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Full Length Article

In vitro and Field Evaluation of Nematicidal Potential of Synthetic Chemicals against Root Knot Nematode *Meloidogyne graminicola* in Rice

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Abstract

Meloidogyne graminicola (Golden and Birchfield) is one of the most important nematode threatening rice productions in Pakistan. In this study, nematicidal potential of five synthetic chemicals *i.e*., Furadan Rugby, Match, Polo and Matanza were tested against *M. graminicola* at their standard (S), half (S/2) and double dose (2S) levels. Results of *in vitro* study disclosed that all chemicals except Polo significantly inhibited egg hatching and J2s mortality of *M. graminicola*. Furadan, Rugby, Match and Matanza were found effective to inhibit 88.4, 86.3, 89.7 and 81.0% egg hatching, respectively as compare to control while Polo inhibited only 32% egg hatching. Moreover, Furadan and Rugby significantly inhibited the invasion and development of nematodes on rice compared to control. In field study, all chemicals were applied either as root dip or soil application. Match, Furadan and Matanza treated plants observed significantly lower number of eggs and J2s against control. Both applications methods were effective, but soil application was the best. All the chemicals applied either as root dip or soil application improved plant height, shoot weight, grain weight by inhibiting nematode reproduction *i.e*., gall formation, development of adult females, eggs and nematode population in soil except Rugby that caused phytotoxic effects on plants. All the chemicals were found more effective at their standard dose than double or half dose. In conclusion, soil application of all tested chemicals in this study except Rugby seemed viable option for commercial control of *M. graminicola* in rice fields of Pakistan. © 2019 Friends Science Publishers

Keywords: Inhibition; Mortality; Polo; Juveniles; Hatching; Inhibition

Introduction

Rice (*Oryza sativa* L.) is the second cash crop of Pakistan and a major source of foreign exchange earnings. Rice contributes 0.6% to the gross domestic product (GDP) and 3.1% as a value added in agriculture of Pakistan. During 2017, cultivated area under rice crop was 2.9 million hectares compared to 2.7 million hectares from last year, reported by government of Pakistan (GOP, 2017-18). Different biotic and abiotic factors *i.e*., irrigation water, soil fertility, insect population and diseases reduce rice production including plant parasitic nematodes. Plant parasitic nematodes are very important pathogen of crop plants and result in huge crop losses when their population is significantly high in the rhizosphere (Ali *et al*., 2017a, b). Plant parasitic nematodes affect wide range of economically important crops. Due to nematodes annual losses reached up to 100 billion US$ in the world and 500 million US$ are spent on management of nematodes (Keren-Zur *et al*., 2000). Root-knot nematodes belonging to genus *Meloidogyne* are the notorious pests infecting more than 3000 plant species in many crop plants (Ali *et al*., 2015, 2017c). *M. incognita* causes 34% losses to vegetables in farmer's fields in Punjab, Pakistan particularly in tunnel farming (Javed *et al*., 2010).

Rice root-knot nematode, *M. graminicola*, considered as an emerging threat in many rice cultivated areas of South East Asia, especially Pakistan (Gaur and Pankaj, 2010; Jabbar *et al*., 2015). It caused 16-32% yield losses in India (Rao and Biswas, 1973). However, *M. graminicola* has been reported as potentially threatening pathogen of rice in Nepal, Bangladesh and India, it is also considered as an emerging threat to cereal crops in Pakistan. *M. graminicola* was reported to infect rice in Faisalabad and Chiniot districts (Jabbar *et al*., 2015). Different strategies used for root-knot nematodes management which includes biological control, cultural practices, soil amendments, sanitation and resistant plants. Due to extra labor these practices are not cost-effective in fields that make it difficult to protect the crop (Kerry, 1990).

In case of *M. graminicola* on cereals, it is not feasible to tackle through conventional practices like crop rotations of rice with wheat in the rice-wheat cropping system of Pakistan (Jabbar *et al*., 2015). Therefore, under rice-wheat cropping system the most suitable alternative is the chemical control.

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The use of chemical control (nematicides) is time saving strategy to minimize the root-knot nematode population in fields. Due to health concerns in humans and environment, the use of some fumigants and nematicides have been banned (Rich *et al.*, 2004). However, chemical control is still considered as key approach for nematode management. Due to high rate of nematode suppression, chemicals are preferred by the farming community. Abamectin was used as seed treatment on cotton against root knot nematode and reduced the density of final population in treated plants (Monfort *et al*., 2006). Similarly, antagonistic bacteria are also good mean of controlling nematodes in plants but farmers may not be ready to use sometimes (Din *et al*., 2018).

The mode of action of nematicides can be contact or systemic. Application could be through soil application, seed treatment, bare root dip and nursery bed treatment (Jain and Bhatti, 1988; Jain and Gupta, 1990). Nematicdes have basipetal systemic movement used as foliar spray on plants (Johnson *et al.*, 1995). The use of carbofuran in rice fields successfully controls the *M. graminicola* (Soriano and Reversat, 2003). Seed lings treated with carbofuran suppressed the nematode population in fields (Prasad, 2006). Carbosulfan and chlorpyriphos at 0.1 and 0.2% concentration respectively as root-dip treatment of rice seedlings for 6 h significantly inhibit the rice root-knot nematode in controlled conditions and enhance the rice growth and decrease the galling and nematode final population in soil (Deka and Das, 2002). Single treatment of nematicide was not found reliable control of root-knot nematode in rice due to quick dilution under submerged conditions (Khan and Jairajpuri, 2010; Prasad *et al.*, 2010).

Furthermore, the true nematicides are not available in agriculture product market of Pakistan, especially for rice nematodes. Carbofuran is used as insecticides against rice borers and leaf minors. Therefore, present study was conducted to discover the nematicidal potential of available synthetic insecticides (Carbofuran, Rugby, Match, Polo and Matanza) against *M. graminicola*. Moreover, the effect of above said chemicals on rice growth and yield was also evaluated.

Materials and Methods

Evaluation of Inhibitory Effect of Chemicals on Egg Hatching of *M. graminicola*

Collection of diseased plants: Rice roots infected with root-knot nematode *M. graminicola* were collected from rice production area of University of Agriculture Faisalabad. Roots were processed to evaluate root-knot nematode population. The roots were gently washed to separate soil and weighed. The whole root system was sliced and transferred into mist chamber for incubation of 5 days for eggs hatching.

Identification of *M. graminicola*

The identification of species was done by making perineal patterns of mature females (Jepson, 1987). The confirmation was done by examination of at least 20 perineal patterns.

Mass Culturing of *M. graminicola*

Sandy loam soil was obtained and sterilized in oven at 120°C for 20 min and after that it was stored at least two weeks at 25°C (Talavera and Mizukubo, 2003). Seeds of rice variety 'PK-386' were collected from Rice Research Institute, Kala Shah Kaku, Lahore, Pakistan. Rice seeds were planted in seedling trays filled with sterilized soil. After four weeks of nursery age, seedlings were transplanted in plastic pots (30-cm diam.). Pure cultures were maintained from field population by taking single egg mass of *M. graminicola* and inoculated into pots around young rice seedlings. For mass culturing at least 15 egg masses obtained from pure culture were inoculated to new seedlings in order to establish sufficient nematode culture for further studies.

Evaluation of Inhibitory Effect of Chemicals on Egg Hatching of *M. graminicola*

Three concentrations (S = Recommended dose, S/2 = Half dose and 2S = Double dose) of each chemical were prepared followed by recommended dose and required volume of distilled water was added. Pure populations of *M. graminicola* for hatching test were used. *M. graminicola* eggs were isolated by using Hussey and Barker method (Hussey and Barker, 1973). An egg mass containing 250 eggs of uniform size was employed in each Petri dish. In each Petri dish three concentrations of individual chemical were added. Whole procedure was replicated and repeated five times at 28 ± 2°C incubation under completely randomized design (CRD). Data was recorded after 24, 48 and 72 h of incubation (Abbas *et al*., 2015). Percent inhibition of egg hatching was calculated by Abbott's formula (Abbott, 1925):

$$\frac{t-c}{100-c} \times 100 = \text{Hatching inhibition}(\%)$$

Where, **t** = Egg hatching inhibition in the treatment; **c =** Egg hatching inhibition in the control.

Egg masses were washed with 1 mL of distilled in their separate plate after each count and shifted to new fresh chemical concentrates.

Evaluation of Synthetic Chemicals on J2s Mortality of *M. graminicola*

In mortality test, experimental protocols and conditions were same as hatching inhibition test except freshly hatched second stage juveniles (J2s) of *M. graminicola*. Juveniles were extracted from the eggs and 100 μL suspension containing 100 J2s was pipetted in each petri dish. Data were recorded after 24, 48 and 72 h of incubation at 26 ± 2°C. Effect of chemicals on J2s mortality was calculated by Abbott's formula (Abbott, 1925):

$$\frac{t-c}{100-c} \times 100 = \text{Mortality (\%)}$$

Where, **t**= Percent mortality in treated; **c**= Percent mortality in the untreated control.

Juveniles were considered dead if they did not move when probed with a fine needle (Abbasi *et al*., 2008).

Effect of Chemicals on Invasion and Development of *M. graminicola*

Four weeks old seedlings of rice cv. PK-386 were transplanted in 1000 mL plastic pots filled with sterilized soil amended with Furadan 3G (8 g/100 mL), Rugby 4G (12 g/100 mL), Match (0.08 mL/100 mL) and Matanza (0.4 mL/100 mL) at their recommended dose. The liquid chemicals were thoroughly mixed at volume of 100 mL in each pot before filling. Untreated pots were served as control. After one month of transplantation when plants established, 500 J2s of *M. graminicola* were inoculated in each pot (Din *et al*., 2018). The experiment was conducted with four replications (10 plants per replicate) under Completely Randomized Design (CRD). Data of nematode invasion and different development stages like J2, J3, J4, adult females and eggs were recorded after 7, 14, 21 and 28 days. At harvesting, plants from each treatment were soaked separately for 3-4 h in a container and then their roots were gently washed under running tap water. The roots were carefully handled to avoid any damage. The females attached inside the roots were recorded after staining with acid fuchsin under a stereomicroscope (Olympus SZ 61).

Evaluation of Chemicals in Field Conditions (Micro plots) Against *M. graminicola*

Four-week-old seedlings of rice were transplanted in micro plots (2.4 × 0.9 m) (Department of Plant Pathology, University of Agriculture Faisalabad). The micro plots were already infested with *M. graminicola* and infestation level was 310 J2s per 100 cm^3 soil. Infestation level was assessed by taking five soil cores from each micro plot. Soil cores were mixed thoroughly and a 100 cm^3 sub sample was used for nematode extraction on sieving-cum-modified Baermann funnel technique (Thistlethwayte, 1970). Plant to plant and row to row distance was 22.5 cm and 30 cm respectively and Randomized Complete Block Design (RCBD) was used and data was analyzed under two factor factorial.

Experiment was conducted in two sets, 1- Soil application 2- Root dip application. Chemicals were applied before transplantation of plants in soil and seedlings were soaked for 6 h as root dip before transplantation. For root-dip treatment, seedlings of PK-386 were dipped in 20 ppm solution of each treatment. Furadan, Match, Rugby and Matanza were applied at their standard concentration whereas the Polo was excluded due to non-significant effects in *in vitro* assay. Untreated plots served as control. After 105 days of transplantation data were recorded. The plant growth responses viz., plant height (cm), shoot weight (g), root weight (g), grain weight (g) and reproduction parameters viz., galling index, female count, eggs/5 g galled roots, J_2s/100 cm^3 of soil were recorded using standard procedures (Dushyant *et al*., 2017; Narasimhamurthy *et al*., 2017).

Statistical Analysis

Data was subjected to statistical analysis using statistical package Statistix (ver. 8.1). Significance of means was evaluated using LSD test at $P \leq 0.05$ after ANOVA.

Results

Egg Hatching of *M. graminicola*

All treatments showed egg hatching inhibition. Out of five chemicals four (Furadan, Rugby, Match and Matanza) led to maximum hatching inhibition after 24, 48 and 72 h at standard dose, while, Polo showed minimum inhibition (Table 1). The doses, S and 2S were more effective then S/2. The time of exposure, dose of chemicals has significant interaction effect on hatching such as increase in exposure time and doses increase the hatching inhibition. However, egg hatching decreased as incubation time increased in case of Polo. Furadan, Rugby, Match and Matanza showed almost equal egg hatching inhibition effect and statistically non-significant but these were significantly different from egg hatching inhibition due to Polo.

Mortality of *M. graminicola*

All treatments showed significant results on juvenile's mortality. Overall, Furadan, Rugby and Match, Match was the best, enhanced the mortality of *M. graminicola* compared with Matanza and Polo. However, Match showed higher mortality as compared to Polo (Table 1). Juvenile mortality was influenced by chemical's concentration and time of exposure. Maximum mortality was at S and 2S concentration as compared to S/2. All treatments equally increased the mortality with time of exposure. Furadan, Rugby, Match and Matanza caused maximum mortality at 2S concentration at 72 h of incubation and minimum mortality at S/2 concentration after 24 h of incubation.

Effect of Chemicals on Invasion and Development of *M. graminicola*

Effect of chemicals on invasion and development of *M. graminicola* was determined at 7, 14, 21 and 28 days of harvesting intervals (Table 2). After 7 days of application, Rugby inhibit maximum invasion of J2s as compared to control followed by Furadan, Matanza and Match. After 14 days the minimum number of J2s were observed in all treatments but number of new J2s were also invaded. Number of J3s increased in all treatments as compared to the first

Table 1: Interactive effect of synthetic chemicals and their concentrations on egg hatching and larval mortality of *M. graminicola*

Chemicals	Dose	Egg hatching inhibition (%)			Juvenile's mortality (%)		
		24 h	48 h	72 h	24 h	48 h	72 h
Furadan	**S**	65.20 klm	70.36 jk	100.00 a	86.8 c	89.0 bc	100.0 a
	S/2	89.52 defg	59.27 mn	93.54 abcdef	30.0 kl	41.0 j	76.6 d
	2S	83.27 gh	100.00 a	100.00 a	100.0 a	100.0 a	100.0 a
Rugby	**S**	73.09 ij	96.27 abcde	100.00 a	51.6 i	100.0 a	100.0 a
	S/2	56.67 no	91.62 bcdef	97.41 abc	24.6 m	41.6 j	58.4 gh
	2S	92.00 bcdef	100.00 a	100.00 a	92.4 b	100.0 a	100.0 a
Match	**S**	91.37 bcdef	94.29 abcdef	100.00 a	50.2 i	54.8 hi	68.4 e
	S/2	62.50 lmn	88.49 efg	97.49 ab	26.2 lm	31.8 k	41.4 j
	2S	92.86 abcdef	100.00 a	100.00 a	61.6 fg	90.0 bc	66.2 ef
Polo	**S**	32.00 qr	27.83 rs	22.78 st	16.4 n	53.6 i	21.8 m
	S/2	11.80 u	15.76 tu	12.57 u	14.6 n	16.2 n	15.8 n
	2S	40.38 p	41.03 p	39.21 pq	30.6 kl	34.2 k	70.0 e
Matanza	**S**	70.00 jkl	87.14 fgh	94.24 abcdef	70.8 e	87.0 c	100.0 a
	S/2	50.18 o	72.36 ijk	89.58 cdefg	50.4 i	79.8 d	85.6 c
	2S	80.00 hi	96.58 abcd	95.89 abcde	86.4 c	100.0 a	100.0 a
LSD ($P \leq 0.05$)		7.83**			4.76**		

The means followed by the same letters are not significantly different by LSD test at $P \leq 0.05$ level S=Standard/recommended dose, S/2=Half dose, 2S=Double dose

Table 2: Invasion and development of *M. graminicola* influenced by different chemicals

Days	Treatment	J2s/P	J3/P	J4/P	Adult female	Eggs/P
	Furadan	71.9 d	-	-	-	-
	Rugby	23.2 e	-	-	-	-
7 days	Match	101.6 b	-	-	-	-
	Matanza	89.0 c	-	-	-	-
	Control	225.0 a	-	-	-	-
	LSD	9.7724				
	Furadan	26.4 c	40.0 c	15.5 b	-	-
	Rugby	22.6 c	52.8 b	5.8 d	-	-
14 days	Match	36.3 b	55.4 b	12.2 bc	-	-
	Matanza	23.4 c	57.3 b	8.3 cd	-	-
	Control	85.3 a	90.2 a	62.5 a	-	-
	LSD	6.3171	8.2660	5.6782		
	Furadan	-	58.5 a	13.4 d	5.6 c	-
	Rugby	-	62.6 a	18.6 d	3.8 c	-
21 days	Match	-	13.5 c	76.7 b	15.5 b	-
	Matanza	-	12.8 c	69.3 c	6.9 c	-
	Control	-	42.1 b	139.3 a	46.8 a	-
	LSD		7.9049	6.6639	7.1745	
	Furadan	-	0.0 c	46.4 c	25.5 c	3825.0 c
	Rugby	-	4.4 b	55.1 c	26.1 c	3915.0 c
28 days	Match	-	16.5 a	77.7 b	23.9 c	3585.0 c
	Matanza	-	0.0 c	10.1 d	67.2 b	10080 b
	Control	-	0.0 c	173.1 a	145.8 a	18990 b
	LSD $P \leq 0.05$		1.48	19.56	17.43	2337.7

Means with same letters are not significantly different by LSD test at $P \leq 0.05$
J2s = second stage juvenile, J3s = Third stage juvenile, J4 = Fourth stage Juvenile, P= Plant

harvest and J4s were also observed after 14 days. The J2s (Swollen) were recorded maximum in control while the minimum was observed in Furadan. The J4s were maximum in control and minimum in Rugby treated plants. Rugby and Matanza caused significant reduction of J4s development as compared to control.

J3s were not recovered from infected roots after 21 days while few J4s were observed from Furadan and Rugby treated plants as compared to control. Small number of J3s was recovered from Matanza and Match treated plants while J4s were maximum from these. The recovery of mature females was maximum in control after 21 days as compared to treated plants. J4s were not recorded after 28 days of harvesting only negligible number of J2s were observed. Among the chemicals Furadan showed maximum inhibition. The number of mature females was significantly reduced by Match, Furadan and Rugby as compared to control. After 28 days of inoculations maximum number of eggs were recovered from control as compared to treated plants while significantly lower number of eggs was recovered from Match, Furadan and Matanza, respectively.

Management of *M. graminicola* through Soil and Root dip Application of Chemicals

Plant growth and yield related traits: Interaction between chemicals and their application methods had significant effect on rice growth attributes (Table 3). Soil application of Matanza and Furadan improved the height of rice plants against all other combinations while soil application of Rugby resulted in minimum plant height, even less than disease control (Table 3). Similarly, for the shoot weigh, root dip method with Furadan, Match and Matanza significantly enhanced shoot weight that was comparable to the healthy control (Table 3). However, on the other hand, Rugby application using both methods led to minimum shoot weight that was non-significantly different from the diseased control (Table 3).

Root dip application of Furadan and Matanza enhanced root weight as compared to all other combinations including healthy control with root dip method whereas soil application of Rugby demonstrated minimum root weight, even less than disease control (Table 3). Likewise, among all the combinations, grain weight was found maximum in root dip method while soil application of Rugby displayed minimum grain weight which was even lower than disease control (Table 3).

Table 3: Interactive effect of soil and root dip application of synthetic chemicals on plant growth

Treatment	PH (cm)		SW/P		RW/P		GW/P (gm)	
	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2
Furadan	64.7 a	75.0 a	49.7 cd	67.6 a	21.3 ef	31.2 b	9.9 de	13.5 b
Rugby	57.6 c	38.0 e	30.8 f	36.0 ef	13.2 h	15.0 gh	6.1 f	7.2 f
Match	62.0 bc	64.5 b	42.0 de	62.6 ab	18.0 fg	26.1 cd	8.4 ef	12.5 bc
Matanza	66.5 b	77.5 a	55.3 bc	70.5 a	23.7 de	29.4 bc	11.0 cd	14.1 b
Control	57.8 c	46.6 d	35.0 ef	37.4 ef	15.0 gh	15.6 gh	7.0 f	7.4 f
Healthy	77.0 a	76.3 a	68.6 a	65.5 ab	36.9 a	27.3 b-d	20.5 a	13.1 bc
LSD ($P \leq 0.05$)	4.65		7.66		3.00		1.61	

Means with similar letters are not significantly different by LSD test at $P \leq 0.05$

Here PH = Plant height; SW = Shoot weight; RW = Root weight; GW = Grain weight; M_1= Soil application; M_2 = Root dip method; P=plant; gm=grams; cm=centimeter

Table 4: Interactive effect of soil and root dip application of chemicals on *M. graminicola* reproduction

Treatment	Galls		Females		Eggs		J2s/100 cm^3 soil		RF_2	
	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2
Furadan	21.3 de	15.6 ef	6.3 de	17.1 d	1067.1 de	2556.8 d	433.8 de	1278.2 c	1.3 d	2.0 b
Rugby	13.2 f	13.5 f	3.9 de	9.4 de	661.3 de	1408.0 de	369.8 de	704.0 cde	1.1 e	2.0 b
Match	18.0 def	31.3 c	5.4 de	65.7 c	901.8 de	9800.0 c	504.0 cde	4900.0 b	1.6 c	2.0 b
Matanza	23.7 d	14.7 f	7.1 de	13.2 de	1187.4 de	1971.3 de	452.6 de	985.6 cd	1.4 d	2.0 b
Control	57.0 a	45.2 b	142.5 a	122.1 b	23798 a	18200 b	891.0 cd	9100.0 a	2.8 a	2.0 b
Healthy	0.0 g	0.0 g	0.0 e	0.0 e	0.0 e	0.0 e	0.0 e	0.0 e	0.0 f	0.0 f
LSD ($P \leq 0.05$)	4.59		6.23		1708.8		559.27		0.05	

Means with similar letters are not significantly different by LSD test at $P \leq 0.05$

RF^2 =Reproduction factor. M_1=Method (Soil application), M_2 =Method (Root dip method)

Nematode Establishment and Reproduction on Rice Plants

Interaction between chemicals and their application methods had significant effect on attributes of nematode development and reproduction (Table 4). Soil and root dip application of Rugby and root dip application of Matanza showed minimum number of galls as compared to all other combinations while root dip application of Match led to the highest number of galls (Table 4). Similarly, soil application of Rugby, demonstrated minimum number of females and eggs followed by soil application Match and Furadan whereas root dipping with Match revealed maximum number of females and eggs in all the combinations (Table 4). However, soil application of Rugby resulted into minimum J2s/100 cm^3 soil followed by soil application of Furadan and Matanza but root dipping with Match revealed maximum number of J2s/100 cm^3 soil in all the combinations which was even more than the nematode control for soil application (Table 4). Moreover, minimum reproduction factor was demonstrated by the soil application of Rugby followed by soil application of Furadan and Matanza (Table 4). Surprisingly, root dip application showed no significant effect on the reproduction factors and all the treatments in the application method were same as nematode control.

Discussion

All the tested chemicals showed hatching inhibition response and J2s mortality after different time intervals under *in vitro* studies. Different researchers have reported the nematicidal potential of chemicals on egg hatching and J2s mortality (Safdar *et al*., 2012). Nematicidal activity of different chemicals was attributed due to their different mode of actions. Rugby and Furadan belong to organophosphate and carbamate group respectively and are very effective against nematodes *in vitro*. Their nematicidal activity could be due to the inactivation of acetylcholinesterase which is critical enzyme in nervous system of nematodes.

Nematode locomotion depends upon the neurotransmission between the motor neurons and interneurons via acetylcholine and acetylcholinesterase stopped the activity of acetylcholine that helps the neurotransmission (reviewed by Ali *et al*., 2019). Similarly, Match (Lufenuron) caused significant inhibition in hatching of *M. graminicola*, its nematicidal activity is attributed to inhibition of chitin production in insects. Without chitin, a larva could not develop a hard-outer shell and its inner organs are exposed after hatching or molting (Meola and Dean, 1999). Another chemical treatment Matanza (Pyriproxyfen) was effective against *M. graminicola* leading to the mortality of juveniles and egg hatching inhibition. Pyriproxyfen is a pyridine-based pesticide which is effective against different arthropods. Pyriproxyfen is a growth regulator of insects and juvenile hormone analog (Hallman *et al.*, 2015). It inhibited the development of different larvae growth stages and hindered the completion of the insects (fleas, cockroaches, ticks, ants, carpet beetles and mosquitoes) life cycle (Szabo and Ede, 2016). So, it also affected the larvae of the nematode and hence could be used as potential control of plant parasitic nematodes.

As first two days of contact with the host are crucial for the penetration of nematodes (Nwauzor and Fawole, 1992), so minimum recovery of juveniles from the chemicals

indicates a population reduction at a critical stage of nematode invasion. In our results, recovery percentage was decreased in all the chemicals tested and it is in accordance with the findings of previous studies of tests of various chemicals on *C. elegans* and *M. javanica* (Lei *et al*., 2010; Moosavi, 2012). Rugby, Furadan, Match and Matanza showed increasing trend of mortality as time of exposure increases, while, Polo showed a decreasing trend in efficacy with the increase in time. Maximum population of nematodes was observed after 28 days interval in all chemicals. Chemicals were applied in soil as a single dose before transplantation of rice plants. A direct relation was observed between efficacy of chemicals and time, with the passage of time, the efficacy of chemicals decreased with the increase in nematode population (Deliopoulos *et al*., 2010). This increase in nematode population could be due to degradation of chemical pesticides in the soil. Galling index, number of females and number of egg masses were higher at third time interval due to decrease in efficacy of chemicals. So, efficiency and exposure time are negatively correlated. It may be concluded from these findings that chemicals have nematiticidal potential against *M. graminicola*.

The nematicide Furadan (Carbofuran) was found most effective against *M. graminicola* that suppress nematode population as compared to Rugby, Match and Matanza. Furadan have already been reported effective nematicide against rice root-knot nematode in India and Philippines (Mohanty *et al.*, 2000; Soriano and Reversat, 2003). Efficacy of chemicals is directly affected by soil chemistry. Light soils do not hold chemicals for long time and the chemical pesticides leach down very fast as compared to heavy soils that decrease the time of exposure of the active ingredient with the nematodes and plant root systems. Therefore, mode of application of nematicides have critical role in light soils. In our findings both root dip and soil application are very effective to suppress nematode population but root dip help to repel primary infection while soil application help to suppress the nematode population in the soil initially.

Root dip and soil application of Furadan after 15 days of transplanting have similar results as compared to the root-dip method. Generally, root-dip treatment in the joint application might be helpful in initial protection of the young plants against nematode invasion. Due to systemic mechanism Furadan and Rugby (Cadusafos) are absorbed by plant roots and their application through root-dip method proved effective against several endoparasitic nematodes (Prasad, 2006). This application kills most infective juveniles present in the root zones. This could describe the reason that why root-dip and soil application showed similar results. Matanza (Pyriproxyfen) was also very effective against *M. graminicola*. However, a contact nematicide, Phorate which is good for controlling root-knot nematodes was not very effective against *M. graminicola* as compared to Furadan when applied as root-dip method (Prasad *et al.*, 2010).

Conclusion

In conclusion, four insecticides Furadan, Rugby, Match and Matanza at their standard dose were affective against *M. graminicola* and Polo was not effective to control nematode. Although, Rugby was very effective in controlling nematode but it caused phytotoxic effect on rice growth, which made it unfit for commercial application. Root dip and soil application both were effective to control nematodes with their implications. Soil application of Carbofuran at its standard dose is very effective to suppress *M. graminicola* population in rice field.

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