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In vitro studies on the effect of rhizobacteria, Phytotherapeutic substances and chemicals on egg hatching of *Meloidogyne graminicola*

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Abstract
Nematicidal activity of rhizobacteria, Phytotherapeutic substances and chemicals on egg hatching of was assayed against *Meloidogyne graminicola*. In laboratory assays extracts from *Azadirachta indica*, *Phyllanthus niruri*, *Achyranthes aspera*, *Eucalyptus globulus* and *Xanthium strumarium*, free culture filtrate from strains of *Pseudomonas fluorescens* (P36), *Gluconacetobacter diazotrophicus* (33-47) and *Azotobacter chroococcum* (Mac 27,HT 54, HT 57) from chemicals, carbosulfan and cartap hydrochloride showed inhibitory effect on the hatching of eggs of *M. graminicola*. The maximum hatching inhibition observed in *Azotobacter chroococcum* HT 54, *A. indica* and carbosulfan with a 19.3 %, 19.0 %, 14.3 % respectively in 1:5 concentration in all exposure period. Minimum hatching was recorded with *A. indica* i.e. 8.0 larvae at 1:5 followed by *Xanthium strumarium* (12.7) at 1:5 after 24 hrs. Among chemicals, carbosulfan showed maximum inhibitory effect followed by cartap hydrochloride. Minimum hatching was recorded with carbosulfan i.e. 14.3 larvae at 1:5 followed by cartap hydrochloride (15.8) at 1:5 after 8 days of exposure. The maximum egg hatching i.e. 94.7 larvae at 8 days was recorded with distilled water check. The rate of hatching was inversely proportional to concentration of substances with of exposure period, as it decreased with increases in concentration viz., 1:5, 1:10, 1:20, 1:40 and 1:80.

Keywords: *Meloidogyne graminicola*, egg hatching, rhizobacteria, Phyto therapeutic substances, chemicals

Introduction
Global crop loss caused by plant parasitic nematodes (PPNs) is more than $100 billion annually and in India, the annual estimated crop losses due to major PPNs have been worked out to be about Rs. 242.1 billion [11]. Rice root-knot nematode, *Meloidogyne graminicola* is one of the most predominant pests associated with rice under upland condition [5] and cause substantial yield losses [21]. It is a serious problem in the nurseries and upland rice but has been recently found to be widespread in the deep-water and irrigated rice also, in many states of India [17]. *M. graminicola* is a pest of international importance and it is reported to cause 17-30% yield loss due to poorly filled kernels [13] while in India nematodes of rice alone cause 10.54 % yield loss which causes monetary losses of 779.30 million rupees [11].

PPNs are difficult to control because of their wide host range and high rate of reproduction, with females capable of producing upto thousand eggs/female [15]. The control of PPNs is a difficult task, and mainly depends on chemical nematicides for decades and remarkable reduction of nematode population has been achieved [1]. As an alternative to chemical pesticides specially for the purpose of protecting crops against nematode and also for the conservation of biodiversity, botanical may stand as the most promising sources of bioactive products of plant origin. Therefore, the use of plant extracts and Phyto-products is gaining attention due to their availability, cost effectiveness, proven nature of specificity, no biodegradability, low toxicity and minimum residual toxicity in the ecosystem [14]. Many botanical extracts have been found to contain phytochemicals such as alkaloids, tannins, saponins, flavonoids, diterpenes, glucosinolates, acetylenes and thinlys[6] which are effective against PPNs [10].

Biological control of nematodes has long been considered as an alternative to managing PPNs with pesticides. Plant growth-promoting rhizobacteria (PGPR) like *Pseudomonas fluorescens*, *Gluconacetobacter diazotrophicus* and *Azotobacter chroococcum* etc., are having protection potential in modern agricultural system. PGPR are capable of improving the plant growth in many plants and they also act as biological control agents against various soil borne plant pathogens including PPNs. In the light of above informations, an *in-vivo* investigation was carried out to evaluate toxic activity of different rhizobacteria, phytotherapeutic substances and chemicals on *M. graminicola* egg hatching.

Materials and Methods

M. graminicola was maintained in rice plants in the infested soil under screen house and infected plants were also uprooted, carefully washed in running tap water and eggs was collected in to Petri dishes containing distilled water. Nematicidal activity of plants *viz*., Neem *(Azadirachta indica), Hazar dana* (*Phyllanthus niruri),* Ulta kanta (*Achyranthus aspera),* Safeda (*Eucalyptus globulus),* and Bhoort *(Xanthium strumarium),* rhizobacteral strains (*G. diazotrophicus* 33-47, *A. chroococcum* Mac 27, HT 54, HT 57 and *Pseudomonas* spp. P36) and chemical, carbosulfan and cartap hydrochloride were evaluated *in vitro* against egg hatching of rice root-knot nematode, *M. graminicola* at various dilutions *viz*, 1:5, 1:10, 1:10, 1:20, 1:40, 1:80. The eggs were kept in 50 mm petri-dish (100 eggs/Petridish) and measured quantity of stock solution was added to each Petri dish to make the resultant dilutions of 1:5, 1:10, 1:20, 1:40 and 1:80. Each dilution was replicated three times. Eggs put in distilled water were treated as control. These Petri dishe were kept in BOD incubator at 25±1^{O}C. The number of juveniles hatched after 1, 2, 4, 6 and 8 days of exposure to different dilutions of different extracts were counted under stereoscopic binocular microscope. Mean larvae hatched was calculated.

Preparation of cultural filtrate

The culture thus obtained was centrifuged at 6000 rpm for 10 minutes. The supernatant were injected through a 0.2 mm filter to remove the bacterial cell and the filtrate was collected.

Preparation of botanical extracts

The leaves of different selected plants *viz*. Neem (*Azadirachta indica*), Hazar dana (*Phyllanthus niruri)*, Ulta kanta (*Achyranthes aspera*), Safeda (*Eucalyptus globulus*), Bhoort *(Xanthium strumarium)*. Leaves of 40 gram each were washed under running tap water and added 100 ml distilled water and grinded in blender for 3 minutes. The mixture was allowed to stand 3 h. filtered through muslin cloth and filter paper to get the extract. The supernatant was removed and stock solution for further studies.

Preparation of chemical concentrate

Stock solutions for chemicals and fractions (30mg/ml) were prepared in their suitable solvents.

Statistical Analysis

The data from an experiment was subjected to analysis of variance (ANOVA) and critical differences (CD) at 5 % were calculated.

Results and Discussion

All cell free culture filtrate (CFs) of rhizobacterial strains showed inhibitory effect on the hatching of eggs of *M. graminicola.* After 24 h of incubation very low level of egg hatching was recorded in all the concentrations. Among all the rhizobacterial strains *A. Chroococcum* (Mac 27) showed most inhibitory effect (19.3 % egg hatching) followed by *Pseudomonas* spp. P36 (19.8 %) in CFs at concentration of 1:5 in all exposure time (Table 1-5). All other treatments also showed low levels of hatching after 24 h while in control 49.6 per cent hatching was recorded. The maximum hatching of eggs (56.1%) was recorded in 1:80 while lowest rate (19.3%) of hatching at 1:5 concentration in all cultures tested. After 48 h of incubation the highest hatching inhibition was recorded for *A. chroococcum* Mac 27 with 9.3 per cent hatching in 1:5 followed by 16.3, 26.3, 36.3 and 41.7 per cent, in 1:10, 1:20, 1:40 and 1:80 respectively. Reducing the concentration of bacterial cell population had positive effect on egg hatching in the case of all rhizobacterial strains. After 96 h of incubation the hatching inhibition was more for *A. chroococcum* Mac 27 (11.0 % egg hatch) in 1:5 concentration followed by 11.3 per cent in *Pseudomonas* spp. After 144 h of incubation a higher hatching inhibition was observed with *A. chroococcum* Mac 27 which was followed by *P. fluorescens* P36 in all the concentrations i. e. 1:5, 1:10, 1: 20, 1:40 and 1:80. After 192 h of incubation the hatching inhibition was also more for *A. chroococcum* Mac 27 (15.3 %) in 1:5 concentration followed by 15.7 per cent in *Pseudomonas* spp. P36. The minimum hatching inhibition was for *A. Chroococcum* HT 57 with a 56.1 per cent hatching in 1:80 concentration in all exposure period, which is near to the hatching per cent in control (60 %). The rate of hatching was inversely proportional to concentration of strains with 8 days of exposure period, as it decreased with increases in concentration viz., 1:5, 1:10, 1:20, 1:40 and 1:80.

Reducing the concentration of the CFs might have diluted the amount of toxic metabolites present exhibiting positive influence on egg hatching of *M. graminicola*. Similar results were observed in studies conducted by [16] with *Bacillus* and *Pseuodomonas* strains. All the bacterial strains produced water soluble compounds that adversely affected the mobility of juveniles and egg-hatch. However, *G. diazotrophicus* Co99-70; *Pseudomonas* spp. RKP-33; *Bacillus* spp. RKB-91 and RKB-68 significantly delayed and decreased nematode egg-hatch within 24 h of exposure. Delayed nematode egg hatch of *Meloidogyne* spp. due to culture supernatants of *Pseudomonas* spp. [18] was also observed. Production of secondary metabolites such as phenazines, pyrolnitrin, tropolone, pyocyanin and 2, 4 diacetylphloroglucinol was reported to be present in the cell free extracts of *P. fluorescens* by [4] Volatile fatty acids produced by *G. diazotrophicus* [2] are known to disrupt [8] and paralyse the movement of PPNs. Moreover, these organic acids also reduce egg hatching by impairing embryogenesis of *M. incognita* [2]. They observed that exposure to a 250 ppm aqueous solution of these volatile fatty acids completely suppressed egg hatching within 48 h. Similar results have been observed in our present *in vitro* studies using CFs obtained from *G. diazotrophicus, A. chroococcum* and *Pseudomonas* spp. The present study also agreed with the findings of [20] that *M. incognita* egg hatching was affected by the toxins produced in bacterial cell free extracts.

The results on effect of aqueous extracts of five plants (*A. indica, P. niruri, A. aspera, E. globulus* and *X. strumarium*) on egg hatching presented in Table (1-5) revealed that in general all the plant extracts recorded to cause significantly inhibition in the nematode egg hatching as compared to the check (water). The effect of different concentration levels (1:5, 1:10, 1:20, 1:40, 1:80) and exposure timings on hatching of eggs varied with material and exposure time. All the treatments (plant leaf extracts) showed inhibitory effect on egg hatching of *M. graminicola.* The rate of hatching (%) was inversely proportional to concentration of extracts, as it decreased with increases in concentration. Among aqueous extracts, maximum inhibition was observed in case of *A. indica* (19.0) followed by *X. strumarium, A. aspera, P. niruri* and *E. globulus* i.e. 24.2, 27.0, 28.8 and 32.7 % respectively at concentration of 1:5. Similar trend was observed with other concentration levels also. Minimum inhibition of hatching (less than 50 %) was recorded with *A. indica* followed by *X. strumarium, A. aspera, P. niruri* and *E. globulus* after 8 days at 1:10 concentration of botanicals.

Table 1: Effect of different rhizobacteria, aqueous extract of phytotherapeutic substances and chemicals on the hatching of eggs of *M. graminicola* (Average of three replicates)

Number of juveniles hatched (%) at concentrations (C)						
Substances (P)	**1:5**					**Mean PxC**
	1st day	**2nd day**	**4th day**	**6th day**	**8th day**	
A. chroococcum (HT 57)	21.0 (27.2)	25.7 (30.4)	27.7 (31.7)	28.0 (31.9)	30.3 (33.4)	(30.9)
G. diazotrophicus (3347)	15.0 (22.6)	18.3 (25.3)	22.3 (28.1)	24.3 (29.5)	26.3 (30.8)	(27.3)
Pseudomonas spp. (P36)	8.3 (16.7)	9.7 (18.0)	11.3 (19.6)	13.3 (21.3)	15.7 (23.3)	(19.8)
A. chroococcum (Mac 27)	14.7 (22.5)	17.0 (24.2)	19.7 (26.3)	21.7 (27.7)	25.0 (29.9)	(26.1)
A. chroococcum (HT 54)	7.7 (15.9)	9.3 (17.6)	11.0 (16.3)	12.7 (20.7)	15.3 (23.0)	(19.3)
Xanthium strumarium	12.7 (20.7)	15.0 (22.7)	17.0 (24.3)	19.0 (25.8)	21.7 (27.7)	(24.2)
Azadirachta indica	8.0 (16.3)	8.7 (17.0)	10.3 (18.7)	12.3 (20.5)	15.0 (22.7)	(19.0)
Achyranthus aspera	15.7 (23.2)	18.7 (25.5)	20.7 (27.0)	23.3 (28.8)	25.7 (30.4)	(27.0)
Eucalyptus globulus	24.7 (29.7)	27.7 (31.6)	29.0 (32.5)	31.7 (34.2)	33.7 (35.4)	(32.7)
Phyllanthus niruri	17.3 (24.5)	21.3 (27.4)	24.0 (29.3)	26.3 (30.8)	28.3 (32.1)	(28.8)
Cartap hydrochloride	3.7 (10.8)	5.3 (13.3)	7.7 (16.0)	10.0 (18.4)	12.7 (20.8)	(15.8)
Carbosulfan	2.3 (8.5)	4.7 (12.4)	6.7 (14.9)	8.7 (17.0)	10.7 (18.9)	(14.3)
Check (water)	49.3 (44.6)	62.0 (52.0)	72.7 (58.7)	84.7 (67.4)	94.7 (77.3)	(60.0)
Mean CxT	(21.8)	(24.4)	(26.6)	(28.8)	(31.2)	
C.D. at 5 %						
Exposure period	(1.3)					
Substances	(2.2)					
Interaction Exposure period v/s Substances	(4.8)					

*Figures in parentheses are arc sine transformed values

Table 2: Effect of different rhizobacteria, aqueous extract of phytotherapeutic substances and chemicals on the hatching of eggs of *M. graminicola* (Average of three replicates)

Number of juveniles hatched (%) at concentrations (C)						
Substances (P)	**1:10**					**Mean PxC**
	1st day	**2nd day**	**4th day**	**6th day**	**8th day**	
A. chroococcum (HT 57)	28.7 (32.3)	34.3 (35.8)	38.7 (38.4)	41.0 (39.8)	48.7 (44.2)	(38.1)
G. diazotrophicus (3347)	24.7 (29.7)	30.3 (33.3)	33.7 (35.4)	36.7 (37.2)	41.7 (40.2)	(35.1)
Pseudomonas spp. (P36)	15.3 (22.9)	19.7 (26.2)	22.7 (28.3)	25.3 (30.1)	28.0 (31.9)	(27.9)
A. chroococcum (Mac 27)	19.3 (25.9)	23.3 (28.7)	26.7 (30.9)	30.0 (33.0)	34.7 (35.9)	(30.9)
A. chroococcum (HT 54)	12.3 (20.4)	16.3 (23.7)	19.3 (25.9)	22.7 (28..3)	25.7 (30.3)	(25.7)
Xanthium strumarium	18.7 (25.5)	21.0 (27.1)	26.3 (30.8)	29.7 (32.9)	34.3 (35.8)	(30.4)
Azadirachta indica	15.7 (23.2)	19.3 (26.0)	23.3 (28.8)	27.7 (31.7)	30.0 (33.2)	(28.6)
Achyranthus aspera	24.7 (29.7)	27.7 (31.6)	30.7 (33.6)	34.0 (35.6)	36.7 (37.2)	(33.5)
Eucalyptus globulus	37.0 (37.4)	44.0 (41.5)	51.3 (45.7)	57.7 (49.4)	63.0 (52.6)	(45.3)
Phyllanthus niruri	25.7 (30.4)	29.7 (32.9)	34.7 (36.0)	40.3 (39.4)	46.3 (42.9)	(36.3)
Cartap hydrochloride	16.0 (23.5)	17.7 (24.8)	22.0 (27.9)	24.3 (29.5)	27.3 (31.4)	(27.4)
Carbosulfan	12.3 (20.4)	14.0 (21.8)	16.7 (24.0)	21.0 (27.2)	22.7 (28.4)	(24.3)
Check (water)	49.3 (44.6)	62.0 (52.0)	72.7 (58.7)	84.7 (67.4)	94.7 (77.3)	(60.0)
Mean CxT	(28.1)	(31.2)	(34.2)	(37.0)	(40.1)	
C.D. at 5 %						
Exposure period	(1.7)					
Substances	(2.8)					
Interaction Exposure period v/s Substances	N.S.					

*Figures in parentheses are arc sine transformed values

Table 3: Effect of different rhizobacteria, aqueous extract of phytotherapeutic substances and chemicals on the hatching of eggs of *M. graminicola* (Average of three replicates)

Number of juveniles hatched (%) at concentrations (C)						
Substances (P)	**1:20**					**Mean PxC**
	1st day	**2nd day**	**4th day**	**6th day**	**8th day**	
A. chroococcum (HT57)	34.0 (35.6)	39.0 (38.6)	47.3 (43.4)	54.0 (47.3)	60.7 (51.2)	(43.2)
G. diazotrophicus (33-47)	29.7 (32.9)	34.3 (35.8)	41.3 (40.0)	49.3 (44.6)	56.3 (48.6)	(40.4)
Pseudomonas spp. (P36)	25.0 (29.9)	29.7 (32.9)	35.3 (36.4)	40.7 (39.6)	50.7 (45.4)	(36.8)
A. chroococcum (Mac27)	25.3 (30.1)	30.0 (33.1)	37.3 (37.6)	42.7 (40.7)	52.3 (46.3)	(37.6)
A. chroococcum (HT54)	20.7 (26.8)	26.3 (30.8)	29.3 (32.7)	32.7 (34.8)	37.7 (37.8)	(32.6)
Xanthium strumarium	28.0 (31.9)	31.7 (34.2)	41.0 (39.8)	46.7 (43.1)	54.3 (47.5)	(39.3)
Azadirachta indica	25.7 (30.3)	31.0 (33.8)	36.3 (37.0)	39.0 (38.6)	48.3 (44.0)	(36.7)
Achyranthus aspera	29.3 (32.7)	36.3 (37.0)	48.7 (44.2)	51.3 (45.8)	58.3 (49.8)	(41.9)
Eucalyptus globulus	39.3 (38.8)	45.7 (42.5)	55.3 (48.1)	63.7 (53.0)	71.7 (57.9)	(48.0)
Phyllanthus niruri	32.7 (34.8)	37.7 (37.8)	49.3 (44.6)	53.0 (46.7)	61.0 (51.4)	(43.0)
Cartap hydrochloride	19.3 (26.4)	25.0 (29.9)	29.3 (32.8)	34.3 (35.8)	36.7 (37.2)	(32.4)
Carbosulfan	15.3 (23.0)	17.3 (24.5)	21.3 (27.5)	24.0 (29.2)	28.7 (32.3)	(27.3)
Check (water)	49.3 (44.6)	62.0 (52.0)	72.7 (58.7)	84.7 (67.4)	94.7 (77.3)	(60.0)

Mean CxT	(32.1)	(35.6)	(40.2)	(43.6)	(48.2)	
C.D. at 5 %						
Exposure period	(1.7)					
Substances	(2.7)					
Interaction Exposure period v/s Substances	N.S.					

*Figures in parentheses are arc sine transformed values

Table 4: Effect of different rhizobacteria, aqueous extract of phytotherapeutic substances and chemicals on the hatching of eggs of *M. graminicola* (Average of three replicates)

Number of juveniles hatched (%) at concentrations (C)						
Substances (P)	**1:40**					**Mean PxC**
	1st day	**2nd day**	**4th day**	**6th day**	**8th day**	
A. chroococcum (HT 57)	44.0 (41.5)	47.3 (43.4)	53.0 (46.7)	58.3 (49.8)	69.3 (56.4)	(47.6)
G. diazotrophicus (3347)	37.3 (37.6)	43.3 (41.3)	50.0 (45.0)	57.7 (49.4)	64.7 (53.6)	(45.4)
Pseudomonas spp. (P36)	32.7 (34.8)	38.0 (38.0)	41.7 (40.2)	48.7 (44.2)	55.7 (48.3)	(41.1)
A. chroococcum (Mac 27)	36.7 (37.2)	41.3 (40.0)	48.3 (44.0)	56.3 (48.6)	63.3 (52.8)	(44.5)
A. chroococcum (HT 54)	32.0 (34.4)	36.3 (37.0)	37.7 (37.8)	42.7 (40.7)	52.3 (46.3)	(39.3)
Xanthium strumarium	31.0 (33.8)	36.3 (37.0)	44.3 (41.7)	49.3 (44.6)	60.0 (50.8)	(41.6)
Azadirachta indica	30.0 (33.1)	34.7 (36.0)	41.3 (40.0)	47.0 (43.3)	55.7 (48.2)	(40.1)
Achyranthus aspera	37.3 (37.6)	44.7 (41.9)	49.7 (44.8)	54.3 (47.5)	64.0 (53.1)	(45.0)
Eucalyptus globulus	43.0 (41.0)	53.7 (47.1)	62.7 (52.4)	70.7 (57.2)	79.7 (63.3)	(52.2)
Phyllanthus niruri	39.7 (39.0)	45.7 (42.5)	53.3 (46.9)	58.7 (50.0)	66.7 (54.8)	(46.6)
Cartap hydrochloride	26.0 (30.6)	30.7 (33.5)	34.0 (35.6)	41.7 (40.2)	48.3 (44.0)	(36.8)
Carbosulfan	24.7 (29.7)	28.7 (32.3)	31.3 (34.0)	36.0 (36.8)	40.3 (39.4)	(34.4)
Check (water)	49.3 (44.6)	62.0 (52.0)	72.7 (58.7)	84.7 (67.4)	94.7 (77.3)	(60.0)
Mean CxT	(36.5)	(40.2)	(43.7)	(47.7)	(52.9)	
C.D. at 5 %						
Exposure period	(1.7)					
Substances	(2.7)					
Interaction Exposure period v/s Substances	N.S.					

*Figures in parentheses are arc sine transformed values

Table 5: Effect of different rhizobacteria, aqueous extract of phytotherapeutic substances and chemicals on the hatching of eggs of *M. graminicola* (Average of three replicates)

Number of juveniles hatched (%) at concentrations (C)						
Substances (P)	**1:80**					**Mean PxC**
	1st day	**2nd day**	**4th day**	**6th day**	**8th day**	
A. chroococcum (HT 57)	50.3 (45.2)	62.3 (52.1)	69.0 (56.2)	76.3 (60.9)	83.3 (66.1)	(56.1)
G. diazotrophicus (3347)	40.7 (39.6)	49.0 (44.4)	57.0 (49.0)	68.0 (55.7)	77.0 (61.4)	(50.0)
Pseudomonas spp. (P36)	39.0 (38.6)	44.3 (41.7)	52.3 (46.3)	58.7 (50.0)	67.7 (55.4)	(46.4)
A. chroococcum (Mac 27)	39.3 (38.8)	46.3 (42.9)	56.3 (48.6)	62.0 (52.0)	72.3 (58.4)	(48.1)
A. chroococcum (HT 54)	33.7 (35.4)	42.7 (40.2)	46.0 (42.7)	54.0 (47.3)	61.3 (51.6)	(42.1)
Xanthium strumarium	40.0 (39.2)	46.3 (42.9)	54.7 (47.8)	61.0 (51.4)	69.7 (56.6)	(47.5)
Azadirachta indica	35.3 (36.4)	39.7 (39.0)	48.3 (44.0)	55.7 (48.3)	63.7 (53.0)	(44.1)
Achyranthus aspera	42.7 (40.7)	50.3 (45.2)	66.0 (49.8)	63.7 (53.0)	74.7 (59.9)	(49.7)
Eucalyptus globulus	45.0 (42.1)	57.3 (49.2)	58.3 (54.4)	79.3 (63.2)	88.3 (70.3)	(55.9)
Phyllanthus niruri	43.0 (40.9)	53.7 (47.1)	63.0 (52.6)	71.7 (57.9)	79.3 (63.2)	(52.3)
Cartap hydrochloride	23.0 (28.6)	32.3 (34.6)	40.7 (39.6)	51.0 (45.6)	59.0 (50.2)	(39.7)
Carbosulfan	19.3 (26.0)	25.0 (29.9)	32.7 (34.8)	42.0 (40.4)	52.7 (46.5)	(35.5)
Check (water)	49.3 (44.6)	62.0 (52.0)	72.7 (58.7)	84.7 (67.4)	94.7 (77.3)	(60.0)
Mean CxT	(38.2)	(42.7)	(48.0)	(53.3)	(59.2)	
C.D. at 5 %						
Exposure period	(1.8)					
Substances	(2.9)					
Interaction Exposure period v/s Substances	N.S.					

*Figures in parentheses are arc sine transformed values

All the concentrations of *A. indica* inhibited the hatching except the lowest level of concentration (1:80) wherein hatching was found to be 35.3 % at 24 h and it increased upto 63.7 % at 192 h. Aqueous extract of *X. strumarium* was found to be even better in comparison to *A. aspera* in delaying the hatching, as there was only 12.7 % hatching at 24 h. The aqueous extract of *A. indica* shows marked nematostatic as well as nematicidal activity due to the presence of certain chemical constituents in the extract. Present studies of delayed hatching with *A. indica* leaf extract are supported by [19], who also reported that fresh extracts of fruit, leaf, bark, root and gum inhibited hatching of *M. incognita*. *A. indica* contains a number of alkaloids and lipid associates such as nimbidol, nimbidin, nimbdin, nimbin, nimbinin, vepanin, pyronimin etc. in various tissues in various concentrations [9]. The neem constituents such as nimbin, salanin, thionemone, azadiractin and various flavonoids have been reported to have nematicidal action [22, 12]. tested different concentration of shade dried leaves, bark and kernel of neem for their inhibitory role in egg hatching of *M.*

incognita. Pronounced inhibition in egghatching and may be possible due the presence of secondary metabolites such as phenols, alkaloids, flavonoids, phlobatannins, saponins, tannins, steroids and glycosides. Neem producte are known to possess nematicidal activity against nematode population in [4]. The perusal of data on effect of chemicals on hatching of *M. graminicola* larvae revealed that both chemicals (carbosulfan and cartap hydrochloride) showed inhibitory effect on hatching of *M. graminicola*. The rate of hatching was inversely proportional to concentration of chemicals and exposure period as it was decreased with increase in concentration. The highest rate of hatching was observed at 1:80 dilution while lowest rate at 1:5 dilution in both chemicals tested (Table 1-5). Among chemicals, carbosulfan showed maximum inhibitory effect followed by cartap hydrochloride. Minimum hatching was recorded with carbosulfan i.e. 14.3 per cent larvae followed by cartap hydrochloride (15.8 %) at 1:5 after 8 days. As the concentration of the chemicals decrease the egg hatching increase. Both the chemical showed a good inhibition of egg hatching at concentration of 1:5 and 1:10. It is revealed that all three factors *viz*, substances, their concentrations and exposure time significantly affected hatching individually as well as in combination with one another. Maximum inhibition in egg hatching was observed after 8 days exposure. Irrespective of exposure period, maximum hatch was observed in distilled water (check).

Conclusion

Many plants are known to have nematicidal properties which may be utilized as organic amendments or bio-pestcides. These findings provides valuable data on wasteland plants provide a wide support for nematicidal activity and suggest that the addition of botanicals to soil has potential for development as novel nematicides for the control of the rice root-knot nematodes. Plant growth-promoting rhizobacteria (PGPR) are capable of improving the plant growth in many plants and they also act as biological control agents against plant parasitic nematodes. However, more field trials need to be carried out on these materials to test their efficacy under natural conditions.

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