5	38-42	CON	SMO	-	-
paranudus	0.59-0.78	25-30	4.8-5.8	12-16	2.5-3.7	51-56	OFF	O	-	18-21	4	14-25	CLA	SMO	23-27	12-14
*penneseti**	0.53-0.67	-	-	-	2.0-3.2	-	CNT	3	-	15-17	4	13-17	CYL	SMO	-	-
persicus	0.71-0.73	35-41	4.9-5.8	13-17	-	50-55	OFF	-	MSC	14-16.5	-	-	CON	BLUNT	22-24	12-14
*phaseoli**	0.61-0.77	28-35	4.0-5.4	17-20		54-58	OFF	6	LSC	19-26	4	22	CON	SMO	23.5	11.5
*punensis**	0.60-0.75	31-41	4.6-5.5	12-15	3.7-4.8	53-59	CONROU	2-3	WSC	16-18	4	24-27	CYL	SMO	20-22	9-10
*sacchari**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
sanwali	0.45-0.71	21.5-23.7	7.5-10.3	16.3-18.7	2	-	OFF	2	-	19.5	4	30-31	CYL	ANN	-	-
savourensis	0.49-0.70	30-36	4.8-5.5	13-17	3-3.5	53-58	SEMOFF	5	LSC	-	3	20	-	SMO	21-22	8-9

Species	L (mm)	a ratio	b	c	c'	V %	Lip region	Lip annules	Frame-work Sclerotization	Stylet (µm)	Lateral incisure	Tail annules	Tail shape	Tail terminus	Spicule (µm)	Guberna-culum (µm)
shivanandi	0.48-0.64	26-35	4.5-5.7	12-16	3-4	52-59	CNT	4	MSC	16-19	4	21	SCYL	SMO	-	-
*solani**	0.60-0.70-	-	-	-	-	-	-	5-6	-	16-17	4	34	CON	SMO	-	-
spinaceae	0.60-0.81	-	-	-	-	-	-	4	-	14-18	4	12-18	CYL	SMO	-	-
*swarupi**	0.42-0.54	-	-	14-15	2.9	-	OFF	5-6	-	13-15	4	-	CON	-	-	-
usmanensis	0.55-0.65	25.7-30.2	5.0-5.6	12.2-14	2.3-3.0	52.7-56	OFF	5-6	LSC	14-16	4	34-40	CON	SMO	16-24	11-11.5
varicaudatus	0.50-0.56	28-33	4.3-5.2	16-19	2.3-2.8	57-59	CNT	2	LSC	17-18	4	14-15	CON	SMO	-	-
vishwanathensis	0.53-0.67	25-26.6	4.8-5.7	9.5-28.8	-	55.4-57.8	OFF	-	MSC	15-20	4	FINE	CON	ANN	-	-
*vulgaris**	0.56-0.67	25-30	4-6	14-20	-	52-57	OFF	6-7	MSC	14-16	4	35-42	CON	SMO	22-25	13-16
zeae	0.53-0.64	26-34	4.8-6.0	14-20	2.6	57-61	CNT	4	LSC	17-20	4	16	CON	SMO	20	9

Shape of lip region: CNT= Continuous; CON = Conoid; OFF = Offset; SEM-OFF = Semi-offset; ROU = Round.
Frame-work Sclerotization: LSC= Lightly Sclerotized; MSC = Moderately Sclerotized; WSC = Weakly Sclerotized; INC = Inconspicuous.
Shape of tail: BLR = Bluntly-rounded; CLA = Clavate; CON = Conoid; CYL = Cylindrical; SCLA = Sub-clavate; SCYL = Sub-cylindrical; ROU = Rounded; BLP = Bluntly-pointed; SHM = Semi-hemispherical.
Shape of tail terminus: SMO= Smooth; ANN = Annulated.
* Transferred/ Synonymized with other genera/ species.

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Role of Organic Carbon on the Efficacy of Carbofuran Against Root-Knot Nematode on Tomato

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ABSTRACT: Investigation was carried out to study the efficacy of carbofuran (1 and 2 kg a.i/ha) with the varying levels of FYM 5,10 and 20%w/w in tomato infected with root-knot nematode under green house conditions. Results on number of root-knot galls indicated that carbofuran efficacy increased with the levels of FYM. Plant growth such as shoot wt. and shoot length improved in treatment while root wt. was decreased amongst treatments.

Key words: Root-knot nematode, tomato, FYM, carbofuran

Nematicides form an important component of nematode management, applied to soil against soil born nematodes parasitic to crops. These chemicals come in contact with soil particles as well as with organic matter present in soil. Thus, affecting the overall activity of biological entities in soil. The quality of organic matter also has implication on chemical activity. Together, soil particle do affect the chemical dynamics of the compound. The organic matter and chemical interaction phenomenon involved adsorption and desorption of chemical compound which determine the persistence and decomposition of molecule. This determine availability of chemical in soil water interphase. It is well known that there is a direct correlation of amount of organic matter and persistence of chemical compound and also amount of organic matter has inverse relation with uptake of chemical by plant. Thus, quantity of FYM plays a major role in the pesticide efficacy. Beside, it may also affect the pesticide by having good growth of soil microflora that also has bearing on pesticide. The systemic nematicides are generally adsorbed on FYM which can affect the availability of pesticide in a soil environment (Bansal, 2009, Bansal, 2010). The organic matter and pesticide interaction is well reviewed by Gabriela *et al.,* 2007) Management of root knot nematode quite often is achieved with the chemical nematicides like carbofuran. The significance of organic matter coupled with carbofuran in the management of important nematode pest like root-knot nematode infecting tomato has been investigated to study the role of organic matter for the better efficacy of carbofuran.

MATERIALS AND METHODS

An experiment was conducted to study the role of farm yard manure (FYM) on the efficacy of carbofuran against root-knot nematode, *Meloidogyne incognita* infecting tomato in 15 cm earthen pots in completely randomize design with three replication under green house conditions. Healthy seedlings of tomato cv. Pusa Ruby were raised in sterilized soil and sand mixture (1:1) in earthen pan. Sterilized sandy loam soil was filled in each pot and thirty days old seedlings were planted in pots. FYM was applied @ 0, 5, 10 and 20% w/w per pot at the time of filling the pots. Freshly hatched juveniles (J2s) were collected from infected tomato roots, maintained in the Division of Nematology, IARI. These J2s were inoculated @ 2 J2/g soil after 7 days of transplant. Subsequently, carbofuran 3G @ 1 and 2 kg a.i./ha was applied per pot 24h after nematode inoculation. Inoculated control was kept as check. Observations on plant growth parameters (such as root and shoot weight

and shoot length), nematode multiplication (number of galls and egg mass) and soil population were observed after 60 days of treatment. Soil population was assessed by washing 200 cc soil collected from the rhizosphere of the treated and control plants. The soil was processed as per the procedure by Cobb (1918). Further, reproduction factor was calculated by using formula Pf/Pi (Pf= final nematode population, Pi= Initial nematode population).

RESULTS AND DISCUSSION

Number of galls caused by root-knot nematode in tomato under different treatments indicated that efficacy of carbofuran was altered by varying dose of FYM (Table 1). Root-knot galls progressively decreased with an increase of FYM (5-20%) with carbofuran 1 kg a.i/ ha, as the nematode galls decreased from 30 to 82% over control. However, in comparison to carbofuran alone the reduction ranged between 5 to 74% clearly suggests, the role of FYM in influencing the efficacy of chemical. At higher carbofuran concentration, reduction in nematode galls was 79% which further increased to 84% at highest level of FYM over control. Similarly, in contrast to carbofuran 2 kg a.i./ha the reduction in nematode galls between ranged of 15-22%. Therefore, significant effect of FYM on the carbofuran efficacy against nematode observed at lower concentration of carbofuran rather than higher level of carbofuran. In general, there was significant reduction in number of galls by different treatments in comparison of control. The number of gall reduced by carbofuran 1 kg a.i/ha at FYM 20% do not vary significantly with carbofuran 2kg a.i./ha at any level of FYM.

Similar observations were recorded on number of egg mass and soil population of nematode in various treatments. Reproduction factor was lower in combined use of carbofuran and FYM than the carbofuran alone application.

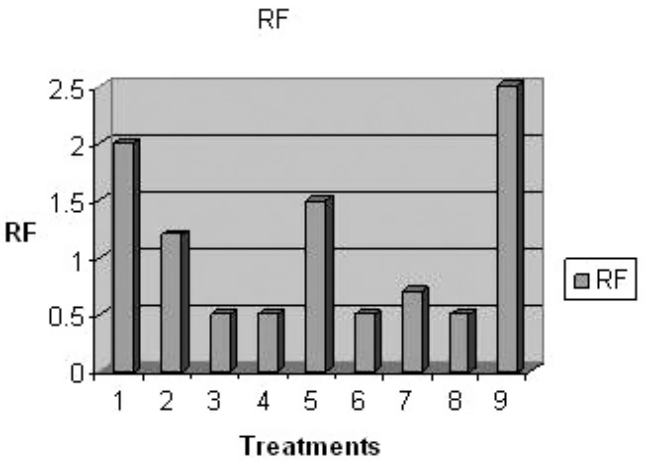
With regards to plant growth of tomato i.e shoot wt. enhanced with both the concentrations of carbofuran with the rise in FYM levels. The increase was observed to be about 72% at low levels of carbofuran over control. Combined application of FYM and carbofuran improved the shoot wt compared to control and when carbofuran was applied alone. Similar observation was made at higher level of carbofuran. The shoot weight improved by the application of carbofuran alone compared to control. Shoot length also showed similar pattern as that of shoot wt. Shoot length was not significantly affected in carbofuran 1 kg a.i/ha applied in 10 and 20% FYM or carbofuran 2 k.g a.i/ha in FYM 5%. Shoot length show

Table 1. Effect of carbofuran in presence of varying dose of FYM on root knot nematode multiplication on tomato.

Treatments	Number of galls	Number of egg mass	Soil population/ 200cc soil
Carbofuran 1kg	220	40	400
Carbofuran 1kg a.i/ha+ FYM 5%	200	30	250
Carbofuran 1kg a.i/ha+FYM 10%	110	20	100
Carbofuran 1kg a.i/ha+FYM 20%	57	10	100
Carbofuran 2kg	77	12	300
Carbofuran 2kg a.i/ha+ FYM 5%	50	15	100
Carbofuran 2kg a.i/ha+FYM 10%	65	8	150
Carbofuran kg a.i/ha+ FYM 20%	52	7	100
Control	320	60	500
CD(P=0.05)	11.3		

Table 2. Effect of carbofuran in presence of varying dose of FYM against root knot nematode infected tomato plant growth.

Treatments	Shoot wt (g)	Root wt (g)	Shoot length (cm)
Carbofuran 1kg	15.2	5.1	23.3
Carbofuran 1kg a.i/ha+ FYM 5%	11.9	5.0	22.3
Carbofuran 1kg a.i/ha + FYM 10%	18.9	4.5	30.8
Carbofuran 1kg a.i/ha FYM 20%	19.2	3.6	30.5
Carbofuran 2kg	15.8	3.9	30.5
Carbofuran 2kg a.i/ha+ FYM 5%	21.8	3.5	27.0
Carbofuran 2kg a.i/ha + FYM 10%	26.0	4.4	39.5
Carbofuran kg a.i/ha+ FYM 20%	25.4	4.1	32.0
Control	11.4	5.7	18.8
CD(P=0.05)	2.6	0.6	3.3



1.Carbofuran 1kg, 2.Carbofuran 1kg a.i/ha+ FYM 5%, 3.Carbofuran 1kg a.i/ha+FYM 10%, 4.Carbofuran 1kg a.i/ha+FYM 20%, 5.Carbofuran 2kg, 6.Carbofuran 2kg a.i/ha+ FYM 5%, 7.Carbofuran 2kg a.i/ha+FYM 10%, 8.Carbofuran kg a.i/ha+ FYM 20%, 9.Control

Fig. 1. Reproduction factor of root knot nematode as influenced by different treatment on tomato

significant improvement with an increase in FYM levels at both the concentrations of carbofuran. Maximum plant growth was recorded in carbofuran 2 kg a.i/ha and FYM (10-20 %) treatment. Improvement was more than 71%. Shoot length increased with the increase in FYM levels.

In contrast, root weight of tomato decreased with the increase in FYM doses with carbofuran 1kg a.i/ha while at higher dose (2 kg a.i/ha) of carbofuran there had been little improvement even though it was lower than control. Root weight was not affected significantly in the treatments however in comparison to control it was affected.

The variation in root-knot galls in tomato plant in response to systemic nematicide has to do with adsorption and desorption of chemical on organic matter which actually suggest whether the chemical is available in soil water phase to be effective against pest. (Durovic *et al.,* 2009;Osborn *et al.,* 2009; Villavarde *et al.,* 2008). The adsorption increased with the increase in organic manure. The adsorption capacity was significantly positively correlated with soil organic carbon and CEC and negatively correlated with soil pH. Desorption of systemic carbamates was more in unamended soil compared to manure amended soil and decreased with the increase in amount of organic manure (Bansal, 2010). Adsorption of carbamate pesticides by clays have shown that they are adsorbed by co-ordination and/or protonation at the carbonyl oxygen by exchangeable cations of clays (Bansal 1983, 2009; Li *et al.,* 2003). Shamalie *et al.* (2011) reported no effect of organic matter on the efficacy of carbofuran either on plant growth or nematode development, may be because doses of carbofuran and organic matter were too low. The high amount of residue of carbofuran linked to high amount of organic matter (Khuntong *et al.,* 2010) and at high pH degradation is faster. This has implication on nematode control. In the present study carbofuran 2 kg a.i/ha do not have effect varied with organic matter probably that this concentration was too high for organic matter (5-20%) to hold this and thus more carbofuran might be available in soil water phase that is why it is more effective against nematode. The study find that carbofuran efficacy increased with FYM dose point out that carbofuran was adsorbed more and released for longer period by FYM and hence its effect as nematocide was more pronounced. Carbamate pesticides has been reported to be adsorbed by organic material amended soil may be retained for a longer time in the soil and control the pesticidal activity effectively (Bansal, 2010). This property also has significance on the efficacy of pesticides and that suggest that it is better to apply pesticide as per the FYM levels in soil. It could be inferred that FYM does play a role in the effectiveness of carbofuran against root-knot nematode and accordingly reflected in plant growth as well. Beside, shoot weight and length improved in treatments over control but root weight was lower in treatments in comparison to control.

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Evaluation of *Paecilomyces lilacinus* for the Management of Root-Knot Nematode, *Meloidogyne incognita* in Flue Cured Virginia (FCV) Tobacco Nursery

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ABSTRACT: Flue Cured Virginia (FCV) tobacco is a major commercial crop grown in light soil regions of Karnataka. Root-knot nematodes, *Meloidogyne incognita* is a major threat to the successful production causing heavy yield and quality loss. Nematicide like carbofuran is being used against this nematode, but with limited efficacy. Extensive use of pesticides of chemical origin especially in higher doses for nematode control has to be avoided due to higher costs and associated hazards to the environment. Replicated trials were conducted with varied dosage levels of *Paecilomyces lilacinus* against the nematode in FCV tobacco nursery raised in root-knot nematode sick plots. Results revealed that at the time of final pulling, *P. lilacinus* @ 30g/m^2 recorded significantly increased total healthy transplants count of 857.5/m^2 and was on par with *P. lilacinus* @ 25g/m^2 (845.1/m^1. Similarly at 60 DAS, applications of *P. lilacinus* @ 30g/m^2 and @ 25g/m^2 were on par with each other in recording the significantly reduced RKI of 1.81 and 1.88 respectively. Both the treatments were also on par with combined application *P. lilacinus* @ 30g/m^2 + Carbofuran @ 10g/m^2, which recorded the significantly reduced RKI of 1.81 compared to untreated check (3.86).

Key words: *Paecilomyces lilacinus,* carbofuran, *Meloidogyne incognita,* FCV tobacco nursery.

Flue-Cured Virginia (FCV) tobacco is an important commercial crop grown in Karnataka light soils (KLS) as rainfed crop has lot of export potentiality. KLS tobacco is preferred internationally due to its ideal chemistry with below detectable levels of TSNA (Tobacco specific nitrosamines) compounds and pesticides residues. Among the various pest and diseases associated with the crop, plant parasitic nematodes and particularly the root-knot nematode, *Meloidogyne incognita* pose serious threat for the production and productivity causing significant reduction in terms of both yield and quality of the tobacco crop in nursery and main field to the tune of 59.4% and 52.9% respectively (Hussaini, 1983; Ramakrishnan *et al.,* 2001). Root-knot nematode infested seedlings, when transplanted in main field, exhibit stunted growth and may even collapse tesulting in gaps. Losses caused by this nematode are very high, especially when they interact with other disease causing pathogens. Ramakrishnan *et al.* (2008) had reported that root-knot nematode, *M. incognita* predispose FCV tobacco crop to wilt disease caused by *Fusarium oxysporum f.* sp. *nicotianae* contributing to significantly reduced yields. Both fumigant and non-fumigant nematicides such as dazomet and carbofuran have been successfully used against this nematode in nursery (Ramakrishnan *et al.,* 1998). But excessive use of synthetic pesticides is not cost effective and moreover many effective nematicides were withdrawn from the market due to their ill-effects and hazards they pose to environment. FCV tobacco. grown in Karnataka being an exportable commodity, presence of chemical residues is a great concern and hence use of such nematicides is highly discouraged. Bio-management of root-knot nematode with antagonistic organisms is an alternative, cost effective and eco-friendly approach. Biological control of plant parasitic nematodes is important in view of long-term advantage of management. *Paecilomyces lilacinus* is the potential egg parasitic fungus, which is highly safe and has been used successfully to manage root-knot nematodes in various crop plants (Ekanayake & Jayasundara, 1994; Jonathan *et al.,* 1995;

Ramakrishnan & Nagesh, 2011). The fungus affects the egg masses, engulfs and penetrates the eggs and proliferates within them by consuming the egg contents (Zaki & Bhatti, 1990). Hence, an attempt was made to evaluate commercial formulation of *Paecilomyces lilacinus* (2 x 10^6 spores/g) in varied dosage levels and also in combination with carbofuran under replicated trials for the management of root-knot nematode, *M. incognita* in FCV tobacco nursery.

MATERIALS AND METHODS

The replicated nursery trials for two seasons were conducted during 2011 & 2012 in the root-knot nematode infested nursery site of CTRI Research Stattion, Hunsur. Raised nursery beds each of 1.2 m X 1.2m size were prepared to fine tilt and the mean initial population of infective juveniles of *M. incognita* was 171 J2/100g soil. The commercial formulation of the egg parasitic fungi, Jai V Jai -*P. lilacinus* formulated as wettable powder with a spore load 2 x 10^6 spores/g formulations was incorporated into the raised nursery beds as per treatment schedule. The treatments incorporated includes, Jai V Jai -*P. lilacinus* at varied dosage levels (ie) @ 5, 10, 15, 20, 25 & 30g/m^2, Neem cake @ 400g / m^2 + Soil Solarization, carbofuran @ 10 g/m^2, Jai V Jai -*P. lilacinus* @ 30g / m^2 + carbofuran @10g / m^2 and with one untreated check. The treatments were replicated thrice in Randomised Block Design. For the treatment Neem cake @ 400g / m^2 + Soil Solarization, where soil solarisation was involved, after the incorporation of neem cake, prepared nursery beds were irrigated and covered with clear low density polyethylene (LDPE) film of 25 μm thickness when the moisture levels in the beds were around field capacity. Edges of the sheets were sealed with mud and left undisturbed for six weeks period. Then the sheets were removed and after two to three days of waiting period, FCV tobacco seed of variety "Kanchan", susceptible to root-knot nematode was sown at the rate 0.3g/ m^2 in all the nursery beds. All the other agronomic practices were followed as recommended. Observations such as Germination count (at 10 DAS), Count of healthy transplants at 60 DAS and at final pulling, root knot index (RKI) at 60 DAS and at final pulling were recorded. Germination count was taken at 15 DAS at random in ten squares, each with dimension of 100 cm^2, from which mean was calculated. Twenty five seedlings were examined for root-knot infection per replication in each treatment and graded under 0-5 scale, from which mean RKI was calculated. In addition, number of galls/g. root, egg mass/g. root and eggs/egg mass were also recorded. Soil samples were drawn before the application of treatments (initial) and at the end of the experiment (final) for the estimation of infective nematode population in the soil. Data gathered were statistically analysed using standard procedure.

RESULTS AND DISCUSSION

All the treatments evaluated under nursery conditions were found to be significantly superior over check in both reducing the root-knot disease incidence and in subsequently improving the seedling growth and root knot free seedlings count. Data from Table 1 revealed that, there was no adverse effect of the product, Jai V Jai -*P. lilacinus* on FCV tobacco seed germination. At 45 DAS, *P. lilacinus* @30g/m^2. significantly differed from the other dosage levels and increased the mean healthy transplantable seedlings count to 316.5/m^2 compared to 216.0/m^2 in untreated check. Whereas, at 60 DAS, *P. lilacinus* @ 30g/m^2 recorded maximum number of mean healthy transplantable seedlings count (320.3/m^2) compared to check (237.1/m^2). But, at the time of final pulling, *P. lilacinus* @ 30g/ m^2 recorded significantly increased mean total healthy transplants count of 857.5/ m^2, which is 53.7 per cent increase over check. It was also on par with *P. lilacinus* @ 25g/m^2, which recorded mean total healthy transplantable seedlings count of 845.1/m^2, which is 51.5 per cent increase over untreated check (557.8/m^2). Similar yield increase in okra plants grown in root-knot sick soil due to soil application of *P. lilacinus* was reported by Dhawan *et al*. (2004). Similar to the present results, Nagesh *et al.* (2001) had obtained significantly increased yield of chrysanthemum flowers to the tune of 23 -28% by use of talc-formulation of *P. lilacinus* @ 4 -6 kg/ac against root-knot nematodes. In the present investigations, carbofuran @ 10g/m^2, the recommended nematicide increased the total healthy transplantable seedlings count to the tune of 22.8 per cent only compared to check. Whereas its combined application with *P. lilacinus* @ 30g/m^2 recorded the maximum total healthy transplants count to the tune of

Table 1. Effect of *Paecilomyces lilacinus* on root-knot free and healthy transplants counts in FCV tobacco nursery.

Treatment details	Germ. count	Healthy transplants count (45 DAS)	% inc. over check	Healthy transplants count (60 DAS)	% inc. over check	Total healthy transplants count	% inc. over check
Paecilomyces lilacinus (2 x 10^6 cfu/g) @5g/m^2	21.1	235.6	9.0	292.8	23.5	658.0	18.0
P. lilacinus (2 x 10^6 cfu/g) @10g/m^2	21.8	240.0	11.1	281.5	18.7	655.5	17.5
P. lilacinus (2 x 10^6 cfu/g) @15g/m^2	21.6	235.1	8.8	294.0	23.9	682.3	22.3
P. lilacinus (2 x 10^6 cfu/g) @20g/m^2	20.3	273.6	26.6	313.1	32.1	759.1	36.1
P. lilacinus (2 x 10^6 cfu/g) @25g/m^2	21.2	307.5	42.3	336.1	41.7	845.1	51.5
P. lilacinus (2 x 10^6 cfu/g) @30g/m^2	21.1	316.5	46.5	320.3	35.1	857.5	53.7
Neem cake @400g/m^2 + Soil Solarization	21.7	278.0	28.7	303.0	27.7	676.0	21.1
Carbofuran @10g/m^2	21.2	279.0	29.1	295.5	24.6	685.0	22.8
P. lilacinus (2 x 10^6 cfu/g) @30g/m^2 + Carbofuran @ 10g/m^2	20.5	313.1	44.9	336.8	42.0	860.8	54.3
Control	20.3	216.0	-	237.1	-	557.8	-
S.Em	0.78	1.84		3.92		3.16	
CD (P=0.05)	NS	5.10		10.86		8.77	

54.3 per cent and it was on par with both the best dosage levels (25 &30 g/m^2) of *P. lilacinus.*

At the time of final pulling, data from table 2 revealed that application of *P. lilacinus* @ 30 g/m^2 and 25 g/m^2 were on par with each other in significantly reducing the number of egg mass/g. root to the tune of 31.0 and 26.5 per cent respectively and eggs/egg mass to the tune of 27.0 and 24.6 per cent respectively and final soil population/100 g soil to the tune of 52.5 and 49.6 per cent respectively compared to un treated check. In general, the application of *P. lilacinus* at varied dosage levels from 5 to 30g/m^2 in FCV tobacco nursery against root-knot nematodes caused 16.5 to 31.0 per cent reduction in number of egg mass/g root, 13.7 to 27.0 per cent reduction in number of root-knot nematode eggs/egg mass and 30.2 to 52.5 per cent reduction in final soil nematode population as compared to un treated check. Similar decrease in root-knot nematode soil population due to application of *P. lilacinus* in banana was also reported earlier by Jonathan & Rajendran (2000). At 45 DAS, application *P. lilacinus* @ 30g/m^2 in FCV tobacco nursery beds significantly reduced root-knot index (RKI) to 1.30 on 0-5 Scale, which is 50.1% decrease over untreated check (2.61) and was found on par with *P. lilacinus* @ 25g/m^2 (1.31). Similarly at 60 DAS, applications of *P. lilacinus* @ 30g/m^2 and @ 25g/m^2 were on par with each other in recording the significantly reduced RKI of 1.81 and 1.88 respectively. Decrease over untreated check was 53.1 and 51.3 per cent respectively. Both the best treatments were also on par with combined application *P. lilacinus* @ 30g/m^2 + Carbofuran @ 10g/m^2, which recorded the significantly reduced RKI of 1.81 compared to untreated check (3.86). Similarly Reddy & Khan (1988) reported 76% reduction in population of reniform nematode, *Rotylenchulus reniformis* with combined application of carbofuran at 2 kg a.i/ha and *P. lilacinus* @ 2g on tomato. Saikia & Das (2001) got highest reduction in galls (60%) with combined application of *P. lilacinus* @ 2g/kg soil and carbofuran @ 1kg. This clearly exhibits the compatibility of *P. lilacinus* with the chemical

Table 2. Effect of *Paecilomyces lilacinus* on root knot nematode multiplication in FCV tobacco nursery .

Treatment details	RKI at 45 DAS	% Dec. over check	RKI at 60 DAS	% Dec. over check /g. root	No.of Egg masses	% Dec. over check	No.of Eggs/egg mass	% Dec. over check	Soil population (/100 g. Soil)	% Dec. over check
Paecilomyces lilacinus (2 x 10^6 cfu/g) @5g/m^2	2.30	11.8	3.03	14.5	16.3	18.5	250.0	14.8	99.5	31.8
P. lilacinus (2 x 10^6 cfu/g) @10g/m^2	2.38	8.8	3.13	18.9	16.7	16.5	253.0	13.7	101.8	30.2
P. lilacinus (2 x 10^6 cfu/g) @15g/m^2	2.33	10.7	2.95	23.5	16.0	20.0	245.5	16.3	101.0	30.8
P. lilacinus (2 x 10^6 cfu/g) @20g/m^2	2.30	11.8	2.60	32.6	15.4	23.0	242.5	17.4	87.3	40.2
P. lilacinus (2 x 10^6 cfu/g) @25g/m^2	1.31	49.8	1.88	51.3	14.7	26.5	235.0	19.9	73.5	49.6
P. lilacinus (2 x 10^6 cfu/g) @30g/m^2	1.30	50.1	1.81	53.1	13.8	31.0	214.1	27.0	69.3	52.5
Neem cake @400g/m^2 + Soil Solarization	2.26	13.4	2.45	36.5	16.5	17.5	242.5	17.3	93.8	35.7
Carbofuran @10g/m^2	2.00	23.4	2.25	41.7	15.7	21.5	242.0	17.5	82.6	43.4
P. lilacinus (2 x 10^6 cfu/g) @30g/m^2 + Carbofuran @ 10g/m^2	1.27	51.3	1.81	53.1	13.5	32.5	214.5	26.9	69.0	52.7
Control	2.61	-	3.86	-	20.0	-	293.5	-	146.0	-
S.Em	0.06		0.03		0.54		1.62		1.80	
CD (P=0.05)	0.16		0.08		1.49		4.49		4.98	

nematicide, carbofuran. Moreover, both the best treatments in the present study were also significantly superior to sole application of carbofuran @ 10g/m^2 with RKI of 2.25 in reducing the root-knot disease incidence in FCV tobacco nursery. The experimental results clearly indicate that *P. lilacinus* @ 30g/m^2 was on par with *P. lilacinus* @ 25g/m^2 in reducing the root knot nematodes incidence in FCV tobacco nursery and in subsequently increasing the total root-knot free and healthy seedlings count. Hence, it is concluded that, application of *P. lilacinus* in talc-formulation with spore load of (2 x 10^6 cfu/g) @ 25g/m^2 (ie) 3 kg/unit nursery (120m^2) is an ideal dosage for the effective management of root-knot nematodes in FCV tobacco nursery.

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Effect of Phorate 10G and Neem Cake on *Tylenchulus semipenetrans* in a Declining Nagpur Mandarin Orchard in Central India

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ABSTRACT: A field experiment was conducted during the year 2008-10 to assess the effect of phorate 10G @ 1.0, 1.5 and 2.0 kg *a.i.* per ha and neem cake @ 10, 15, 20 kg/ tree on population behavior of *Tylenchulus semipenetrans* in a declining Nagpur mandarin orchard in central India. Nematode population levels were assessed before treatment and after 4, 8 and 12 months following treatment. Application of phorate 10G and neem cake beneath the tree canopy resulted in significantly higher % reduction (44.2% in soil and 48.4% in roots in case of phorate 10G @ 2.0 kg / ha and 39.2% in soil and 32.8% in roots in case of neem cake @ 15 kg/ tree) in nematode population compared to untreated control plants (22.6% in soil and 20.59% in roots) twelve months after treatment. The results indicated that in general the percent reduction over initial population was significantly more in trees treated with phorate 10G in comparison to trees treated with neem cake. Percentage reductions in population of females per gram fresh root weight were approximately double in plants receiving phorate 10G compared to untreated control plants. Plants receiving phorate 10G supported minimum nematode reproduction (Rf = 0.55) compared to all treatments and control plants. Maximum increase in canopy volume (9.54%) was observed in the treatments where phorate 10G was applied @ 2 kg per ha.

Key Words: *Tylenchulus semipenetrans*, Nagpur mandarin.

In India, main fruits of *Citrus* group include lime, lemon, mosambi and orange (mandarin). Nagpur mandarin (*Citrus reticulata* Blanco) is a major fruit crop grown in central India. In India, *Citrus* is cultivated over an area of 846 thousand hectares with a total production of 7464 thousand MT and productivity of 8.8 MT/ ha during the year 2011 (Anon, 2011). The citrus nematode, *Tylenchulus semipenetrans*, has been a problem in both established and replanted orchards and its infection leads to slow decline of citrus (Du Charme, 1969). Rough lemon (*Citrus jambhiri*), the most commonly used rootstock for Nagpur mandarin is highly susceptible to citrus nematode. Survey of various citrus growing areas of Vidarbha showed that Citrus nematode (*T. semipenetrans*) is a major nematode problem of Nagpur mandarin orchards which require immediate attention for its management (Bamel 2006 and 2009). Several management options have been tried in India (Ahmad, 1985; Alam *et al.* 1977, Mani *et al.* 1986). Commonly available nematicide, phorate 10 G and neem cake were tested for controlling the population of citrus nematode in an established mandarin orchard heavily infested with this nematode.

MATERIALS AND METHODS

Investigations were carried out on a sixteen-years-old Nagpur mandarin orchard (block No. 30) with a total area of about 2304 m^2 situated at National Research Centre for Citrus, Nagpur with *Citrus jambhiri* (rough lemon) rootstock. The orchard was on drip irrigation system. Trees were growing at a spacing of 6x6 m.

Soil samples were collected individually from all 64 plants of the block. Each composite sample consisted of four sub-samples from four sides beneath the canopy of each tree at a distance of 1.5 m from tree trunk and up to 40 cm depth in the fibrous root zone with the help of

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soil auger. Each composite sample was mixed well and a 100 cc working sample was drawn and washed as per Cobb's sieving and decanting technique and further processed for 48 h on a modified Baermann funnel. The J2s of *T. semipenetrans* were collected after 48 h and counted under stereoscopic binocular microscope. Fibrous roots collected from each composite sample were washed and processed to recover females of *T. semipenetrans*. One gram roots from each composite sample were weighed and stained in boiling cotton blue lectophenol for 30 seconds and then placed in plain lectophenol for 48 h. Females were counted under stereoscopic binocular microscope. Reproduction factor was calculated by dividing the nematode final population in soil and roots by the initial nematode population in soil and roots.

Trees were treated during the month of December when nematode population is much higher (Singh, 1997) with phorate 10G @ 1.0, 1.5 and 2.0 kg *a.i.* per ha and neem cake @ 10, 15, 20 kg/ tree beneath the canopy about 1 m away from tree trunk and in a band of about 1 m wide in the feeder root zone and incorporated mechanically after applying light irrigation. All sides of tree were treated, and untreated trees served as controls.

All the treatments were replicated 8 times and arranged in a randomized complete block design. Nematode population levels in the soil as well as roots were assessed after 4, 8 and 12 months following treatment. The treatments and observations were repeated after one year.

The canopy volume was calculated as per the formula $\pi/ 6 \times HD^2$, where H is the height of the plant above tree trunk and D is the average diameter of the canopy in East-West and North-South direction

RESULTS AND DISCUSSION

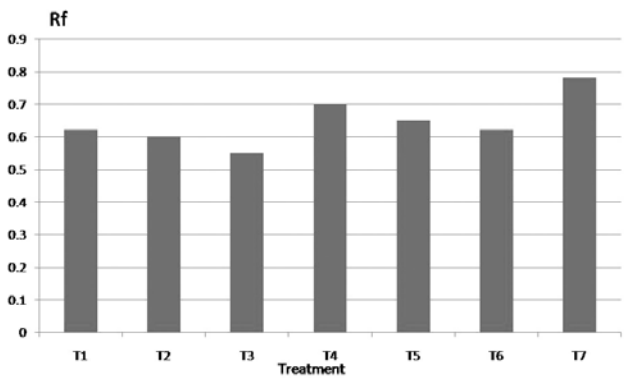
The results presented in Table 1 clearly indicated that application of phorate 10G and neem cake beneath the tree canopy resulted in significant reduction in nematode population compared to untreated control plants. The results indicated that in general the percent reduction over initial population was significantly more in trees treated with phorate 10G in comparison to trees treated with neem cake. Four months following treatment, maximum reduction in soil (73.2%) and root (74.1%) population of the nematode were recorded in plants receiving phorate 10G @ 2.0 kg *a.i.* per ha. With an increase in dose of phorate 10G, there was a corresponding higher decrease in soil as well as root population. In case of neem cake, the % reduction in population was found to be significantly less compared to

Table 1: Effect of phorate 10G and neem cake on canopy volume and population of *Tylenchulus semipenetrans* in soil and roots at 4, 8 and 12 months after treatment in Nagpur mandarin

Treatment	*% reduction in nematode population after						% Increase in canopy volume (m^3) after two years
	4 months		8 months		12 months		
	Soil (/100cc)	Root (/g)	Soil (/100cc)	Root (/g)	Soil (/100cc)	Root (/g)	
Phorate 10 G @1 kg *a.i.*/ ha	62.4	55.4	67.3	61.4	37.4	38.5	4.93
Phorate 10 G @1.5 kg *a.i.*/ ha	67.4	67.7	70.0	66.5	39.5	41.5	6.74
Phorate 10 G @2.0 kg *a.i.*/ ha	73.2	74.1	73.6	71.1	44.2	48.4	9.54
Neem cake @ 10 kg/ tree	53.7	39.5	39.8	33.6	31.9	26.0	4.87
Neem cake @ 15 kg/ tree	54.3	42.7	44.4	36.4	35.5	31.5	4.06
Neem cake @ 20 kg/ tree	62..8	47.5	51.9	41.0	39.2	32.8	6.87
Control	40.2	28.2	34.4	22.8	22.6	20.59	2.96

(*Pooled data of 2008-09 and 2009-10)

trees receiving Phorate 10G after twelve months of application (39.2% in soil and 32.8% in roots in case of neem cake @ 15 kg/ tree and 44.2% in soil and 48.4% in roots in case of phorate 10G @ 2.0 kg / ha). Reduction in root population showed similar trend to soil population. The reduction was also observed in soil (40.2%) and roots (28.2%) after four months in control plants. This may be probably due to natural decline of nematode population during summer (Singh, 1997). The estimation of nematode population after 8 month of treatments showed that the nematode population decreased further. However, the % reduction in population decreased with time. There was a decrease in soil and root population after one year of treatment in all the treatments and population were lower compared to initial population. Subsequent measurements demonstrated that application of nematicide or neem cake reduced population levels to approximately half the level of those in untreated control. Phorate 10G or neem cake applied under canopy reduced adult female nematode population. Twelve months after treatment, percentage reduction in population of females per gram fresh root weight were approximately double in plants receiving phorate 10G at all doses compared to control plants. Nematode reproduction factor at the end of second year (Fig. 1) showed that plants receiving phorate 10G supported minimum nematode reproduction (Rf = 0.55) compared to other treatments. With an increase in dose of nematicide or neem cake, there was a corresponding decrease in nematode reproduction factor. Maximum value of reproduction factor was observed in control plants (Rf = 0.78). At the end of second year, maximum increase in canopy volume in the treatments was observed where phorate 10G was applied @ 2 kg per ha (9.54%) followed by neem cake @ 20 kg per tree (6.87%). During the course of experimentation, the plants were not given the stress treatment for flowering. Fruit set due to natural flowering were recorded and found to be non-significant.



Rf: Reproduction factor; T1: Phorate 10 G @1 kg *a.i.*/ ha; T2: Phorate 10 G @1.5 kg *a.i.*/ ha; T3: Phorate 10 G @2.0 kg *a.i.*/ ha; T4: Neem cake @ 10 kg/ tree; T5: Neem cake @ 15 kg/ tree; T6: Neem cake @ 20 kg/ tree; T7: Control

Fig. 1: Effect of Phorate 10G and neek cake on reproduction factor of *Tylenchulus semipenetrans* infecting *Citrus reticulata* (Blanco)

Singh, B. (2004) observed in a field trial for the control of the citrus nematode, *Tylenchulus semipenetrans* in a ten year old Nagpur mandarin orchard on rough lemon rootstock that the application of carbofuran 3G and phorate 10G, each at 1, 3 and 5 kg *a.i*/ ha reduced the nematode *(Tylenchulus semipenetrans)* populations in soil and on roots significantly within one month of nematicide application. The repeated application of the nematicides after one year kept the namatode populations significantly lower as compared to non-repeated and control treatments. Tiwari & Vadhera (1999) found that application of phorate @ 4 kg a.i./ ha reduced 48% nematode population in roots and increased the plant yield by 49% compared to untreated plants. Mc Clure & Scmitt (1996) also observed the effect of Rugby 10G up to 12 months for suppression of the citrus nematode in lemon trees. Philis (1993) observed the effective control of nematodes and increase in grapefruit yield by 74.2% and 53.2% with fenamiphos and aldicarb, respectively. Philis (1997) achieved effective control of nematode with application of Rugby (cadusafos) for consecutive three years.

It may be concluded that application of phorate 10G @ 2.0 kg/ ha may be used for reducing the population of citrus nematode in citrus orchards, as the chemical reduced the nematode population in soil (44.2%) as well as roots (48.4%) and increased the canopy volume by 9.54%.

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Biodiversity of Plant Parasitic Nematodes in Tea Nurseries and Plantations in Tripura

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ABSTRACT: One hundred and thirty sites at thirteen widely separated tea nurseries and plantations of different age groups were sampled for qualitative and quantitative analysis of plant parasitic nematodes communities during March to October, 2001. A total of 14 species of plant parasitic nematodes within eight genera, were isolated and identified from roots and rhizosphere soil of tea plants. All species of plant parasitic nematodes associated with tea are first records of nematodes associated with tea from Tripura. The most frequently encountered nematode species includes: *Helicotylenchus* spp., *Meloidogyne* spp., *Pratylenchus brachyurus* (Pb), *Rotylenchulus reinformis* (Rr), *Hoplolaimus* spp. and *Hemicriconemoides mangiferae* (Hm), which were detected in 85%, 70%, 68%, 55%, 53% and 41% of the sites. respectively. Nematode communities were analysed by mean abundance, prominence value, importance value and diversity index based on nematode numbers and biomass. Analysis showed that *Meloidogyne hapla* ranked first in importance value followed by *M. incognita,H, dihystera R. reniformis* and *H. erythrinae.* A linear increase in the index of diversity (H') of the nematode communities with increase in the age of plantation up to eight years was observed and with the further increase in the age of plantation there is a gradual decline in the diversity index. This indicated that with an increase in the age of plantations up to certain age parasitic species have become more abundant. This is a distinct sign of gradual establishment of these species.

Key words: Biodiversity index, Community analysis, Plant parasitic nematode, Tea, Tripura, India.

Tea (*Camellia sinelisis* L.) occupies a prominent place as a cash crop as well as an export crop in India. In Tripura, there are 59 active tea plantations (Numbers mentioned in parenthesis with each sub-division) : Agartala Sadar (25), Kailasahar (16), Dharmanagar (8), Kamalpur (5), Khowai (2) Sabroom (2) and Belonia (1), subdivisions of the state (Anonymous, 2001). The agroclimatic conditions in Tripura are suitable for development of tea plantations. The fertile soil, without major problems of toxicity or deficiency, high annual rain fall (2400mm) makes Tripura the fifth largest in average among the 14 tea producing states after Assam, West Bengal, Tamil Nadu and Kerala. This is cultivated in the high slopes, hillocks and in plain lands with diversified agroclimatic conditions covering a land area of 6124,22 ha. under the cultivation. The average production of tea is 2749 kg/ ha against the all India average 1000 kg/ ha (Bhattacharjee & Singh, 1995)

Nematode damage to tea plantations in India has been well documented only from Darjeeling District, West Bengal (Mukherjee and Dasgupta, 1982) and catelogued 19 species of plant parasitic nematodes within 13 genera from the state. The serious pathogenic nematodes of tea including *Pratylenchus loosi, P. brachyurus, Meloidogyne brevicauda* and *Helicotylenchus erythrinae* were detected from the soil and root samples. They concluded that with an increase in altitude, diversities of nematode communities declined, Association of *P. loosi* with devastation and debilitations in Sri Lanka. Japan and Bangladesh have been noted (Campos *et al*., 1990). Records of plant

nematode infestation of tea plantation in Assam included *Meloidogyne incognita, M. hapla* and *P. brachyurus* (Phukan, 1998). Subsiquently M. *brevicauda* has been recorded from matured tea plants in Coonor, Tamil Nadu (Mehta & Somasekhar, 1998). Although a large number of plant parasitic nematodes have been encountered from soil around the tea plants, the known and suspected pathogens are *Pratylenchus* spp., *Meloidogyne* spp., *Radopholus similis*, *Helicotylenchus* spp., *Rotylenchulus reniformis*, *Hoplolaimus* spp., *Rotylenchus* spp., *Xiphinema* spp. and *Hemicriconemoides* spp. through out the world (Koshy, 2002). Since no information is available about the plant parasitic nematodes associated with tea in Tripura, an intensive nematological survey for qualitative and quantitative analysis of pJant parasitic nematodes associated with tea nurseries and plantations situated in widely separated geographical locations in the state.

MATERIALS AND METHODS

During March to October, 2001, an intensive survey of plant parasitic nematodes asociated with tea plants was conducted in nurseries at 3 locations (6 months to 2 years old) and plantations at 10 locations (3 to 25 years old) in the main tea producing areas in Tripura state. A total of 130 composite soil and root samples were collected. At each location, 10 sites were fixed oil a given sampling date. At each site, within a radius of 100 meters, 20 mature tea bushes were randomly selected and soil and root samples were collected. Each sample consisted of 20 sub-samples of rhizosphere soil and associated roots in a sample area of $30m^2$ of nursery and 1ha. of plantation up to 40 cm depth with the help of a GI pipe (2.5 cm dia). The age of the nurseries and plantations were recorded as reported by the estate managers and government officials at the tea estate concerned.

Nematodes were extracted from 250 cm^3 composite soil sub-samples by modified Baermann funnel technique (Whitehead & Beming, 1965). The root samples were washed in running tap water, cut into smaller pieces, which were thoroughly mixed. Five gm of these composite root masses were macerated in 100 ml tap water in a blender for two min. The resultant root suspensions were incubated for three days over double layered facial tissue paper on coarse wiremesh placed in water using 1-3% hydrogen peroxide (Gowen & Edmunds, 1973) for extraction of migratory endoparasites. Batches of five gms of tender roots were stained by acid fuchsin lactophenol method and nematode population in roots was estimated after extraction through maceration in a blender. Plant parasitic nematodes were identified up to genus and species level in permanent mounts, in dehydrated glycerol. The species of root-knot nematodes was identified on the basis of perinneal pattern, male and juvenile characters (Eisenback, 1985).

Population density (mean ± SE) with range and frequency of ocurrence of each nematode genus was determined at each location and for total samples collected from the area. Nematode cornrnunites were analysed by prominence value (Absolute density X V Absolute frequency) and importance values (Relative density = Relative frequency + Relative biomass) for each nematode species following the formula given by Norton (1978). Biomass was determined by morphometric measurement of 20 females collected from each location.

Shannon -Weiner diversityindex, H' =*L* Pilog Pi, where's' is the number of species at each location and 'Pi' is the relative abundance of the 'i'th species, was used to determine the diversity of nematode community. Diversity of nematode species was measured by Shannon-Weiner information measure to base 'e'.

Physicochemical analysis of soil samples

The representative soil samples collected from the root zones of tea at different locations were labelled and transported to the laboratory .These samples were air dried in the lahoratory for 10 days, grinded and passed through coarse sieves and then stored in polythene bags. Mechanical analysis of soil samples were carried out by International pipette menthod (Piper, 1966) to differentiate the sand, silt and clay content (%). Determination of pH of solid samples were conducted by a glass electrode pH meter (Blackman) as described by Jackson (1967). Determination of organic carbon was done by Walkely and Black's rapid titration method (Piper, 1966). Estimation of available nitrogen by alkaline permanganate method, available phosphorus byBray and Kurtz method and available potassium by Flame Photometric method (Jackson, 1967) were carried out.

RESULTS

Fourteen species of plant parasitic nematodes within eight genera were isolated and identified from roots and rhizosphere soils around tea plants. The species were *Helicotylenchus dihystera,* (Cobb, 1893) Sher, 1961, *H. microcephalus* Sher, 1966, *H. erythrinae* (Zimmerman, 1904) Golden, 1965, *Hemicriconemoides mangiferae,* Siddiqi, 1961, *Hoplolaimus columbus,* Sher, 1963 *H. indicus* Sher, 1963, *Meloidogyne hapla* (Chitwood 1949). *M. incognita* (Kofoid & White, 1919) Chitwood, 1949. *Pratylenchus brachyurus* (Godfrey, 1929) Filip jev & Sch. 1941, *Rotylenchulus reniformis* Linfored & Olivera, 1940 *Tylenchorhynchus brevilineatus* Williams, 1960, *T. Mashhoodi* Siddiqi & Basir, 1959 *Xiphinema elongatum* Sch. Stek & Teunissen, 1983 and X, *insigne* Loos , 1949. Among these, *Helicotylenchus* spp. (85%), *Meloidogyne* spp. (70%), *Pratylenchus brachyurus* (68%), *Rotylenchulus reniformis* (55%), *Hoplolaimus* spp. (53%) and *Hemicriconemoides mangiferae* (41%) were most abundant and predominant nematodes effecting both nurseries and plantations (Table 1). All species of plant parasitic nematodes associated with tea plants are being reported for the first from Tripura.

The Physicochemical properties of tea soils showed that the soils were sandy clay type in all locations, acidic (pH 4.3-6.8) with high clay (34-65%), higher sand (24-48%) and low silt (9-17%) content. Organic carbon varied from 0.38 to 1.5% and a wide variation in NPK status (Table 1). Diversity of nematode community based on nematode numbers (H'n) was highest in the plantation located at Mahespur (8 years old), than either the younger or older plantations (Fig. 1). According to Niblack and Bernard (1985), correlations were found between sand, silt or clay content of soil and densities of several nematode species but densities of these species were not related to soil texture class.

Nematode abundance

The mean density and frequency of occurrence of different nematode differed in nurseries and plantations. The spiral nematodes, *Helicotylenchus* spp. were represented by three different species, viz. *H. dihystera,*

Table 1: Physicochemical properties of tea soils in Tripura[1]

Location	Sand (%)	Silt (%)	Clay (%)	Organic matter (%)	Organic carbon (%)	Nitrogen kg/ha	Phosphorus kg/ha	Potassium kg/ha	pH
Nursery									
Durgabari	30	10	34	0.654	0.380	0.03	0.5	11	6.2
Harendranagar	25	9	47	0.909	0.529	0.05	0.5	12	6.4
Gokulnagar	27	9	51	0.923	0.537	0.05	0.6	11	6.2
Plantation									
Mekhlipara	48	12	37	1.245	0.724	1.3	1.5	15	6.1
Durgabari	43	12	46	0.964	0.561	0.05	0.7	16	4.6
Maheshpur	34	14	53	1.252	0.728	0.04	0.6	17	6.7
Sabroom	24	11	65	1.985	1.154	0.03	0.5	12	6.5
Harendranagar	40	17	43	0.938	0.545	1.2	1.0	15	4.4
Mahabir	43	11	47	1.378	0.811	0.07	0.3	25	6.3
Golakpur	37	11	52	1.203	0.7	0.06	0.2	24.4	5.7
Lakhilunga	48	14	38	2.027	1.185	0.06	0.3	14	6.8
Gokulnagar	44	16	40	0.929	0.540	0.5	1.6	17.4	4.3
Rangung	40	11	49	2.56	1.5	0.8	0.5	22	6.3

[1]Soil type (Sandy clay in all locations)

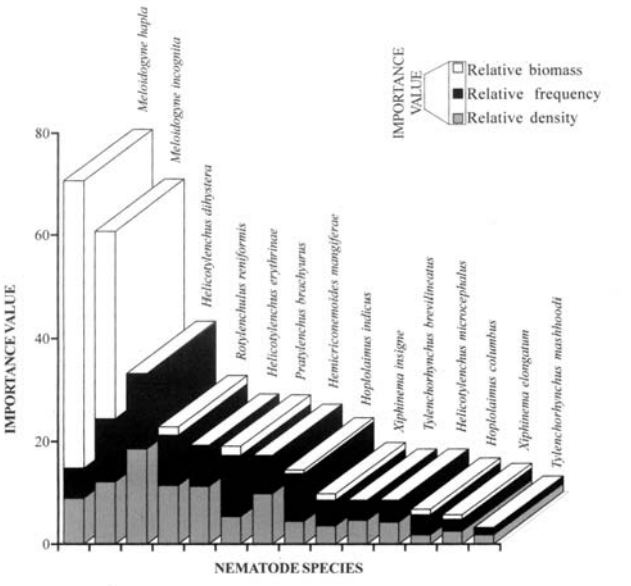

Fig. 1. Community analysis showing importance values and their components for 14 plant parasitic nematodes associated with tea in Tripura state, India

H. erythrinae and *H. microcephalus,* among which *H. dihystera* was the most prominent. The mean densities of the special nematodes, *Helicotylenchus* spp. were 93 and 182 nematodes per 250 cm^3 soil and the population ranged from 10 to 189 per 250 cm^3 in nurseries and 12 to 516 in plantations respectively.

Root-knot nematodes, *Meloidogyne* spp. were uniformly present in all nurseries and plantations surveyed. The frequency was 80% in nurseiies and 70% in plantations. Their population ranged from 6 to 272 (mean 96) nematodes in nurseries and 10-376 (mean 124) nematodes in plantations. Root-knot disease caused by *M. incognita* and *M. hapla* were most common in nursery and young plants below 5 years of age. The sizes of the root galls varied widely in different plantations. The larger sized root-galls (2-16 mm dia) were recorded in younger plantations (3-5 years of age) at Mekhlipara and Durgabari in West Tripura district. The sympatric prevalence of *M. hapla* and *M. incognita* in tea roots were found. Lesion nematode, *Pratylenchus brachyurus* was more abundant in plantations (53 nematodes) than in nurseries (34 nematodes per 250 cm^3 soil). The frequency of occurrence was also higher (68%) in plantations than in nurseries (50%). The presence of dark red cortical lesions on the epidermal layer of the feeder roots indicated the involvement of the pathogen in different plantations. The mean abundance of *P. brachyurus* was highest (98 nematodes/g) on root at Harendranagar (12 years old) and the population ranged from 53 to 118 nematodes per g of root samples. The species has been recorded long back from tea plants of the adjoining state of Assam (Basu, 1968) and from Darjeeling, West Bengal (Mukharjee & Dasgupta, 1982).

The reniform nematode, *Rotylenchulus reniformis,* was present both from nurseries and plantations. The mean density of *R. reniformis* was 88 nematodes ranging from 28 to 320 nematodes per 250 cm^3 soil in nursery. The species was also uniformly present in different plantations except Mekhlipara although the density varied. The mean density of the species was 104 nematodes per 250cm^3 soil in plantations, which was much higher than that in nurseries (Table 1). The reniform nematode was much more prevalent in nursery plants, causing 100% infection by the juveniles and young females which were observed in soil samples too. The same species has caused casualities in young tea fields in Indonesia and widely prevalent in different tea estates of Sri Lanka in the elevation range 200 to 900m amsl (Campos *et al*., 1990).

Three nurseries (6 months to 2 years old) and ten plantations (3-25 years old) surveyed varied greatly with respect to age and in nematode abundance. Population densities of all identified species also varied widely among nursery sites and plantations (Table 1).The incidence of *Hemicriconemoides mangiferae, Hoplolaimus indicus, H. columbus, Tylenchorhynchus brevilineatus, T. mashoodi, Xiphinema elongatum* and *X. insigne* were observed in nurseries and plantations alike in Tripura. The population load of different nematode pathogens at higher age groups of tea plantations become less severe because with the increase in age of the plants, the tannin content of the roots increased. The concomitant occurrence of *M. incognita, M. hapla, Pratylenchus brachyurus* and *R. reniformis* suggested a multiple species infection in Tripura. It appears that the root-knot nematodes and lesion nematodes are a growing threat and a potent major constiaint in tea nurseries of the state, where sanitation and othe management practices are advisable.

Nematode community structure

Nematode communities were analysed by absolute density, relative density, absolute frequency, relative frequency, prominence value, importance value and diversity index. Analysis of the nematode communities showed that *M. hapla* ranked first in importance value (70.64) followed by *M. incognita* (60.85), *H. dihystera* (33.27), *R. reniformis* (22.77) *and H. erythrinae* (19.28). As the importance values of *M. hapla* and *M. incognita* were more than two to three times those of other prominent nematode species viz. *H. dihystera R. reniformis* and *H. erythrinae.* However. *H. dihystera* occupied the first rank in relative density (18.50) and relative frequency (14.56) followed by *M. incognita* (12.07), *R. reniformis* (11.48) *and H. erythrinae* (11.19) in the community indicating the significance of prominence and improtance values (Fig. 1) in determining the relative ecological role in terms of energy transfer played by nematode species in a diverse community. The pathological significance of the *M. incognita* and *M. hapla* are the most important in tea because it causes severe damage of tea seedlings and younger plants throughout the. world. Pathogenic significance of *P. brachyurus* comes next to *Meloidogyne spp.* (Table 2). However, the relative pathogenic role played by *R. renifonnis* has been documented from Sri Lanka (Campos et al, 1990) which is similar in the present findings.

Nematode community diversity

Diversity of ptant parasitic nematode community was measured by the index of diversity (H') using nematode numbers (H'n) and biomass (H'b) of individual species in different tea plantation. These values were correlated with the age of plantations (Fig. 2). Diversity index ranged from 1.288 to 1.901 in different nurseries and plantations when the index was based on nematode numbers. However, diversity index rangedfrom 0.459 to 0.952 when nematode biomass of respective species was used (Table 3). It was observed that nematode co~unity diversity (H') was less when nematode biomass of respective species was used instead of numbers. These findings are in agreement with Norton and Edwards (1988) in the study of age structure and community diversity of nematodes associated with maize in Iowa, USA. Nematode community diversity was the highest in

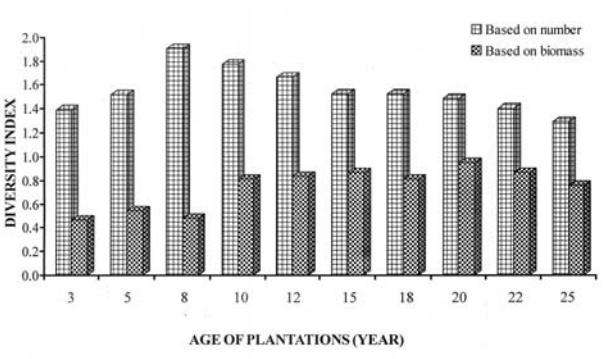

Fig. 2. Nematode community diversity in relation to age of tea plantations in Tripura, India

the plantation located at Maheshpur (8 years old) than either the younger or older plantations when the diversity was based on nematode numbers. The plantation located at Rangrung (25 years old) was least diverse in the regard (Fig. 2). The plantation location at Lakhilunga (20 years old) was most diverse when diversity was based on nematode biomass. This shows that there was a linear increase in the index of diversity of the nematode communities with an increase of the age of plantations, which reaches its highest upto 8 years of age and with further increase in the age of plantation, there was a gradual decline in the diversity index in the higher age groups. This indicated that with an increase in the age of plantation up to certain age, parasitic species have become more abundant (Table 3). This is a distinct sign of gradual establishment of these species. Beyond certain age of monoculture, perennial plantation crops do not support a very diverse pathogenic community of phytonematodes. Only the serious and specific plant parasites prevail on a particular crop, as in banana in West Bengal (Mukherjee & Dasgupta, 1983), in dogwood nurseries in Tennesse, USA (Niblack & Bernard, 1985).

Similar findings have been made in the diversity and community structure of plant parasitic nematodes in pineapple plantations (Nath *et al.*, 1997), in rubber nurseries and plantations (Mukherjee, *et al*., 2000) and jackfruit orchards ofTripura (Mukherjee *et al*., 2001). Because plant related inputs provide the resources for nematode communities, soil texture is related to suitability of cropping and affects nematode communities through crop specific infestations (Yeates, 1999).

Table 2: Population density with ranges and frequency of occurrence of plant parasitic nematodes

Nematode species	Population density (Mean ± SE)														
	NURSERY				PLANTATION										
	Durga-bari	Harendra-nagar	Gokul-nagar	Mean	Mekhli-para	Durga-bari	Mahesh-pur	Sabroom	Harendra-nagar	Mahabir	Golak-pur	Lakhil-unga	Gokul-nagar	Rangung	Mean
Helicotylenchus spp. (*H. dihystera, H. erythrinoe H. microcephalus*)	98±20.8 (10-145)	112±77 (35-189)	82±13.2 (16-156)	93±14 (53)[3]	150±41 (32-415)	217±45 (38-450)	89±23 (22-262)	264±24.5 (55-326)	174±46.7 (42-430)	378±78 (24-516)	130±44 (12-252)	276±52.5 (92-382)	142±34.6 (25-276)	84±22 (36-158)	182.3±15 (85)[3]
Hemicriconemoides mongiferoe	10±2.7 (6-14)	28±10.1 (10-45)	-	21±7.2 (16)	44±13.5 (8-115)	-	162±84 (78-246)	136±42.4 (42-278)	218±43 (94-360)	82±26 (35-196)	48±18 (12-110)	94±12 (82-106)	26±15.5 (4-72)	102±37 (65-139)	92±13 (41)
Hoplolaimus spp. (*H. columbus, H. inducus*)	22±3.8 (8-46)	40±10.4 (22-74)	17±10.4 (6-38)	26.1±4.3 (60)	14±7.6 (5-82)	45±11 (30-66)	74±19.1 (28-120)	92±14.5 (10-134)	38±8.4 (12-77)	-	30±11 (17-128)	46±11.3 (25-64)	24±2 (22-26)	-	46±5.6 (53)
Meloidogyne spp. (*M. hapla M. incognita*)	15±8.2 (6-48)	98±16.7 (35-162)	135±24 (64-272)	96.1±15 (80)	60±10 (24-110)	210±50 (82-354)	113±18.1 (44-180)	178±29.1 (36-220)	280±34.3 (92-376)	47±11.4 (12-94)	124±13.2 (60-175)	142±27.6 (86-190)	38±23.1 (10-84)	55±13 (18-76)	124.3±11.2 (70)
Pratylenchus brochyurus	-	27±6.5 (12-80)	48±13 (15-76)	34±6.4 (50)	28±4.3 (16-60)	55±8.6 (20-86)	42±9.1 (22-117)	86±15 (44-158)	135±24 (62-198)	44±6 (26-90)	15±4.6 (6-32)	36±11 (25-47)	82±26 (56-108)	20±6.4 (10-32)	53.1±5.1 (68)
Rotylenchulus reniformis	54±9 (28-126)	135±31 (66-320)	86±17.4 (54-235)	88.5±12.5 (93)	-	104±23 (72-218)	217±52.7 (96-344)	358±55.6 (120-482)	50±7.1 (35-94)	156±5.1 (105-207)	34±14 (18-160)	67±15 (52-82)	45±8 (22-110)	37±16.3 (20-86)	53.1±5.1 (55)
Tylenchorhynchus spp. (*T. brevilineatus, T. mashhoodi*)	-	16±6.5 (6-42)	28±8.5 (12-55)	22±5.4 (33)	-	-	32±7.7 (8-74)	-	47±9.1 (15-80)	62±20.5 (20-115)	-	-	-	-	43.6±6.5 (22)
Xiphinema spp. (*X. elongatum, X. insigne*)	-	16±5.3 (8-26)	23±11 (12-34)	19±5 (16)	42±8.1 (18-86)	38±18 (20-56)	60±16.4 (38-92)	-	34±12 (10-48)	26±10 (6-52)	-	54±42 (12-96)	13±4 (8-21)	30±8 (16-44)	37±5 (28)

[1]Districts (WT = West Tripura, NT= North Tripura, ST = South Tripura, D = Dhalai); [2]Age of plantations; [3]Frequency of occurrence (%)

Table 3: Community analysis of plant parasitic nematodes associated with tea plantations of Tripura.

Nematode species (1)	Relative density (%)	Relative frequency (%)	Relative biomass (%)	Prominence value	Importance value	Plantations code(3)	Pathogenic significance ranking(2)
Meloidogyne hapla	8.95	5.86	55.83	528.4	70.64	3-8	2
M. incognita	12.07	12.43	36.35	1037.4	60.85	1-10[4]	1
Helicotylenchus dihystera	18.50	14.56	0.21	1720.5	33.27	1-10[4]	-
Rotylenchulus reniformis	11.48	9.76	1.53	875.1	22.77	2-10[4]	4
Helicotylenchus erythrinae	11.19	7.99	0.10	771.4	19.28	1,2,4,5,8,9[5]	-
Pratylenchus brachyurus	5.25	12.07	1.70	445.2	19.02	1-10	3
Hemicriconemoides magniferae	9.83	7.28	0.14	646.7	17.25	1-10[4]	-
Hoplolaimus indicus	4.38	9.41	0.71	327.6	14.50	1-5,7-9	-
Xiphinema insigne	3.60	4.97	1.25	195.7	9.82	2,3,5,8,9	-
Tylenchorhynchus brevilineatus	4.57	3.90	0.07	220.4	8.54	3,5,6	-
Helicotylenchus microcephalus	4.28	4.08	0.09	211.0	8.45	3,6,7,10	-
Hoplolaimus columbus	1.65	4.08	1.00	81.5	6.73	1,3,6,8,9	-
Xiphinema elongatum	2.43	2.30	0.86	90.1	5.59	1,6,8,10	-
Tylenchorhynchus mashhoodi	1.75	1.24	0.14	47.6	3.13	3	-

1. Nematodes are arranged in the descending order of their importance values; 2. Based on biological understanding (Rama & Dasgupta, 1987); 3. Plantations code: (i) Mekhlipara, (ii) Durgabari, (iii) Maheshpur, (iv) Sabroom, (v) Harendranagar, (vi) Mahabir, (vii) Golokpur, (viii) Lakhilunga, (ix) Gokulnagar, (x) Rangrung; 4. Most widely distributed; 5. Least widely distributed

DISCUSSION

This result is indicated that with an increase in the age of plantations up to eight years, parasitic species have become more abundant. This is a distinct sign of gradual establishment of these species. Diversity of plant parasitic nematode communities was measured by the index of diversity (H') using nematode numbers (H'n) and biomass (H'b) of individual species of diferent tea plantations. H'n was at its highest in the plantation located at Maheshpur (8 years of old). H'b was most diverse at Lakhilunga (20 years of old).

Meloidogyne hapla, M. incognita, Pratylenchus brachyurus and *Rotylenchulus renifonnis* have been recorded in tea nurseries and plantations in Tripura state. Severe infestations by *M. hapla, M. incognita* and *P. brachyurus* have been encountered throughout the state and also for the first time from North Eastern states of India which required immediate attention for nematode

Table 4: Nematode community diversity indices based on nematode number and biomass in tea plantations of Tripura, India

Index based on nematode number		Index based on nematode biomass	
Age of plantations[1] (Years)	Index (H'n)	Age of plantations (Years)	Index (H'b)
08	1.901	20	0.952
10	1.772	22	0.866
12	1.660	15	0.865
18	1.523	12	0.836
15	1.522	10	0.812
05	1.512	18	0.809
20	1.481	25	0.759
22	1.402	05	0.537
03	1.388	08	0.475
25	1.288	03	0.459

[1]Age of plantations arranged by descending diversity of nematode community

management. This result is significant from poor productivity in tea plantations in Tripura.

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Role of Salicylic Acid and Phenols in the Resistance of *Tagetes* species Against *Meloidogyne incognita*

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ABSTRACT: To understand the basis of mechanism of host resistance in marigold spp. against root-knot nematode, *Meloidogyne incognita*, marigold, *Tagetes erecta* cv. Hazara and YD, and *T. patula* cv. Jafri were grown in pots inoculated with 500, 2500 and 5000 juveniles of *M. incognita*/kg soil, and the biochemical (chlorophyll, total phenol and salicylic acid), histopathological (root penetration), and morphological responses of the host plant (length, fresh and dry weight of shoot and root) and soil population of the nematode were determined. The marigold cultivars were found resistant to root-knot nematode and did not supported gall formation or egg mass production. Nematode population drastically decreased over time, and only 3-11% larvae could penetrate the roots, the penetrated juveniles remained confined to cortex and did not attain maturity. Plant growth variables and leaf pigment were not influenced, but total phenol and salicylic acid contents of leaves of marigold cultivars growing in the infested soil showed drastic increase ($P \leq 0.001$) and varied linearly with the inoculum level. The concentration of the biochemicals was relatively greater in the host cultivar which supported lesser root penetration. This resistance was mainly biochemical rather than morphological. The small proportion of nematode population that penetrated into roots was not able to grow normally due to synthesis of greater amount of phenols and salicylic acid. Marigold cultivars not only antagonized the penetrated juveniles, but also suppressed the nematode larvae in soil and caused mortality to them apparently through root exudates.

Key words: *Meloidogyne incognita,* phenols, resistance, salicylic acid, *Tagetes*

Marigold (*Tagetes* spp.) is one of the important ornamental plants grown in India and is widely cultivated in Maharashtra, Gujarat, Andhra Pradesh, Tamil Nadu, Kerala and Karnataka. *Tagetes* spp. possesses repellant and antagonistic character to several pathogens, especially nematodes (Khan *et al*., 2012b). Some important nematoxic compounds such as á-terthienyl have been found in *Tagetes* spp. (Meijneke and Oostenbrink, 1957) which causes toxic effect on plant nematodes (Devakumar, 1994). *Meloidogyne incognita* is an important pest of ornamental crops (Khan and Mustafa, 2005), but most of the *Tagetes* spp have shown complete resistance to the nematode (Tyler, 1938; Oostenbrink *et al.*, 1957). Ample research has been done to use *Tagetes* spp. as green manuring or intercropping to control root-knot and other nematodes (Suatmatdji, 1969; Supratoya, 1993; Khan *et al*., 2012a). The present study is aimed to examine biochemical and histopathological basis of resistance in *T. erecta* and *T. patula* with regard to plant growth, leaf chlorophyll, phenol and salicylic acid and root penetration of *M. incognita* juveniles in marigold using different inoculum levels of *M. incognita* to understand the mechanism of resistance in marigold against the nematode.

MATERIALS AND METHODS

The experiment was conducted in earthen pots (6 x 6 inch size) filled with 1 kg autoclaved soil and farmyard manure (4: 1 ratio). Pure culture of second stage juveniles of root-knot nematode, *M. incognita* (Kofoid and White) Chitwood was prepared from the egg masses collected from the roots of egg plants growing in a pure culture bed. The egg masses were placed on a wire gauge in Petri plates having adequate water and incubated at 27±2°C for a week to facilitate egg hatching (Khan, 2008). The nematode juvenile suspension was collected and standardized to 500 J_2 of *M. incognita*/ml, which was used to inoculate soil in pots to achieve initial population equivalent to 500, 2500 and 5000 J_2/kg soil. A

day after nematode inoculation, 3 week old seedlings of *T. erecta* L. cvs. Hazara and YD and *T. patula* cv. Jafri were planted. Ten replicates were maintained for each treatment, and the pot were placed on the roof top receiving uniform sunlight in a completely randomized designs. Pots were watered regularly to maintain adequate soil moisture.

Five out of ten replicates were used to determine biochemical and root penetration. Fifteen days after planting, a few leaves were carefully collected (1 g/ plant) from the 5 plants to determine total phenol (Zieslin and Ben-Zaken, 1993) and salicylic acid contents of leaves (Shane and Kowblansky, 1968). To estimate chlorophyll (Arnon, 1949), fresh leaves (1 g/plant) were collected from the same five plant 15 days later (total days after transplanting). After collecting leaves, the plants on same day, were carefully uprooted from the pots to determine juvenile penetration in marigold roots (Southey, 1986). The roots were washed and cut into small pieces of 3-5cm. The pieces were plunged into boiling 0.1% acid fuchsine for 3 minutes in a beaker. The pieces were later washed in running water and placed in Petri plates with plain lactophenol for 2-3 days allowing differentiation of nematodes from the root tissue. The small parts of roots were gently pressed between 2 glass slides to make the surface plain and were examined under stereoscopic microscope, and the number of juveniles and adults of *M. incognita* inside the root tissue were counted.

Remaining five pots of each treatment were harvested three months after transplanting and length of root and shoot, fresh and dry weight of root and shoot, soil population of *M. incognita*, galls and egg masses were determined. Soil population of juveniles of *M. incognita* was determined using Cobb's decanting and sieving method (modified) followed by the Baermann funnel technique (Southey, 1986). The data were analyzed by single factor ANOVA and least significant difference (LSD) was calculated at $P \leq 0.05$, 0.01 and 0.001.

RESULTS AND DISCUSSIONS

Marigold cultivars inoculated with 500, 2500 or 5000 J_2/kg soil did not develop any symptoms that could be attributed to the nematode damage. Any swelling that could resemble to gall or egg mass production was not noticed on the root system of any of the three marigold cultivars evaluated (Table 1). Soil population of the nematode at the used inoculum levels significantly decreased over time ($P \leq 0.001$), being 59-95% (*T.*

Table 1. Galling, egg mass production, soil and root population of *Meloidogyne incognita* in *Tagetes* species at different inoculum level of *Meloidogyne incognita*

Tagetes spp.	Inoculation level (J_2/kg soil)	Total chlorophyll (µg/g fresh leaf tissue)	Gall/Egg mass	Soil population of nematodes (J_2kg soil)			Root population (larvae/g root at harvest)
				Months after inoculation			
				One	Two	Three	
T. erecta cv. Hazara	500	2.148	00/00	205^c	100^c	26^c	2
T. erecta cv. YD	500	1.644	00/00	925^c	350^c	102^c	4
T. patula cv. Jafri	500	1.66	00/00	925^c	375^c	88^c	7
T. erecta cv. Hazara	2500	2.107	00/00	950^c	375^c	115^c	4
T. erecta cv. YD	2500	1.59	00/00	1400^c	600^c	150^c	10
T. patula cv. Jafri	2500	1.632	00/00	1450^c	650^c	100^c	13
T. erecta cv. Hazara	5000	2.084	00/00	1500^c	650^c	185^c	8
T. erecta cv. YD	5000	1.562	00/00	2973^c	1308^c	419^c	14
T. patula cv. Jafri	5000	1.603	00/00	3121^c	1393^c	378^c	13

Each value is mean of five replicates. Significantly different from the respective initial inoculum level) at $P \leq 0.05^a$, 0.01^b, 0.001^c

erecta cv. Hazara), 30-94% (*T. erecta* cv. YD) and 25-92% decrease (*T. patula* cv. Jafri) over respective initial populations. Histopathology of the roots showed that 3-11% juveniles of *M. incognita* penetrated the roots (Table 1). Highest root population of *M. incognita* was recorded in *T. patula* cv. Jafri (7-11%) followed by *T. erecta* cv. Yellow Small (5-9%) and *T. erecta* cv. Hazara (3-6%) at 2500 and 5000 J_2 level (Table 1). The root population comprised of only vermiform stage, any saccate stage or egg masses were not observed. Researchers have shown that in resistant plants a small population of nematodes may penetrate but they do not develop further to attain maturity (Gaunguly and Gupta, 1994). Inoculation with *M. incognita* at any inoculum level did not cause a measurable effect on any of the plant growth and dry matter parameters of *T. patula* cv. Jafri, *T. erecta* cvs. YD or *T. erecta* cv. Hazara (data not presented). Marigold is an established antagonistic plant for root-knot nematode and most of the *Tagetes* cultivars are resistant or poor host for this nematode (Tylor, 1938). Apparently, the decrease in population of *M. incognita* in the rhizosphere of marigold cultivars was due to release of some nematoxic compounds such as á-terthienyl along with root exudates (Meijneke and Oostenbrinnk, 1957). Recently Buena *et al.* (2007) screened *T. patula* against some populations of *M. incognita, M. javanica, M. arenaria* and *M. hapla* and concluded that marigold was resistant to all populations except race A of *M. hapla.*

Inoculation with *M. incognita* did not influence chlorophyll contents of any of the marigold cultivars (Table 1). However, total phenol contents of marigold cultivars in response to nematode inoculations increased with the increase in inoculum level of the nematode. At 500 inoculum level, the phenol contents of leaves increased by 24% in *T. patula* cv. Jafri to 32.5% in *T. erecta* cv. Hazara over respective controls (Fig. 1). Same varietal response was recorded at rest of the inoculum levels of the nematode and the increase in the phenolic contents was 60-80% and 90-195% with 2500 and 5000 J_2/pot, respectively. Phenolic compounds are considered important in imparting host resistance and also inducing systemic acquired resistance in plants (Kuc, 1995). Greater synthesis of phenol compounds in chickpea and tobacco plants inoculated with root-knot nematodes has also been reported earlier (Pankaj *et al.,* 2005; Khan and

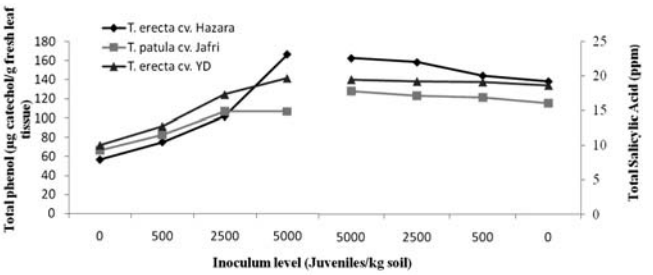

Fig. 1. Phenol and salicylic acid contents of leaves of *Tagetes* spp. in response to inoculations with *Meloidogyne incognita*

Haq, 2011). The drastic increase in the phenol contents in response to nematode inoculation in all three cultivars recorded in the present study supports the role of phenols on nematode resistance in plants.

Salicylic acid contents also got influenced with the nematode inoculation. A significant increase in the salicylic acid content was recorded with 2500 J_2 (*Pd"*0.01) and 5000J_2 (*Pd"*0.001) of *T. erecta* cv. Hazara over uninoculated control (Fig. 1). In other two cultivars increase in salicylic acid due to nematode inoculation was significant at *P* d" 0.05. Salicylic acid is considered as a key factor in inducing systemic acquired resistance in plants against pathogens (Ryals *et al.,* 1996; Wobbe *et al*., 1996). Tolerant or resistant plants synthesize salicylic acid in greater concentrations to avert the pathogen attack (Ryals *et al.,* 1996). Greater concentration of salicylic acid was recorded in the cv. Hazara which supported ingress of juveniles in roots relatively less than other two cultivars.

The present study has demonstrated that marigold cultivars tested were found resistant to root-knot nematode, *M. incognita* and did not developed any galling or egg mass production. This resistance was mainly biochemical rather than morphological. The small proportion of nematode population (3-11%) that penetrated into roots was not able to grow normally and did not attend maturity evidenced by absence of saccate females, galls and egg masses. This has revealed that although a limited number of larvae could penetrate the roots, but because of synthesis of greater amount of phenols and salicylic acid the nematode pathogenesis could not proceed further. Marigold cultivars not only

antagonized the penetrated juveniles, but also suppressed the nematode larvae in soil and caused mortality to them apparently through root exudates.

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Efficacy of Essential Oil Extracts of Medicinal Plants Against Rice Root-Knot Nematode *Meloidogyne graminicala* in Pots

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ABSTRACT: A tub experiment was conducted to study the efficacy of essential oil extracts of medicinal plants against *Meliodogyne graminicola* on common rice variety Dharam.Essential oil extracts obtained from five selected medicinal plants viz. *Parkia javanica, Zanthoxylum acanthopodium, Jatropha curcas, Vitex negundo* and *Adatoda vasica* were tested as seed soaking against rice root knot nematode. The seeds were soaked in 100ppm concentration for 24 hours. The plants treated with essential oil extract of *Jatropha curcas* showed improvement in plant growth and reduction in diseases incidence when compared with other treated plants. Essential oil extracts of *Zanthoxylum acanthopodium* was found to be least effective among five tested plants.

Key words: Essential oil, *Meloidogyne graminicola, Oryza sativa(local variety Dharam.)*

Rice is believed to have first been domesticated in India, although the oldest surviving written record telling of its existence 500 years ago is from China.It is staple food for about half of the population of our planet and 80% or more of the world rice production is reported to be cultivated in the highly populated southern and eastern parts of Asia. Rice is grown in almost all the states of India as a principal food crop. Root knot nematode, *Meloidogyne graminicola* is recognized as one of the serious nematode pest of rice that causes root knot diseases characterised by the presence of root galls or knot on the roots, yellowing, stunting and wilting of the plant is related to nematode-to-root biomass ratio (Singh *et al.,* 2006). Nationally *M. graminicola* is reported to cause upto 50% loss in grain yield (Rao & Biswas, 1973) and in severe cases it may go upto 64% (Phukan, 1995) Losses in grain yield were also estimated to range from 16-32 % due to this nematode (Rao & Biswas, 1973). This diseases is widely distributed in rice growing areas of the world. Occurrence of the diseases was reported from different states of India by several workers including Western and Central part of Uttar Pradesh (Kamalwanshi *et al.,* 2002 and Pankaj *et al.,* 2010).Considering the damaging potential and heavy economic losses caused by this nematode on rice, the present investigation were undertaken to evaluate essential oil extracts of medicinal plants against root knot nematode on local breed Dharam (local rice variety) in tub experiments.

MATERIALS AND METHODS

Healthy leaves of *Parkia javanica, Zanthoxylum acanthopodium, Jatropha curcas, Vitex negundo* and *Adatoda vasica* were collected, washed with water and oven dried at 58±2°C for 48 hours before making into powder with the help of a domestic grinder. For extraction of oil 50g dry weight of each plant porduct was taken and the essential oil extracts was done with the help of Clevenger apparatus. The solvent was distilled off and transferred into a separate beaker, which was completely evaporated from the extract in oven till it become semi solid material. The essential oil extracts was emulsified with triton X-100 as emulsifier. The seeds of rice plant (Dharam local variety) were soaked in stock solution of different oil extract mention above for 24 hours. Then, the seeds were spread and allow to dry under shade condition before sowing. The seeds were soaked in distilled water served as control. The seed from each

treatment were sown in 30 cm tub containing 5 kg soil. Each treatment were replicated three times. After three weeks of sowing, plants were inoculated with freshly hatched 1000 J2 of *Meloidogyne graminicola.* The control was also inoculated and replicated three times.

Thirty five days after inoculation of J2 the mature plants were uprooted with the help ofhoe and gently washed with running tap water. The plants were cut at the margin ofroot and shoot. Length of the root and the shoot was measured with the help of meter scale. Fresh weight of the root and the shoot was determined by physical balance. Root and shoot were kept separately in paper envelopes and kept in an incubator maintained at 58°C for 5 days. Dry weight of root and shoot was determined. The number of grains were also counted.Number of galls, root knot index (0-5 scale) and final nematode population (both in soil and root) were also recorded.

RESULTS AND DISCUSSION

The germination percentage of rice seeds was not affected by seed treatment with oil extract of medicinal plant and it was similar and better in all the treatments (Table 1). In general, all the treatment showed better plant growth but also reduced the damage by root knot infestation of rice as compared to control. It is clearly indicated from the data in Table-1 that shoot and root length of rice plant treated with *Jatropha curcas* was maximum followed by rice plant treated with *Adhotoda vasica.* The highest number of grains was also recorded in *Jatropha curcas* treated plant. The essential oil extract of *Zanthoxylum acanthopodium* was found to be least effective among the plant extracts.

In case of the incidence of diseases, there were reduction in infestation rate in *Jatropha curcas* oil extract treated plant by recording 7 galls and total polulation of only 580 indicating nearly half times reduction from initial inoculum level whereas in case of untreated one 23 number of galls with l690 nematode population were recorded indicating one and half times increased in population level (Table 2). The results supported works of Stirpe *et al.,* 1976 who reported that *Jatropha* oil cake is toxic to mammals due to presence of curcin and alectin which is conformity with the present findings. The seed oil of *Jatropha* is also reported to possess insecticidal, mollucicidal ,fungicidal and nematicidal properties (Chitra & Dhyani, 2006). Similar results was obtained on reduction of root knot galls and total population with the use of botanicals as reported by Verma & Khan (2004). Plant treated with the oil extract of *Zanthopodium acanthopodium* was found to be least effective with the 16 number galls and 1310 nematode population. From the above observations, it can be concluded that treated plants had low infestation rate and improvement in overall growth parameters in comparison to control but inoculated one (Table l).

The present investigation is in conformity with those reported by Kumar *et al.* (2011) they also reported management of root knot and reniform nematode in pots and suggested the effectiveness of the oil extracts of

Table 1. Effect of oil extract of medicinal plants against rice root-knot nemoatode infecting rice plant Dharam. in plant growth parameters

Medicinal Plants	Root Length (cm)	Shoot Length (cm)	Fresh Shoot wt. (g)	Fresh Root wt. (g)	Dry Shoot wt. (g)	Dry Root wt. (g)	Number of grains
P. javanica	5.96	30.5	8.99	3.01	6.77	1.45	41
X. acanthopodium	5.76	30.46	8.82	2.9	6.50	1.02	39
J. curcas	6.54	33.7	11.9	4.18	9.67	2.86	56
V. negundo	5.52	31	10.5	3.56	8.09	1.07	45
P. thyrsiflorus	5.99	32.1	10.9	3.98	8.87	1.91	48
Control	3.76	29	7.99	2.04	5.77	1.01	35

Table 2. Effect of oil extract of medicinal plants on nematode population against root knot nematode infecting Dharam local rice variety.

Medicinal Plants	Number of Galls/plant	Soil pop. (500 g of soil)	Root pop. /plant	Root-knot Index	Total Pop.	Rf
P. javanica	13	580	460	2	1040	1.04
X. acanthopodium	16	880	430	2	1310	1.31
J. curcas	7	360	220	1	580	0.58
V. negundo	10	730	320	2	1110	1.11
P. thyrsiflorus	9	430	390	1	820	0.82
Control	23	920	770	3	1690	1.69

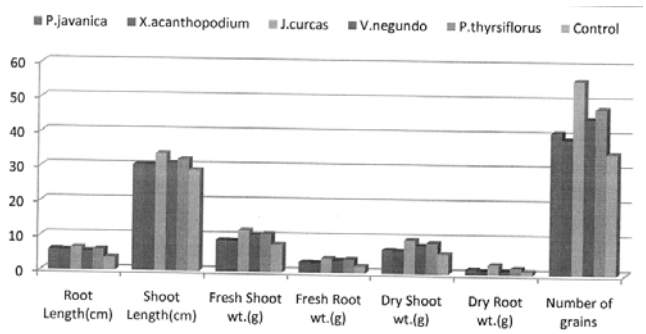

Fig. 1. Effect of oil extract of medicinal plants against rice root-knot nematode infecting rice plant Dharam. in plant growth parameters

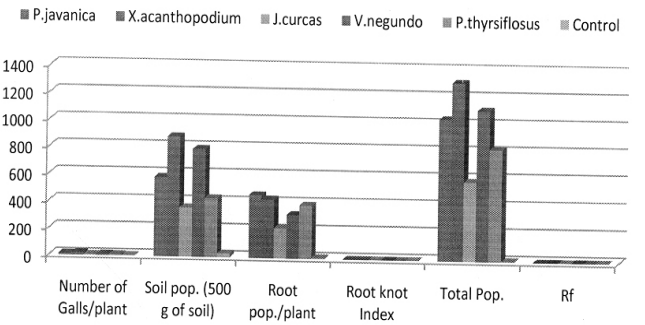

Fig. 2. Effect of oil extract of medicinal plants on nematode population against root-knot nematode infecting Dharam. local rice variety

sacred basil over the other extracts. This study was supported by Dora and Neog (2006) who tested oil cakes against root knot nematode larvae in terms of their mortality rate. The work also supported the finding of Raider & Askary (2011) investigated seed extracts of 17 plant extracts against *M. incognita* juvenile for their larval mortality and found inhibitory effects. Rena *et al.* (2009) tried out different extracts of plants against *M. incognita* and found effective. The above investigation supported works of Joymati *et al.,* 2012 who reported the essential oil extract of medicinal plants was found to be effective against the root knot nematode on kidney bean. Prasad *et al.* (2002) reported some plant extracts were toxic to root-knot nematode and further application in pots enhanced plant growth significantly with reduction in the nematode population.

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SHORT COMMUNICATION

Management of Root-Knot Nematode, *Meloidogyne incognita* Race-II (Kofoid and White, 1919) Chitwood, 1949 infesting Brinjal (*Solanum melongena* L.)

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Brinjal or egg plant, *Solanum melongena* (Lin.) belonging to family solanaceae, is a native of India. It is grown in all South-East Asian countries. Brinjal is cultivated throughout the warmer regions of the world except in the regions having severe winter. Besides, its nutritional value, it is well known for its medicinal value and is useful against liver complaints, toothache, diabeties etc. It is also a good appetizer (Chaudhari, 1977).

In India, brinjal is being cultivated round in a year in *kharif, rabi* and summer season with area 6.07 lakh hectares and production 10.563 million tonnes. In addition to pest and diseases, plant parasitic nematodes have also become limiting factor in successful cultivation of brinjal. Many species of phyto nematodes have been found associated with rhizosphere of brinjal. Amongst, the root-knot nematode, *Meloidogyne incognita* race-II is considered to be of great economic importance.

The experiment was conducted during summer, 2011 on brinjal (Cv. Ankur) in the field of AICRP on Nematodes, Department of Agril. Entomology, M.P .K. V., Rahuri. The seedlings of brinjal grown in nursery were transplanted in a root-knot infested field at 60 x 45 cm spacing having 4;20 x.2.25 m gross plot size. The bioagents, organic amendments and nematicides were applied at the time of transplanting. There were eleven treatments *viz.,* neem cake @ 2 t/ha; *Pseudomonas fluorescens, Paecilomyces lilacinus, Trichoderma plus* and *Trichoderma viride,* @ 20 kg/ha alone and @ 10 kg/ha in combination with ne em cake @ I t/ha, carbofuran 3 G @ I kg a.i./ha including an untreated control replicated three times at Randomized Block Design. The observations at initial and final root-knot nematode population, number of root galls and egg masses, gall index/plant and yield at termination were recorded. The data obtained were statistically analysed and presented in Table 1 to 3.

The initial root-knot nematode population in the field plots was within the range of 480 to 620 J_2/200 cm^3 of soil.

It could be seen from the data presented in Table 1 that all the treatments were found significantly superior to an untreated control in reducing the root-knot nematode population number of root galls/egg masses and gall index and increasing the yield ofbrinjal.

However, the treatment of *P. lilacinus* at 10 kg/ha + neem cake at 1 t/ha was found to be most effective in reducing root-knot nematode population (51.28 %), number of root galls/egg masses (71.87 %) and gall index (37.33 %) and increasing the yield (34.67 %) of brinjal with 1 :5.24 ICBR at termination. This was followed by the treatment of *P. fluorescens* at 10 kg/ha + ne em cake at 1 t/ha, *Trichoderma plus* at 10 kg/ha + neem cake at 1 t/ha and *T. viride* at 10 kg/ha + neem cake at 1 t/ha which were equally effective and on par with each other in reducing root-knot nematode population (49.28 to

Table 1. Effect of different treatments on root galls/egg masses and gall index of root-knot nematode, *M. incognita* race-II infesting brinjal

Treatment	Decline in nematode population (%)	Number of root galls egg masses/ plant at termination	Gall index/ plant	Yield (q/ha)	ICBR
Soil application of neem cake at 2 t/ha at time of transplanting	32.17	69.74	4.60	175.00	1:1.05
Soil application of *Pseudomonas fluorescens* at 20 kg/ha at time of transplanting	36.37	61.54	4.06	184.44	1:4.67
Soil application of *Paecilomyces lilacinus* at 20 kg/ha at time of transplanting	37.21	58.60	3.86	189.73	1:5.64
Soil application of *Trichoderma plus* at 20 kg/ha at time of transplanting	34.55	70.07	4.53	182.01	1:4.23
Soil application of *Trichoderma viride* at 20 kg/ha at time of transplanting	35.83	65.94	4.30	179.89	1:3.85
Soil application of carbofuran 3G at 1 kg a.i./ha at time of transplanting	45.67	52.87	3.67	192.91	1:11.49
Soil application of *Pseudomonas fluorescens* at 10 kg/ha + neem cake at 1 t/ha	49.28	37.47	3.33	207.41	1:4.64
Soil application of *Paecilomyces lilacinus* at 10 kg/ha + neem cake at 1 t/ha	51.28	33.40	3.13	213.76	1:5.24
Soil application of *Trichoderma plus* at 10 kg/ha + neem cake at 1 t/ha	48.31	39.67	3.40	201.37	1:4.056
Soil application of *Trichoderma viride* at 10 kg/ha + neem cake at 1 t/ha	47.24	47.34	3.53	200.70	1:4.00
Untreated control	0.00	118.73	5.00	158.73	-
S.E.±	0.96	1.66	8.85	4.44	-
CD (P=0.05)	2.83	4.91	0.26	13.12	-

*Figures in parentheses are arcsin transformed values

Market Rates: 1. Brinjal fruits-Rs. 1000/q, 2. Bioagents-Rs. 250/kg, 3. Carbofuran-Rs. 75/kg, 4. Neem cake-Rs. 7.5/kg, 5. Labour charges- Rs. 500/ha (Soil application)

47.24 %), number of root galls/ egg masses (68.44 to 60.06 %), gall index (33.33 to 29.33 %) and increase in yield (30.67 to 26.44 %) over an untreated control with 1 : 4.64 to 4.00 ICBR.

The effectiveness of soil application of *P. lilacinus* + neem cake for management of root-knot nematode is in conformity with that of Cannayane and Rajendra (2001) on brinjal and Rao (2008) on acid lime for the management of root-knot nematode.

Hence, in the integrated management of root-knot nematode population, the soil application of *P. lilacinus* with neem cake was found most effective for the management of root-knot nematode.

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Management of Root-Knot Nematode, *Meloidogyne incognita*, Race-II Infesting Pomegranate by using Bioinoculants

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Pomegranate (*Punica granatum* L.), belongs to family Punicaceae is one of the important fruit crops of tropical and subtropical regions. This ancient world fruit originated in Persia, Iran, Afghanistan and Baluchistan (de Candolle, 1967).

Extensive cultivation of pomegranate has been reported in Mediterranerul countries like Spain, Egypt and Morocco. It is also grown to some extent in Burma, China, Japan, Russia, California (USA) and India. In India, it is grown over an area of 1.13 lakh hectares with an annual production of about 7.92 lakh metric tonnes.

Such an important fruit crop is attacked by several insect and non insect pests as well as diseases. Diseases caused by nematodes are of economic importance. Darkekar *et at.* (1990) reported ten species of plant parasitic nematodes associated with this crop in the Maharashtra state. The root-knot nematode, *Metoidogyne incognita* is one of the them causing considerable yield losses in pomegranate. Besides, the direct damage caused to the plant, the root-knot nematodes are notorious for disease complexes involving fungi, bacteria, virus, mycoplasma, insects and other nematodes (Dasgupta & Gaur, 1986).

The experiment was conducted continuously for three years during *kharif* , 2008-09 to 2010-11 at Central Campus, Mahatma Phule Krishi Vidyapeeth, Rahuri on pomegranate (cv. Mrudula) planted at 4.5 x 3 m spacing. The bioagents and nematicides were applied in the rhizosphere soil by ring method at 'Bahar' (Bloosom). There were five treatments viz., *Pseudomonas fluorescens* and *Trichoderma viride* at 20 kg/ha, cartap hydrochloride (4 G) at 75 kg/ha and carbofuran (3 G) at 100 kg/ha including an untreated control replicated four times at Randomized Block Design. The observations at initial, intermediate and termination on root-knot nematode population and number of root galls/5 g roots and yield/ tree were recorded. The pooled data obtained were statistically analysed and presented in Table 1.

The average initial root-knot nematode population in the field ranged from 360 to 600; 400 to 620 and 480 to

Table 1. Effect of different treatments on population of root-knot nematode, *M. incognita* race-II on pomegranate

Treatment	Decline in population (%)	Root galls/ 5g roots (%)	Yield (t/ha)	B:C Ratio
P. fluorescens at 20 g/m^2	31.2	29.2	18.4	1:2.37
T. viride at 20 g/m^2	28.3	23.9	18.0	1:2.33
Cartap hydrochloride 4 G at 0.3 g a.i./m^2	27.2	20.4	17.6	1:2.27
Carbofuran 3 G at 0.3 g a.i./m^2	29.8	25.1	17.8	1:2.27
Untreated control	-	-	15.4	1:2.27
S.E.±	1.03	0.80	0.36	-
CD (P=0.05)	2.99	2.30	1.17	-

640 12/200 cm3 of soil during 2008-09, 2009-10 and 2010-11, respectively.

It could be seen from the pooled data presented in Table 1 that all the treatments were significantly superior to an untreated control in reducing the root- knot nematode population and number of root galls/5 g root and increasing the yield of pomegranate.

However, the treatment of *P. fluorescens* at 20 kg/ ha was found to be most effective in reducing the root-knot nematode population (31.28 %)and number of root galls/5 g roots (29.28 %) and increasing the yield of pomegranate (18.99 %) with 1:2.37 B:C ratio. It was followed by the treatments of *T. viride* at 20 kg/ha and carbofuran (3 G) at 100 kg/ha. The reduction in root-knot nematode population, number of root galls/5 g roots and increase in yield over an untreated control recorded in these treatments were 23.28 and 29.81; 23.90 and 25.07 and 16.78 and 15.75 per cent with 1:2.33 and 1:2.27 B:C ratio, respectively.

The reduction in nematode population as a result soil application of carbofuran (3 G) may be due to inhibition of root-knot nematode. This is in conformity with that of reported by Hashim (1983), Siddiqui and Khan (1986), Darekar *et al.* (1989) in pomegranate.

The results of the present investigation of effectiveness of *P. fluorescens* is in collaboration with those reported by Anonymous (2008). *T. viride* was also found to be effective for the dontrol of root-knot nematode by Goswami & Singh (2004). As regards the additional yield and cost: benefit ratio, the treatment of *P. fluorescens* and *T. viride* is in conformity with that of reported by Tadavi (2008). Thus, effectiveness of *P. fluorescnes* and *T. viride* by lowering down the nematode population is extending the scope of biological control o phytonematodes .

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Induction of Defence Enzymes in Tomato by Plant Growth Promoting Rhizobacterium, *Pseudomonas fluorescens* against Root-Knot Nematode, *Meloidogyne incognita*

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Tomato (*Lycopersicon esculentum* Mill.) is one of the major vegetable crops grown in India. Root-knot nematodes *(Meloidogyne* spp.) are major pathogens of tomato throughout the world, affecting both the quantity and quality of marketable yields. Success in nematode management over a long term requires several alternatives and combinations of approaches to be economically feasible. Currently both naturally occurring and applied biological controls are being utilized to control plant parasitic nematodes. Biological control of nematodes has long been considered as an alternative to managing nematodes with pesticides.

Plant growth promoting rhizobacteria *Pseudomonas fluorescens* which live in close proximity of plant roots, help in boosting the plant growth and vigor by different mechanisms are deleterious to the plant pathogens. The bacteria achieve this mainly by bio-stimulation, bio-control, bio-fertilization and bio-remediation.

Induced systemic resistance (ISR) of plants against pathogens is a widespread phenomenon that has been intensively investigated with respect to the underlying signaling pathways as well as to its potential use in plant protection. Changes in cell wall composition, *de novo* production of pathogenesis - related-proteins such as chitinases, glucanases, peroxidase, polyphenol oxidase, phenylalanine ammonia-lyase and synthesis of phytoalexins are associated with resistance due to application of *P. fluorescens.*

Peroxidases are involved in the regulation of plant cell elongation, phenol oxidation, IAA oxidation, oxidation of hydroxy cinnamyl alcohols into free radical intermediates and wound healing. Polyphenol oxidase accumulation takes place upon wounding by pathogens in plants. PPO is induced *via*. octadecanoid defense signal pathway which is usually associated with feeding by insects or similar physical trauma. It involves jasmonic acid as an intermediate signal and culminates in the production of proteins such as PPO and proteinase inhibitors (Schaller & Ryan, 1995). Phenylalanine ammonia lyase (PAL) catalyzes the deamination of L-phenylalanine to trans-cinnamic acid, which is the first step in the biosysthesis of large group of plant products such as lignin monomers and phytoalexins. Induction of PAL, peroxidase and the accumulation of such phenolics occur in response to pathogen attack. Therefore, a study was conducted to assess the induction of the defense enzymes by applying talc-formulation of *P. fluorescens* against root-knot nematode in to tomato.

A field experiment was conducted in Coimbatore, India on tomato cv.PKM-1 infested with *M. incognita* the talc-based formulations of promising *P. fluorescens* isolates were tested against *M. incognita* infesting tomato. The talc-based formulation of the promising *P. flourescens* isolates Pft 18, Pft 20 and Pft 25, and standard Pf 1 were applied to the soil at two doses *viz.,* 2.5 g/pot and 3.0 g/pot and chemical carbofuran at 1 g a.i./pot. Untreated control was also maintained (Table 1). The plot size of 10 m^2 was maintained for all the treatments. The formulation was applied 30 days after planting of the tomato seedlings of cv. PKM-1. The population densities of *P. fluorescens* in the formulations were 2.5 x 10^8 cfu/g. The study was undertaken with the following treatments by using randomized design with ten treatments each replicated three times. The treatments are as follow T1: Pft 18 @ 2.5 kg / ha; T2: Pft 18 @3.0 kg / ha; T3: Pft 20 @ 2.5 kg / ha; T4: Pft 20 @ 3.0 kg / ha; T5: Pft 25 @ 2.5 kg / ha; T6: Pft 25 @ 3.0 kg / ha; T7: Pf 1 @ 2.5 kg / ha; T8: Pf 1 @ 3.0 kg / ha; T9: Carbofuran @ 1kg a.i./ ha; T10: Control

The activity of defense enzymes were analyzed in the leaf samples 30 days after application of *P. fluorescens*. Peroxidase activity was assayed spectrophotometrically (Hartee & Keilin, 1955). The reaction mixture consisted of 1.5 ml of 0.05 M pyrogallol, 0.5 ml of the enzyme extract and 0.5 ml of 1 percent H_2O_2. The reaction mixture was incubated at room temperature (28 ± 1 ° C). The change in absorbance was recorded at 30 sec interval for 3 min. The boiled enzyme preparation served as blank. The enzyme activity was expressed as change in the absorbance at 420 hM/min/g on fresh weight basis (Hammerschmidt *et al*., 1982). Polyphenol oxidase activity was determined as per the procedure given by Mayer *et al.,* (1965). One g of leaf was used for polyphenol oxidase estimation. The reaction mixture consisted of 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5) and 200 ml of the enzyme extract. To start the reaction, 200 ml of 0.01 M catechol was added and the activity was expressed as changes in absorbance at 495 hM/min/g fresh weight of tissue. The assay mixture containing 100 ml of enzyme, 500 ml of 50 mM Tris HCl (pH 8.8) and 600 ml of mM L -phenylalanine

Table 1. Effect of *Pseudomonas fluorescens* isolates on peroxidase, polyphenol oxidase and phenylalanine ammonia lyase activities in leaves of tomato cv. PKM-1

Treatment	Peroxidase*	Per cent increase over control	Poly phenol oxidase**	Per cent increase over control	Phenylalanine ammonialyase***	Per cent increase over control
Pft 18 (2.5 kg/ha)	0.45	67.1	0.07	16.9	0.31	7.5
Pft 18 (3.0 kg/ha)	0.42	54.3	0.06	4.6	0.31	6.5
Pft 20 (2.5 kg/ha)	0.77	181.0	0.09	27.6	0.44	52.2
Pft 20 (3.0 kg/ha)	0.76	179.9	0.09	24.6	0.44	51.2
Pft 25 (2.5 kg/ha)	0.32	17.5	0.07	12.3	0.29	34.0
Pft 25 (3.0 kg/ha)	0.31	13.8	0.07	9.2	0.28	32.9
Pf 1 (2.5 kg/ha)	0.68	151.4	0.07	21.5	0.43	49.1
Pf 1 (3.0 kg/ha)	0.57	108.0	0.07	16.9	0.40	38.1
Carbofuran 3G	0.37	36.8	0.07	10.7	0.31	7.9
(1 kg a.i./ha)						
Control	0.27	-	0.06	-	0.25	-
CD (P=0.05)	0.17	-	0.01	-	0.05	-

*Changes in the absorbance/min/g leaf tissue at 480 nm, ** Changes in the absorbance/min/g leaf tissue at 495 nm, *** Changes in the absorbance/min/g leaf tissue at 290 nm

ammonia lyase was incubated for 60 min. The reaction was arrested by adding 2 N HCl. Later 1.5 ml of toluene fraction containing trans – cinnamic acid was separated. The toluene phase was measured against the blank of toluene. Standard curve was drawn with graded amounts of cinnamic acid in toluene as described earlier. The enzyme activity was expressed as hM of cinnamic acid at 290 hM min $^{-1}$ g^{-1} of fresh tissue.

The results showed an enhanced activity of defense enzymes in tomato plants treated with *P. fluorescens*. The Pft 20 @ 2.5 kg/ha treated tomato leaves recorded 0.770 followed by Pft 20 @ 3.0kg/ha which recorded 0.767 and the control being 0.274. A significant increase in polyphenol oxidase activity was observed in the Pft 20 treated plants followed by the application of Pf1, Pft 18 and Carbofuran. Resistant plants of tomato when inoculated with nematode *M. incognita* showed higher concentration of peroxidase (Zacheo *et al.,* 1982). Sujatha and Mehta (1998) found that there was an increase in enzyme levels of peroxidase and polyphenol oxidase in sugarcane roots infested with *M. javanica*. Galled roots contained twice the amount of simple sugars and insulin than that of the healthy roots (Epstein, 1972). Quantitative increase in peroxidase activities was obvious throughout the period of observation in the resistant tomato in the susceptible tomato plants (Ganguly and Dasgupta, 1979).

Phenylalanine ammonia lyase activity significantly increased in the Pft 20 treated plants by 0.443 when compared to the untreated control which recorded 0.251. PAL plays a significant role in regulation and accumulation of phenolics, which directly related to the degree of plant resistance to pathogens. Increased phenolic synthesis is observed in the *P. fluorescens* treated plants (Graham & Graham, 1991).Induction of systemic resistance was correlated with the accumulation of defense enzymes PO, PPO and PAL upon treatment with plant growth promoting rhizobacteria, *P. fluorescens* and there by reduced the infection by root-knot nematode.

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Quantitative Estimation of Free Amino Acids and Amides in Okra Inoculated with *Meloidogyne incognita*

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Stock cultures of the root-knot nematode, *Meloidogyne incognita* for the present study was obtained from a single egg mass progeny, maintained and multiplied on susceptible brinjal variety, Pusa Purple

Long. Earthen pots of 15 cm dia were surface sterilized with formaldehyde solution (1.0 %) and filled with autoclaved soil @ 1 kg/pot. Surface sterilized seeds of okra variety L.B.H-55 were planted in pots. One month after germination second stage juveniles of *M. incognita* were inoculated @ 1000 J2 ± 20 per kg soil. Side by side a healthy uninoculated check was also maintained. After 45 days of nematode inoculation the plants were uprooted and the roots were thoroughly washed with tap water and double distilled water. Then the roots were surface sterilized in 0.1% Mercuric chloride solution followed by repeated washing with distilled water and taken for analysis of amino acids and amides. Root samples of both healthy and inoculated plants were taken, ground separately in 80 percent ethyl alcohol, boiled for 5 to10 minutes in a hot water bath and filtered through a double layered fine cheese cloth. The extracts were centrifuged at 5000 rpm for 10 minutes. The supernatants carefully decanted into separate watch glasses and allowed to evaporate till a slight yellowish colour syrupy material obtained. This syrupy material was dissolved in 1 ml of 10 % isopropyl alcohol and transferred to separate glass vials representing for a single lot and stored in the freezer compartment of refrigerator. The above solution was used for estimation of amino acids and amides quantitatively by paper chromatographic technique and subsequently by using TLC scanner (densitometer). After measuring the density of various amino acids and amides from developed chromatograms, their concentrations were calculated with the help of standard graph of known concentration. The results were expressed in terms of milligrams of amino acids and amides per gram fresh roots.

Results presented in Table-1 revealed 9 amino acids viz. L-cystine, L-arginine, L- serine, L-glutamic acid, L-

Table 1. The Rf value of ninhydrin positive spots on chromatogram of inoculated and un-inoculated okra variety L.B.H-55

Rf. Value of inoculated cultivars	Amino acids and amides identified	Rf. Value of un-inoculated cultivars	Amino acids and amides identified
1.4/22.5=0.06xI00=6	L-cystine	1.4/22.5=0.06xI00=6	L-cystine
2.9/22.5=0. 13x100=13	L-arginine	2.9/22.5=0.13xI00=13	L-arginine
4.1/22.5=0.18xI00=18	L-serine	4.1/22.5=0.18xI00=18	L-serine
5.3/22.5=0.24xI00=24	L-glutamic acid	5.3/22.5=0.24xI00=24	L-glutamic acid
2.6/22.5=0. 12x100=12	L-aspargine*	2.6/22.5=0. 12x100=12	L-aspargine*
7.4/22.5=0.33xI00=33	L-alanine	4.8/22.5=0.21xI00=21	L-glycine
2.3/22.5=0. 10x100=10	L-Iysine	7.4/22.5=0.33xI00=33	L-alanine
9.5/22.5=0.42x100=42	L-tyrosine	9.5/22.5=0.42xI00=42	L-tyrosine
8.2/22.5=0.36xI00=36	L-proline	3.7/22.5=0.16x100=16	L-glutamine*
11.5/22.5=0.5IxI00=51	L-tryptophan	2.1/22.5=0.09xI00=9	L-histidine
14.4/22.5=0.64xI00=64	L-valine	11.5/22.5=0.51xI00=51	L-tryptophan
13.7/22.5=0.61xI00=61	L-phenyl alanine	13.7/22.5=0.61x100=61	L-phenyl alanine
		16.3/22.5=0. 72x1 00=72	L-Ieucine
		17. 1/22.5=0.76xI00=76	L-isoleucine
		4.3/22.5=0. 19x100=19	L-aspartic acid
		6.0/22.5=0.27xI00=27	L-threonine

* amide

Table 2. Quantity of amino acids and amides in healthy and *M. incognita* inoculated okra (var- L.B.H-55) root

Name of amino acids and amides	Healthy mg/g	Inoculated mg/g	% increase(+) or decrease(-) over healthy
L-cystine	0.052	0.069	+32.69
L-arginine	0.098	0.087	-11.22
L-serine	0.049	0.056	+14.29
L-glutamic acid	0.075	0.089	+18.67
L-aspargine	0.038	0.048	+26.31
L-alanine	0.073	0.091	+24.66
L-phenyl alanine	0.072	0.089	+23.61
L-tyrosine	0.061	0.049	-19.67
L-tryptophan	0.046	0.024	-47.83
L-glycine	0.058	-	-
L-glutamine	0.044	-	-
L-histidine	0.029	-	-
L-aspartic acid	0.056	-	-
L-Ieucine	0.047	-	-
L-isoleucine	0.054	-	-
L-threonine	0.051	-	-
L-Iysine	0.056	-	-
L-proline	0.041	-	-
L-valine	0.082	-	-

aspargine, L-alanine, L-phenyl alanine, L-tyrosine and L-tryptophan common to both healthy and inoculated roots. However, 7 amino acids specific to healthy roots namely L-glycine, L-glutamine, L-histidine, L-aspartic acid, L-Ieucine, L-isoleucine and L- threonine were identified. Similarly amino acids specific to inoculated root were L-Iysine, L- proline and L-valine. Perusal of data of both qualitative and quantitative study (Table-2) indicated increase in most of the amino acids and amide common to healthy and inoculated roots after post infection period except L-arginine, L-tryptophan and L-tyrosine. Similar increase in various amino acids was also recorded by Mohanty and Pradhan, 1990; Mohanty *et al,* 2001; Mishra and Mohanty, 2007; Tripathy and Mohanty, 2008 in different plants. The increase in various amino acids in infected plant may be due to progressive hydrolysis of plant proteins by the proteolytic enzymes secreted by root-knot nematode, *M. incognita.* The reduction of L- tryptophan (47.83%) in nematode inoculated samples might be attributed to the diversion and synthesis of IAA for gall formation as reported by Krusberg, 1961. Accumulation of L-proline in nematode inoculated roots as observed in the present investigation is in agreement with Lewis and McClure, 1975; Mohanty and Das, 1976; Mishra and Mohanty, 2006; Tripathy and Mohanty, 2008. Proline accumulation may be attributed as a mechanism in plants to overcome nematode induced stress as reported by Epstein, 1972. Further the reduction of L-arginine in nematode inoculated sample may be due to conversion of L-arginine to L-proline through Ornithine cycle. Decrease in aromatic amino acid L-tyrosine in nematode infected samples in the present study have significant role in resistance. It is also interesting to note that during post infection period some of the amino acids appeared and some other disappeared along with an increase in certain amino acid accompanied by concomitant decrease in another amino acid as reported by Epstein and Cohn, 1971. Such phenomenon suggests possible interconversion of one amino acid from another.

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Distribution of Root-Knot Nematode in Ornamentals of Jorhat district, Assam

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The ornamental plants and flowers are high valued cash crops when grown commercially. The income per unit area from floriculture is much higher than any other agriculture products. The economic importance of ornamentals has been increasing day by day and so is the international demand and market. The recent statistics shows that the area under cut flowers in our country is 64,768 ha (Dadlani, 1999). Five hundred hectare climatically controlled greenhouses are available for growing quality flowers for export. Though the production of ornamental is increasing day by day still the total yield is significantly less as compared to the demand. One the main causes is the pest and diseases. Pest attack not only reduces the production of ornamentals quantitatively, but also deteriorate the quality of produced thereby reducing the market value drastically. Among the major constrains in flower production, plant parasitic nematodes are found to have deleterious affect on quantitative yield and quality of flowers.

Sasser and Freckman (1987) reported 11 per cent losses in ornamental plantations throughout the world due to plant parasitic nematodes. Nematodes are reported to be major constraint in ornamental production by various workers in India (Jayaraman *et al*. 1975, Khan & Pal, 2001).

Among the plant parasitic nematodes, root knot nematode, *Meloidogyne incognita*, is a major nematode pest infesting almost all the ornamentals.

To study the occurrence and distribution of root-knot nematode in ornamental plants of Jorhat district, a random roving survey was carried out during 2009-2011, covering seven blocks of Jorhat district. Soil and root samples were collected from different nurseries, homestead ornamental gardens, parks, farms and from green houses, distributed randomly throughout the district. A total of 174 Soil and root samples were collected from the rhizosphere of different ornamental plants up to a depth of 15-30 cm depending on the depth of the roots. Each bulk sample was constituted of several sub samples and portion of roots collected randomly from different sites of each locality. Samples were collected with the help of a khurpi or a hoe from the rhizosphere of the crop and then kept in polythene bags and tightly tied with a twine thread. An aluminium tag containing information like host crop, locality, date of collection was tied with the polythene bag carrying the soil and root samples. The samples were then taken to the laboratory and stored in refrigerator at about 10º C till the extraction of nematodes was made.

Concentrated nematode suspension was taken in a beaker and left undisturbed for few minutes allowing the nematodes to settle down at the bottom. An equal amount of double strength boiling formalin solution (8%) was added to the suspension in the beaker. The nematode suspensions were stored in small glass bottle with proper label. The root samples were taken out from the polythene bags to observe for the presence or absence of galls and eggmasses on the roots. For staining of nematodes within the plant tissue, Acid Fuchsin in lactophenol method was followed (Byrd *et al*. 1983). Cut roots were then wrapped with a muslin cloth and tied with a twine thread. The muslin cloth was then plunged into boiling lactophenol containing 0.05-0.1 per cent acid fuchsin stain and boiled for 2-5 minutes.

Preparation of perineal pattern was done for the confirmation of the root-knot species (Taylor and Nester 1974).The species was confirmed to be *Meloidogyne*

incognita. Dorsal arch was high, squared-off and the dorsal arch composed of smooth to wavy striae. Some striae forked near the lateral lines and lateral incisures are not so distinct.

A Green house, growing gladiolus, observations revealed that, the extent of damage caused by this nematode was much higher within the greenhouse as compared to the outdoor plants. Symptoms of infestation was observed in yellowish / brownish patches. Above the ground, the plants were stunted and discoloured, nematodes also affected the size and leaf count of the plants.

A total of 174 samples were collected randomly from different blocks of Jorhat district, *viz*., Baghchung (A.A.U Campus and Jamuguri), Dhekorgorah (Namdeori and Rajpriom nursery), Majuli (Kamalabari), Ujoni Majuli (Maz Deori gaon), Kaliapni (Teok), Titabar (Bebejia and Central Muga Research Station) and Chipahikhola (Kakojan). Root-knot nematode infestation was recorded in most of the root and soil samples. Out of 174 samples, root knot nematode was detected in 103 samples from different places. The frequency of occurrence in Baghchung, Dhekorgorah, Majuli, Ujoni Majuli, Kaliapni, Titabar and Chipahikhola are 56.75, 64.70, 66.66, 63.63, 70.00, 48.38 and 76.92 per cent respectively. The maximum frequency was recorded in Chipahikhola block i.e. 76.92 per cent, whereas the minimum frequency of occurrence was recorded in Titabar block i.e. 48.38 per cent.

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Spectral Signature of Mungbean Infested with Root-Knot Nematode, *Meloidogyne incognita* under Different Management Options

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Spectral reflectance from the plant canopy vary with conditions which actually are govern by different biotic and abiotic factors however these are specific to conditions. These could be part of remote sensing exercise to get the ground truth. It has been seen that chlorophyll content, water and mineral content and overall turbidity of system guide reflectance. In this backdrop an effort was made to get the reflectance pattern from the mungbean plant canopy infested with different level of root knot nematode *Meloidogyne incognita*. Does it discriminate as per the infestation load. Also an attempt has been made to get the reflectance pattern from the mungbean canopy when the management options are implied against root knot nematode. This can form a diagnostic tool for nematode problem identification at field situation.

Experiment was conducted in green house condition in 6" earthen pots filled with 1 kg of steam sterilized soil. Mungbean cv Pusa vishal was used for the experiments. Experiment was done with six replication in completely randomize design. Root-knot nematode culture was isolated from the brinjal seedling and the inoculation was made as 0. 10, 100,1000, 4000, 6000 J2 per pot (T1, T2,T3,T4,T5,T6,T7) along with untreated (Fig. 1). Reluctance was captured after 25 days of inoculation . In another experiment root knot nematode was managed with carbofuran 1 and 2 kg a.i/ha (T1 and T2), Trichoderma harzianum 1 and 2 %w/w (T3 and 4) beside neem oil coating of seed at 1 ad 2% w/v (T5 and 6) of mungbean along with nematode control (T7) and untreated (T 8) (Fig. 2). The reflectance was captured after 25 days of inoculation. Reflectance was measured spectrophotometrically on plant canopies

As shown in figure 1 that reflectance varies with inoculums level. Reflectance has been high (0.3-0.4) with highest inoculums level 6000 J2/pot in the wavelength of 300-700um and lowest reflectance has been with no or 100 J2/pot. However, this trend become opposite at high wavelength of 700-1000.

Reflectance from the plant canopy of mungbean in response to nematode management indicated (fig 2) that at lower wavelength 300-700um reflectance are mix one and could be determined easily which has high or low however at higher wavelength 700-1000um lowest 0.35 and median 0.45 reflectance is observed with low and high conc of carbofuran respectively. High reflectance of 0.5 and low reflectance of 0.4 has been observed with *T. harzianum* high and low conc. respectively. While with neem oil reflectance is similar 0.45 at both the conc. Highest reflectance of 0.6 has been observed with untreated un inoculated. Gausman et al (1975) observed differences between light reflectance from leaves of cotton (*Gossypium hirsutum*) plants grown with a low- or no-nematode (*Rotylenchulus reniformis*) population (nonstressed), and from leaves grown with a high nematode population (stressed), over the 0.5- to 2.5-/ microm waveband, stressed leaves had lower reflectance than nonstressed leaves of the same chronological age for both field- and greenhouse-grown plants. Reflectance differences between stressed and nonstressed leaves in the visible (0.5 to 0.75 microm), near-infrared (0.75 to 1.35 mum) and infrared water absorption (1.35 to 2.5 microm) regions were primarily caused by differences in leaf chlorophyll concentration, mesophyll structure, and water content, respectively. Similarly Palacharia *et al.* (2011) used hyperspectral data for reniform nematode problem detection in cotton fields. Likewise use of remote sensing in detection of soil nematode is highlighted by Chrishtian Hillnhutter *et al.* (2010). Lawrence, *et al.* (2007) reported nematode management using remote sensing technology, self-organized maps and variable rate nematicide applications. Nutter *et al.* (2002) studied

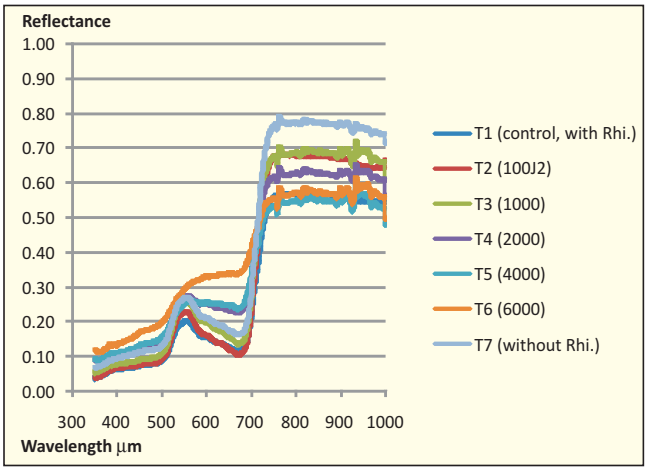

Fig. 1. Effect of inoculum levels of *Meloidogyne incognita*, on the reflective pattern of light from plant leaf canopy in mungbean cv. Pusa Vishal after 25 days of inoculation

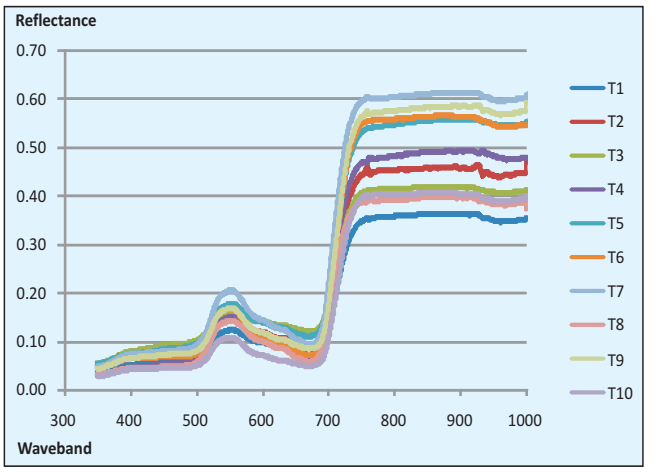

Fig. 2. Effect of management options on the reflective pattern of light from plant leaf canopy in mungbean cv. Pusa Vishal

the use of remote sensing of sybean cyst nematode with infra red reflectance through landsat and observed 60% variation in data.

Results indicate the potential for remotely sensing to detect nematode-infested plants.

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Diversity of Plant Parasitic Nematodes in Banana at Ten Districts of Assam

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Banana and plantains constitutes the fourth most important fruit crops in the tropics and subtropics forming major constituents of diet for millions of people across the globe.

Production of banana is challenged by many pests and diseases, of which plant parasitic nematodes are considered as countervail. Globally, a total of 151 species of nematodes belonging to 51 genera have been documented to be associated with banana rhizosphere (Gowen & Queneherve, 1990; Koshy & Gulsar Banu, 2000). In India, 71 species of plant parasitic nematodes are known to be associated with banana (Krishnappa & Reddy, 1995; Koshy & Sosamma, 2001). Yield loss caused by nematodes to bananas are very high, with average annual yield losses estimated at about 20 per cent worldwide (Sasser & Frackman, 1987), but may reach up to 40 per cent or more when frequent tropical storms topple plants with rotten, nematode infested root system.

Keeping the view of limiting production and productivity of banana by nematodes the present investigation was carried out to examine the biodiversity of plant parasitic nematodes around banana rhizospheres in ten districts of Assam.

Roving survey was conducted at ten banana growing districts of Assam during 2006-2011. Plant as well as root samples were collected from around the rhizosphere of banana at around 30 cm away from the bole of the plant and at a depth of 15-50 cm. From each site a total of 5 subsamples were collected, and then these subsamples were mixed thoroughly to draw a homogeneous sample of 500 gram. 10 gram of root sample also collected from each site. Thus, a total of 150 samples were collected from nine districts of Assam. Soil samples (200cc of soil from each sample) were processed by following Cobb's modified sieving and decanting technique and root samples (5 gm of root from each sample) were processed by Baermann Funnel

Table 1: Plant parasitic nematodes associated with banana rhizosphere at different districts of Assam.

Districts	Population of different nematodes (200cc of soil)					
	Helicotylenchus dihystera	***Meloidogyne incognita***	***Pratylenchus thornei***	***Rotylenchulus reniformis***	***Tylenchorhynchus leviterminalis***	***Macroposthonia sp***
Nalbari	88 – 116 (80.25)	48 – 85 (56.25)	18 – 46 (37.50)	24 – 50 (43.75)	19 – 64 (50.0)	12 (13.33)
Borpeta	52 – 172 (75.0)	52 – 98 (60.0)	25 – 54 (50.0)	28 – 56 (37.5)	16 – 54 (31.25)	7 (6.67)
Cachar	35-160 (80.0)	26 – 52 (55.0)	10-28 (30.0)	10 – 62 (50.0)	23 – 82 (30.0)	5 (11.33)
Karimganj	25 – 170 (70.0)	12 – 40 (45.0)	4 – 16 (35.0)	9 – 18 (50.0)	9 – 46 (35.0)	7 (10.0)
Hailakandi	35 – 138 (75.0)	12 – 52 (50.0)	4 – 18 (40.0)	6 – 22 (45.0)	8 – 60 (35.0)	5-12 (10.0)
Karbi Anglong	56 – 161 (85.0)	43 – 76 (60.0)	34 – 85 (35.0)	77 – 121 (40.0)	32 – 121 (40.0)	13-20 (30.0)
Lakhimpur	85 – 162 (100.0)	25 – 52 (56.0)	38 – 88 (20.0)	—	24 – 50 (43.0)	18-27 (25.0)
Dhubri	52 – 142 (100.0)	46 – 54 (70.0)	32 – 98 (15.0)	25 – 54 (21.0)	28 – 56 (41.0)	7-16 (19.0)
Golaghat	121 – 156 (100.0)	32 – 65 (60.0)	—	33 – 43 (26.33)	21 – 33 (60.0)	12-45 (80.0)
Jorhat	32 – 220 (100.0)	12 – 76 (66.67)	10 – 78 (35.67)	25 – 88 (28.55)	7 – 91 (62.0)	23-35 (26.33)

(Data in parenthesis are the per cent frequency of occurrence)

technique (Christei and Perry, 1951). Nematodes were identified upto generic level under stereozoom binocular microscope and counting of nematodes were made by using a Hawkshly Nematode counting dish. Identification of nematodes up to species level was done under stereozoom research microscope. Frequency of occurrence was calculated following Norton's (1978) formula.

All the 250 soils and root samples were found positive for plant parasitic nematodes. A total of eight different plant parasitic nematodes (Table 1), belonging to eight genera were recorded from the rhizosphere of banana plantations of ten districts of Assam. Plant parasitic species recorded during the present investigation are: *Helicotylenchus dihystera, Meloidogyne incognita, Pratylenchus thornei, Rotylenchulus reniformis, Tylenchorhynchus leviterminalis, Hoplolaimus* sp., *Macroposthonia* sp., *Psilenchus* sp., and *Longidorus* sp. *Psilenchus* sp and *Lonidorus* sp. has been reported for the first time from around the rhizosphere of banana in Assam. Study revealed that *Helicotylenchus dihystera* was the most predominant nematode species in all the districts surveyed, with 80 – 100 per cent frequency of occurrence. The gall forming nematode, *Meloidogyne incognita* was found to occur in 55-70 per cent of the samples. Another important nematode, *Pratylenchus thornei* occurred in 15 – 50 per cent of the samples.

Population of *H. Dihystera* was found more in all the districts, irrespective of the banana cultivar. Singh & Uma (1996) also recoded high population of this nematode irrespective of growing season and variety. Root-knot

nematode, *M. Incignita* was found to produce medium root gall on the root system of banana. During the visual observation, no external plant symptoms were noticed on plants affected by root knot nematode. The attack of *M. Incognita* alone can cause a loss of 30.95 per cent to yield of banana (Jonathan & Rajendran, 2000),while in association with *Fusarium* the loss may be higher.

Population of lesion nematode, *Pratylenchus thornei* was recorded at lower level and this species was not recorded from Golaghat district during the present survey. Characteristic dark lesion on the cortical region of root was observed, mostly on cv. 'Jahaji'. The present investigation revealed the multi nematode species infestation in the banana plantation. Khan and Hasan (2010) also observed similar kind of nematode infestation on banana in West Bengal. They recorded eight different genera of plant parasitic nematodes from the banana rhizosphere.

The investigation on nematode diversity around banana rhizosphere indicated that banana roots are prone to nematode attack and they co-inhibit a large density forming a nematode species complex. The information generated through this investigation will be helpful to provide advisory services and to undertake pest risk analysis.

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Screening of Cucumber Cultivars Against *Meloidigyne incognita*

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Root-knot nematode, *Meloidogyne incognita* is a polyphagus pest of vegetable crops including cucumber (*Cucumis sativus* L.) causing significant reduction in plant growth and fruit yield. Krishnaveni and Subramanian (2002) recorded 69.2 per cent yield loss in cucumber due to *M. incognita*. Therefore, effort was made to screen varieties of cucumber for their resistance against this major nematode pest. Darekar and Bele (1990) observed that with an increase in the inoculums level of *M. incognita*, there was a progressive decrease in growth parameters of cucumber seedlings. In the present investigation, 18 varieties of cucumber were screened. In the present study 7 varieties were supplied from the NBPGR and others were collected from local market.

Three seeds of each varieties were sown in 10 cm diameter earthen pots containing 500 g starilized soil. After one week of germination one healthy seedling per pot was retained. Three week after sowing, 500 freshly hatched second stage juveniles of *M. incognita* were inoculated. Each treatment was replicated five times and pots were arranged in Completely randomized design. After 45 days of inoculation, the plants were uprooted and root system was washed gently. Number of galls and egg masses per root system were counted. The gall index was recorded as per the 1-5 scale as highly resistant (0.00), resistant (0.01-2.00), moderately resistant (2.01-3.00), susceptible (3.01-4.00) and highly susceptible (4.01-5.00).

Out of 18 cultivars, none of the cultivars ranked in resistant reaction. Only one as moderately resistant (EC 641913), eleven were susceptible (EC 641908, EC 641912, EC 641920, EC 641927, EC 641934, Cucumber green long special, Malini, Bankim, Cucumber No. 243, Nandini, Cucumber No-786) and six cultivars were found to be highly susceptible (EC 641925, Kalyan, Cucumber NS-408, Debstar, Alisa, Improve Noori).

Table 1. Screening of cucumber cultivars against *Meloidogyne incognita*

Mean of 5 replications

Cultivars	Mean root-knot index (1-5 scale)	Reaction
EC 641913	2.4	MR
EC 641908	3.6	S
EC 641912	4	S
EC 641920	3.8	S
EC 641927	4	S
EC 641934	3.6	S
Cucumber green long special	3.4	S
Malini	4	S
Bankim	3.4	S
Cucumber No. 243	3.6	S
Nandini	4	S
Cucumber No-786	3.6	S
Cucumber green long (Check)	4	S
EC 641925	4.4	HS
Kalyan	4.6	HS
Cucumber NS-408	4.6	HS
Debstar	4.4	HS
Alisa 4.6	HS	
Improve Noori	4.2	HS
S.Ed 0.29		
CD (P=0.05)	0.58	

MR : Moderately Resistant; S: Susceptible; HS : Highly Susceptible

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Effect of Different Plant Extracts as Root Dip Treatment Against Root-Knot Nematode, *Meloidogyne incognita* Infecting Tomato

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Tomato *[Lycopersicum esculentum* Mill] is one of the most important annual vegetable crop grown throughout the country. Among various pest and diseases, which damage crops, plant parasitic nematodes, present a formidable pest problem for different crops. Most species attack and feed on plant roots and underground plant parts. Vegetable crops are excellent host for a wide range of plant parasitic nematodes. Estimated overall average annual yield losses of the world major vegetable crops by nematodes are 12.3 per cent. Average losses for the 40 crops in developed countries were estimated to be 8.8 per cent compared with 14.6 per cent for developing countries (Ravichandra, 2008).

So far substantial work has been done on various aspects of root-knot nematode, *M. incognita* on tomato but there is not much information available on management of root-knot nematode through plant extracts.

Healthy leaves of neem (*A. indica*), karanj (*P. pinnata*), castor (*R. communis*) and leaves of jatropha (*J. curcas)* were collected from herbal park of RCA, Udaipur (Raj.). The collected plant parts were washed with distilled water. Hundred gram of clean fresh plant leaves were grind with 100 ml absolute alcohol. The mixture was allowed to stand for 48 hrs at room temperature and was subsequently filtered through filter paper. The solvent was completely evaporated from the extract at 70° C till it become a semi-solid material. This semi-solid material becomes stock solution by adding distilled water and makes different respective concentration by different plant extracts stock solution.

In-vivo effect of plant extracts on the root-knot nematode infecting tomato: Tomato seedling were raised in bulk and seedling of uniform size were dipped in alcoholic all there plant extracts along with best two concentration of each with one untreated check and one chemical check *i.e.,* Carbosulfan 25 EC 2 per cent for 4 hrs. The treated seedlings were transplanted in pot. The experiment was laid out in CRD with four replications.

Three tomato seedlings were sown in each pot. After 10 days, one healthy plant in each pot was maintained and others were uprooted carefully. The pots were watered regularly as and when required. Observation on plant growth and reproduction of root- knot nematode on tomato were recorded at 45 days after transplanting or at harvest.

Observation on shoot length (cm), root length (cm), fresh shoot weight (g), and fresh root weight (g) were taken at harvest. The root were washed carefully under tap water and stained with 0.1 per cent acid fuchsin lacto phenol (Mc Beth, 1941) and after wash kept in clear lacto phenol for 24 h. Thereafter the roots were examined

thoroughly under a stereoscopic binocular microscope for counting number of gall per plant, number of egg masses per plant and number of eggs and larvae per egg mass. After removing the plant from the pot, soil was thoroughly mixed and 100cc soil from each pot were taken and processed by Cobb's sieving and decanting technique (Cobb, 1918) followed by Baermann's funnel technique (Christie and Perry, 1951) for estimation of nematode population in soil. The data were subjected to statistical analysis.

Tl= 10% Jatropha plant leaf extract; T2= 20% Jatropha plant leaf extract; T3= 10% Neem plant leaf extract; T4= 20% Neem plant leaf extract; T5= 10% Karanj plant leaf extract; T6= 20% Karanj plant leaf extract; T7= 10% Castor plant leaf extract; T8= 20% Castor plant leaf extract; T9= Chemical check (Carbosulfan) 2%; T10= Untreated check. An experiment with 10 treatments was conducted in CRD with four rel?lications.

The observation on plant growth characters and reproduction of *M. incognita* on tomato are presented in (Table 1).

Results of the experiments conducted during kharif, 2012 at Rajasthan College of Agriculture, Udaipur to see the effect plant leaf extracts of neem (*Azadirachta indica*), karanj (*Pongamia pinnata*), castor (*Ricinus communis*) and jatropha (*Jatropha curcas*) are presented Data pertaining to the effect of plant leaf extracts on plant growth characters (shoot length and weight, root length and weight) and nematode reproduction (number of galls per plant, number of egg masses per plant, number of eggs and larvae per egg mass, soil population per 100cc soil and total population of nematodes) were recorded and statistically analyzed to test the significance of results.

All the treatments were effective in significantly reducing the nematode population and increasing plant growth. Among the individual treatments, significant increase in shoot length (31.61 cm), shoot weight (20.67 g), root length (24.13 cm), root weight (3.38 g) and significant decreases in number of galls plant-l (18.46), no. of egg masses plant-l (21.70), no. ofeggs & larvae eggmass-l (190.75), larval population 100 CC-l soil (233) and total population (6469) was recorded in plants treated with 20% Neem plant leaf extract (T4), followed by 20% Jatropha plant leaf extract (T2), 20% Karanj plant leaf extract (T6) and 20% Castor plant leaf extract (T8) over untreated check (Table 1). The results of the study clearly indicated that the treatments effects were better realized with 20% Neem plant leaf extract.

To find out the eco-friendly and economically feasible method for management of root- knot nematode, *Meloidogyne incognita* infecting tomato. All the four plant leaf extracts namely neem (*Azadirachta indica*), karanj (*Pongamia pinnata*), castor (*Ricinus communis*) and jatropha (*Jatropha curcas*) with two concentrations (10 & 20%) used as root dip treatment found superior over untreated check (control) in improving plant growth and reducing nematode reproduction. However, leaf extract ofneem (*Azadirachta indica*) at 20 per cent was found to be the best treatment in improving plant growth characters and in reducing nematode reproduction on tomato followed by leaf extracts of jatropha (*Jatropha curcas*), karanj (*Pongamia pinnata*) and castor (*Ricinus communis*) at 20 per cent concentration.

This investigation is in adjustable conformity with the finding of Saravanapriya & Sivakumar (2003) that tested the efficacy of leaf extract of some botanicals namely *Calotropis gigantea, Tagetus erecta, Azadirachta indica, Areca catechu* and *Citrullus lantatus* against the root-knot nematode, *M. incognita* infecting tomato. The leaf extract of *C. gigantea* significantly reduced the nematode population both at 45 days af!er transplanting (87.30%) and at harvest (89.96%) over control. The same treatment also increased fruit yield by 23.91 per cent. Reported by Sosamma & Jayasree, (2002). Different plants including jatropha have different type of saponin and exhibits different type of biological activity .These saponins are being successively used as larvicidal and nematicidal because of its ampililic nature. Omar *et al.* (1994) reported that *Q. saponaria* saponin wextract at 260-280 ppm reduced the number of egg masses and viable juveniles of the root-knot nematode, *M. javanica.* Hence, in the present study leaf extract of neem (*Azadirachta indica*), jatropha (*Jatropha curcas*), karanj (*Pongamia pinnata*) and castor (*Ricinus*

Table 1. Effect of Plant Leaf Extracts as Root Dip Treatment against Root-knot Nematode, *Meloidogyne incognita* Infecting Tomato Under Pot Experiment.

Treatments		Plant Growth Characters				Nematode Reproduction				
		Shoot Length (cm)	Shoot Weight (g)	Root Length (cm)	Root Weight (g)	No. of galls/ plant	No. of egg masses/ plant	No. of eggs & larvae/ egg mass	larval population in 100 cc soil	Total population of nematode
10% Jatropha plant leaf extract	T1	24.38	13.66	18.45	1.86	32.83	36.25	217.50	262	10510
20% Jatropha plant leaf extract	T2	29.12	18.25	21.82	2.23	21.83	24.50	191.75	244	7141
10% Neem plant leaf extract	T3	25.79	15.38	19.88	1.94	30.92	35.50	213.00	257	10134
20% Neem plant leaf extract	T4	31.61	20.67	24.13	3.38	18.46	21.70	190.75	233	6469
10% Karanj plant leaf extract	T5	22.96	13.22	16.17	1.61	35.92	36.25	228.75	268	10971
20% Karanj plant leaf extract	T6	29.80	17.25	20.85	2.15	23.10	25.25	207.75	249	7729
10% Castor plant leaf extract	T7	24.23	14.08	18.58	1.83	33.33	35.25	220.00	262	10363
20% Castor plant leaf extract	T8	29.16	18.21	21.92	2.17	21.33	27.25	201.00	245	7923
Chemical check (Carbosulfan) 2%	T9	35.25	23.30	31.89	3.44	16.25	8.50	134.50	222	3363
Nematode alone (Control)	T10	22.25	9.25	14.18	1.39	48.23	46.00	207.75	300	12558
	S.Em±	0.954	0.467	0.513	0.099	0.513	0.798	4.426	6.997	221.623
	CD (P=0.05)	2.756	1.348	1.481	0.285	1.481	2.304	12.783	20.209	640.094

Note: (i) Data are average value of four replication (ii) Initial inoculums 2 larvae/g soil.

communis) at higher concentrations were found most effective in reducing population of *M. incognita* on tomato and in enhancing the plant growth characters.

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Community Analysis of Plant Parasitic Nematodes Associated with Rice in Imphal East District of Manipur (Pourabi)

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Rice is one of the valuable cereal food crop in the world and forms the staple food for more than half of the global population. In India, rice is grown in almost all the state as a principal food crop and occupies about 23.3% of the gross cropped area of the country , covering about 44.8 mha of land. In Manipur, it occupies about the maximum cultivated area (Anon, 2008). Rice contributes 43% of the total food grain production and 46% of the total cereal production in India. Several soil and plant inhabiting parasitic nematodes have been known to be associated with vegetables, crops (Roy, 2005). Plant parasitic and free living nematodes are the important members of the nematodes community and it cause serious damage to many fruits, vegetables, crops etc. The annual yield loss in rice due to plant parasitic nematodes on a world basis is estimated to be 10% (Sasser & Freckrnan,1987). The present survey of rice growing area of Imphal East district of Manipur was taken from August 2012- October 2012 for recording the occurrence of plant parasitic nematodes associated with rice.

The present survey was carried out in eighteen different villages of Pourabi, Imphal East from August 2012-0ctober 2012 during summer season namely: Laijing maya loubuk, Laijing ching maya loubuk, Sinam lok loukon, Yomalok loukon, Ehickon loukon, Tairenpokpi loukon, Kharou loukon, Kanglou loukon, Ikop loukon, Thingel makha loukon, Kongon toubi loukon, Nungoi loukon, Huidrom loukon, Sawombung loukon, Hidumbi loukon, Bamon loukon, Patlou loukon, Wairi loukon, Maniyaiskul loukon and Thamnapokpi loukon. Diseased field were selected on the base of above ground symptoms of the crops such as, wilting, slow growth, stunting and yellowing of leaves. Soil sample from the associated rhizosphere were collected from a depth of 10-15 cm at the rate of one unit sample. Each unit sample was a composite of 20 cores obtained from four comers and

Table 1: Occurrence of plant parasitic nematodes within Pourabi in Imphal east district of Manipur

Village	Crop	Nematode population/200 cm³ soil						
		Meloidogyne graminicola	*Helicotylenchus multieinetus*	*Tylenchorhynchus* sp.	*Pratylenchus* sp.	*Hoplplaimus* sp.	*Xiphinema* sp.	*Criconematode* sp.
Loijing maya loubuk	Thangjing	50	-	87	43	-	-	-
Loijing ching Maya loukon	Kd angoubi	-	946	-	-	-	-	-
Sinam lok loukon	Kd angoubi	44	230	-	54	-	34	-
Yomalok loukon	Phaungang	-	-	115	-	178	-	290
Checkon loukon	Chabokpi	12423	-	-	69	100	-	250
Kanglou loukon	Ayangleima	6614	500	-	45	-	65	-
Ikop loukon	Hemochandra	-	-	700	-	850	-	24
Thingel makha loukon	Dharam	48	-	100	-	-	-	-
Tairenpokpi loukon	Jatra phou	-	4400	-	388	-	450	-
Kharou loukon	Nongin	135	-	550	-	450	-	800
Kongon toubi loukon	Khamba	-	-	-	750	-	500	-
Nungoi loukon	Phoudum	200	100	-	-	250	-	300
Huidrom loukon	Jatra phou	-	2065	-	-	308	-	-
Sawombung loukon	Dharam	85	-	1020	-	-	-	-
Hidumbi loukon	KD angoubi	-	125	-	560	-	625	-
Wairikhul loukon	Phaungang	-	-	-	1012	-	-	560
Mani yaiskul loukon	Chabokpi	3400	-	-	-	540	-	100
Thamnapok pi loukon	Hemochandra	25	-	150	-	-	5	-
Bamon loukon	Thangjing	120	-	-	50	-	-	400
Patlou loukon	K Damubi	-	1150	-	-	50	-	100

centre of the field. Root soil sub sample were stored in polythene bags and kept at 4°C in a refrigerator not more than 7 days. Altogether 180 soil samples and roots were collected, 10 samples from each field. Infected roots were stained in cotton blue-lactophenol and observed for the presence of nematodes. The females of root-knot nematodes were dissected out from the galled roots and perineal section prepared for species identification. The estimation of nematode population per 10 9 root samples and 2009 soil sample was done in a multi-chambered counting dish under a stereoscopic binocular microscope. The population densities of nematode species in the samples were calculated using the formulae (Norton, 1978).

Seven plant parasitic nematode genera *viz*., *Meloidogyne graminicola, Helicotylenchus multicinetus, Tylenchorhynchus* spp., *Pratylenchus* spp., *Hoplolaimus* spp., *Xiphinema* spp. and *Criconematodes* spp. were found associated with different varieties of rice in different localities of Pourabi. Highest number of *Meloidogyne graminicola* (12,423 J2s/500g of soil) was recorded in Chabokpi variety of rice at Checkon loukon of Pourabi with lowest density (44 J2s/500g of soil) was recorded in KD am ubi at Sinam lok loukon of Pourabi (Table 1). The population density of *Helicotylenchus multicintus* was found to be slightly lower as compared to *M. graminicola.* Its highest density (4400 J2s/500g of soil) was recorded in Jatra phou at Tairenpokpi loukon and lowest density (50 J2s/ 500g of soil) in Hemochandra and Khamba at Ikpo loukon and Kongon loukon. *Tylenchorhynchus* spp. showed highest density of 1020 J2s/500g of soil in Dharam at Sawombung loukon and lowest of 87 J2s/ 500g of soil in Thangjing variety of rice at Laijing maya loukon of Pourabi. Among these plant parasitic nematodes, *Hoplolaimus* spp., *Xiphinema* spp. and *Criconematodes* spp. were found to be in more or less same number of population density in different variety of rice at different localities of Pourabi.But for *Pratylenchus* spp. highest density (1012 J2s/500g of siol) was found in Phaungang at Laijing maya loukon of Pourabi.

Table 2: Community analysisi of plant parasitic nematodes in different villages of Pourabi, Imphal east district of Manipur

Nematode	Absolute frequency	Absolute density	Relative frequency
Meloidogyne graminicola	6.11	661.25	18.96
Helicotylenchus multicinktus	5.55	274.74	17.24
Tylenchorhynchus spp.	3.88	77.77	12.06
Pratylenchus spp.	5	90.6	15.51
Hoplolaimus spp.	4.44	77.88	13.79
Xiphinema spp.	3.33	62.11	10.34
Criconematode spp.	5	80.68	15.51

Table 2 shows, analysis of soil and root samples of different rice variety of Pourabi revealed that most of the collected smaples were severely infested with seven nematodes *viz*., *Meloidogyne graminicola, Helicotylenchus multicinktus, Tylenchorhynchus* spp., *Pratylenchus* spp., *Hoplolaimus* spp., *Xiphinema* spp. and *Criconematodes* spp. Among these root-knot nematodes *Meloidogyne graminicola* occurred most frequently followed by *Helicotylenchus multicinktus* with absolute frequency of 6.11 % and 5.55% respectively. The root-knot nematode, *M. graminicola* was found in higher density (18.96%) followed by *Helicotylenchus multicinktus* (17.24%). *M. graminicola* is the most predominant phytonematodes of rice.

The results support the report of Joymati & Thoithoi (20 10) about the incidence of rice root-knot nematode *M. graminicola* at Thoubal district of Manipur. The work also support the of Joymati *et al*., (2011) about the community analysis of plant parasitic nematode in different vegetables field of Bishnupur district of Manipur. The present findings can give important knowledge about plant parasitic nematodes especially rice root-knot nematode which cause serious damage to food crop i.e., rice and leads to totally damaged in the production of rice in the state.

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BOOK REVIEW

Nematodes of Banana: Taxonomy and Ecology of Dorylaimids and Tylenchids by Viswa V.

Gantait, T .Bhattacharya and A.Chatterjee Published by Lambert Academic Publishing Germany, (2010, pp. 300)

The book contains the details of the work done carried out on the survey of soil and plant parasitic nematodes belonging to the order Dorylaimida and Tylenchida associated with Banana plantations(Musa *paradisica L.cv* Kanthali) in Pashim Medinipur District of West Bengal, lndia.The second aspect deals with the study of community structure of durylaimids and tylenchid associated with banana plantation.The third aspect concerns with the temperal variation of four selected edaphic factors. The last one revolves around the population dynamics of nematode population in relation to the edaphic factors.

During the survey a total 46 species of soil and plant parasitic nematodes belonging to 32 genera and 15 families under order Dorylaimida and Tylenchida have been collected and identified amongst dorylaimids, 8 species and amongst tylenchids 4 species are recorded as new. The species key of the five genera *viz*. Dorylaimus Lainydorus, Lagenonema, Makatinus and Discolaimus are provided. Sixteen species (12 dorylaimus and 4 tylenchids) are recorded for the first time from the rhzosphere of banana. Community composition of nematodes was analysed with reference to abundance, frequence of occurrence, relative abundance, mean crowding, dominance index species diversity and species evenness.

Among the tylenchids, *Rotylenchulus reniformis* was the most abundant and *Pratylenchus coffeae* was least abundant species. Among dorylaimids, *Prodorylaimus sukuliwas* the most abundant and *Promuntazium elongatum* was the least abundant species. This book therefore, provides the valuable information about the taxonomy of dorylaimid and tylenchid nematodes of banana in India ,their community structure population dynamics and seasonal population fluctuation in relation to different edaphic factor. Hence, it will serve as reference book for the researchers working in banana.

Nematodes Associated with Guava and Litchi Plantations: Studies on Taxonomy and Ecology of Soil and Phytophagous Nematode by Oebabrata Sen,Amalendu Chatterjee and Buddhadeb Manna; Lambert Academic Publishing Germany (2012, pp. 321).

This book is outcome of comprehensive study on soil and plant parasitic nematodes belonging to the orders Dorylaimida and Tylenchida associated with Guava and Litchi plantations in India. Guava and Litchi are the most economically important fruit crops in West Bengal.This book dealt with taxonomy and ecology of soil and plant parasitic nematodes belonging to the orders Dorylaimida and Tylenchida associated with these crops. Thirty one species of Dorylaimida under 19 genera,14 subfamilies,10 families, 5 superfamilies and 2 suborders have been observed. From the study, one genus and 11 species were added to the science. In addition, two species from India, nine species from West Bengal and eight species from south 24 Parganas has been recorded for the first time. Under the order Tylenchida one genus and 3 species have been proposed as new. Different edaphic factors, like soil moisture, temperature, pH, nitrogen, phosphorus, potassium, organic carbon and soil electrical conductivity were estimated as they are determining factors for the shape and quality of the nematode population. The population fluctuation of phytophagous nematode were observed in relation to predatory and omnivorous nematodes belonging to the orders Mononchida and Dorylaimida. This book therefore provides the valuable information about the taxonomy of dorylaimid and tylenchid nematodes of Litchi and Guava plantations in India, Hence it will serve as reference book for the researchers working on Guava & Litchi.

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NEMATOLOGICAL NEWS

- Dr. H.S. Gaur, former JD (Edn.) & Dean, PG School, IARI, New Delhi has joined as Vice Chancellor, Sardar Vallabh Bhai Patel University, Meerut on 16th Feb., 2013.

- Dr. Viswa Venkat Gantait from Zoological Survey of India, Kolkata participated in the 32nd ISEA as a summer member during November 2012 to April 2013. He was the only Biologist in the expedition group. He was nominated as a Zoologist, especially as a Nematologist, as because the nematodes occupy about 22% of the total invertebrate fauna of Antartica. In the field of Nematology, he is the first in India who attended the Indian Scientific Expedition to Antartica. Dr. V.V. Gantait stayed in both the stations (Dakshin Gangotri and Maitri) and collected more than 100 soil, moss and algal samples from different locations of 'Larshman Hills', 'Schirmacher Oasis'. All the samples have been collected for taxonomic and ecological studies of invertebrate fauna in Antartica.

- Department of Nematology, CCS Haryana Agricultural University and KVK, Fatehabad of this university organised a Molya Gyan Diwas at village Khabra kalan in district Fatehabad on 20th October,2012. Dr Ravi Kumar, AD (ResearchExtension Linkage) of University was the chief guest on this occasion. About 150 farmers of Khabra kalan and surrounding villages and ofificers of agriculture department of Fatehabad district participated in this Gyan Diwas. On 20th March, 2013, a Molya Khet diwas (field day was organised in village Salam Khera (Fatehabad). Dr S.S.Sharma, Associate Director (Farm Advisory Services), CCS HAU Hisar graced this occasion. It was attended by ca. one hundered farmers, agriculture and horticulture officers of the district. Purpose of these programmes was to awaken the farmers and field functionaries about the crop diseases caused by nematodes particularly Molya disease in wheat and barley. Dr K.R. Dabur, Professor and Head , DR.I.J. Paruthi, Dr. R.S.Kanwar (Department of Nematology) and Dr. S.S.Mann (KVK, Fatehabad) delievered expert lectures on nematode problems of cotton, wheat, guar and horticultural crops.

- Dr. (Mrs.) Padma Bohra, a well known taxonomist in Nematology, Desert Regional Centre, Zoological Survey of India, Jodhpur passed away on 27th May, 2013. She was awarded Ph. D. degree from JNV University, Jodhpur on the thesis entitled "Taxonomy of Plant and Soil Nematodes Associated with Cereal Crops in Rajasthan" under the able guidance of Dr. Qaiser H. Baqri in the year 1999. She was selected to the post of Scientist-C in the year 2002 and was subsequently promoted to the post of Scientist-D in the year 2008. She also served as Officer-in-Charge of Desert Regional Centre, ZSI, Jodhpur from August, 2006 till her last breath. During her short carrer in research on taxonomy of plant and soil nematodes (1996-2013), she described one new genus and more than 12 new species, besides recording more than 100 species for the first time from Rajasthan and Gujarat states and several species for the first time from India in the journals of national as well international repute.