Nematicidal effect of fluopyram and fluensulfone against rice root-knot nematode, *Meloidogyne graminicola*

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Summary. The comparative effectiveness of twelve non-fumigant pesticides was evaluated against the rice root-knot nematode, *Meloidogyne graminicola*. *In vitro* screening of pesticides at 50, 100 and 200 ppm exhibited substantial variability to inhibit hatching and to cause mortality of *M. graminicola* juveniles. Thereafter, the six most promising nematicides were selected for pot trials on rice 'Pusa Sugandha-5'. The plant grown in soil infested with *M. graminicola* suffered 32-36% suppression in dry shoot weight and grain yield. However, soil treatment with nematicides reduced adverse effects of nematodes but varied significantly with the treatments. Among the nematicides, soil application with fluopyram proved most effective and reduced the number of galls (76%) and reproduction factor (83%), resulting in 35-41% shoot weight promotion and grain yield production. Carbofuran was next in effectiveness followed by fluensulfone, phorate, carbosulfan and chlorpyriphos. Two years of trials in farmer's fields also confirmed the effectiveness of fluopyram, carbofuran and fluensulfone. We have demonstrated the nematicidal potential of fluopyram and fluensulfone for first time against *M. graminicola* in a series of *in vitro*, pot and field experiments.

Key words: carbamate, organophosphate, reproduction factor, root-knot nematode, soil population.

Meloidogyne graminicola Golden & Birchfield, the causal agent of root-knot disease of rice, is one of the world's most notorious and destructive nematode problems in rice (Peng *et al.*, 2018; Rusinque *et al.*, 2021). *Meloidogyne graminicola* is widespread and poses a constant threat to rice farming in various rice-cultivating nations, particularly in Southeast Asia, where it can reduce the yield of rice by up to 42% (Haque *et al.*, 2018). In India, rice root-knot nematode causes 37-45% yield loss in rice crops and has become one of the leading nematode problem in the last two decades (Khan *et al.*, 2021).

Several nematode management techniques are successful against *M. graminicola* in the rice crop (Peng *et al.*, 2018; Rusinque *et al.*, 2021). However, pesticides are still the most effective way to manage nematodes in rice environments, despite their recognised negative consequences (Khan *et al.*, 2014). Pesticides (nematicides) are favoured by farmers due to the restricted availability of rice cultivars resistant to rice root-knot nematode (Dutta *et al.*, 2012; Haque & Khan, 2021) and their immediate effects, whilst other disease control measures take a long time to show results. However, due to their negative effects on human health and the environment, several synthetic nematicides have been banned or prohibited from the market (Rusinque *et al.*, 2021), further reducing the available alternative chemicals for *M. graminicola* control.

Pesticides can have contact and/or systemic effects and are administered in a variety of ways, including seed dressing, root-dip and nursery bed applications (Khan *et al.*, 2014), although soil treatment is the most common method (Morris *et al.*, 2016). Organophosphate and carbamate nematicides are quite effective against *M. graminicola*; however, their efficiency of control varies significantly depending on the course of treatment (Haque & Khan, 2021). Earlier studies reported that the nematode galling was greatly reduced by seed or soil treatment of carbofuran, carbosulfan, chlorpyriphos and phorate in rice crops (Padgham *et al.*, 2004; Khan *et al.*, 2014). Seeds and seedlings treated with carbofuran have fewer galls and egg masses, and the populations of the nematode were reduced by up to 82% (Prasad *et al.*, 2010). Soil application with phorate and carbosulfan also significantly reduces the number of galls on rice plants (Soriano & Reversat, 2003; Khan *et al.*, 2012).

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A careful study of the available data revealed that the majority of research in this field has used carbofuran, phorate or carbosulfan with numerous treatments schemes, whereas another newly introduced nematicides, fluopyram and fluensulfone, have rarely been evaluated against rice root-knot nematode. Fluopyram is basically a broad-spectrum fungicide that belongs to the succinate dehydrogenase (SDH) group. The active ingredient inhibits Complex II of the mitochondrial respiratory chain (Storelli *et al.*, 2020). After the discovery of the nematicidal property of fluopyram, it has been widely studied against several nematodes (Faske & Hurd, 2015; Khanal & Desaeger, 2020; Storelli *et al.*, 2020) on various crops but a detailed understanding of nematicidal activity is limited. Similarly, fluensulfone belongs to a new chemical group fluoroalkenyle (thioether), exhibits systemic activity and causes irreversible paralysis to the nematode (Kearn *et al.*, 2014). The nematicidal activity of fluensulfone has been studied on various crops against several plant-parasitic nematodes (Morris *et al.*, 2016; Oka & Saroya, 2019; Khanal & Desaeger, 2020). Although a few studies have investigated the effect of fluopyram (Zhou *et al.*, 2018; Xiang *et al.*, 2020) and fluensulfone (Pooja *et al.*, 2021) as components of integrated management against *M. graminicola*, the effectiveness of both pesticides against *M. graminicola* has not been fully explored.

With this background, the present investigation was carried out and initially twelve non-fumigant chemical pesticides, viz. carbofuran, carbosulfan, chlorpyriphos, dimethoate, emamectin benzoate, fluensulfone, fluopyram, imidacloprid, malathion, monocrotophos, phorate and thiamethoxam, were screened against mortality and hatching of second-stage juveniles (J2) of *M. graminicola in vitro*. Thereafter, the evaluations were extended and the six most effective chemicals were evaluated on *M. graminicola* in pot conditions for two consecutive cropping seasons (2017 and 2018). Based on the relative effectiveness in pot trials, the three best-performing nematicides, viz. carbofuran, fluensulfone and fluopyram, were validated in naturally infested fields with *M. graminicola* for two years (2018 and 2019).

MATERIAL AND METHODS

Rice nursery. Seeds of highly susceptible rice (*Oryza sativa* L.) 'Pusa Sugandh-5' ('PS-5') (Haque & Khan, 2018) were water-soaked for a day before being seeded in 25 cm diam. clay pots, filled with 2 kg autoclaved sandy loam soil and farmyard manure (4:1), sterilised in a vertical autoclave at 121°C and 103 kPa (15 psi) for 15-20 min. The seedlings attained the planting stage in 1 month.

Pesticides and their doses. Twelve non-fumigant pesticides (Table 1) were obtained from an approved dealer of pesticides in Aligarh, India to examine their nematicidal activity in terms of inhibition of hatching and mortality of J2 *in vitro*, and three concentrations, viz. 50, 100 and 200 ppm of each pesticide were used. For pot experiments, the nematicides were applied to the soil at 20 days after planting (DAP) at the specific doses presented in the Table 1. In the field trials, carbofuran, fluensulfone and fluopyram were also administered as soil application at 20 DAP @ 2 kg, 0.5 kg and 500 ml a.s. ha^{-1}, respectively.

Nematode inoculum. A pure culture of *M. graminicola* was maintained in a Net-house on a culture bed. Susceptible rice 'PS-5' was planted in the bed on a regular basis to increase nematode reproduction and maintain a large population density of *M. graminicola* (4000-6000 J2 kg^{-1} soil). Using sterile forceps, egg masses were extracted from the galled roots. These egg masses were then surface sterilised with 0.5% sodium hypochlorite for 2 min, rinsed three times in sterile water to remove excess bleach, and put in a double-layered tissue paper held on a coarse sieve in a Baermann's funnel holding distilled water until the hatching started (after 72 h incubation at 25-27°C). Juveniles which had just hatched were collected at 24 h intervals and standardised from the funnel to 100 J2 ml^{-1} of water. The J2 were stored at 25-27°C in a BOD incubator and used within 48 h of the collection. Nematode mortality was checked before inoculation by transferring 1 ml nematode suspension to a counting dish containing 5 ml distilled water. After 2 h, J2 mortality was determined by counting the immobile J2 under stereo microscope, which was 1-2%.

Effects of pesticides on hatching and mortality of the nematode *in vitro*. Five ml of each pesticide at three concentrations (above) were put individually into a glass cavity block (length 5.5 cm, width 5.5 cm, and height 3 cm) in which ten egg masses of uniform size and surface sterilised (0.5% NaOCl for 2-3 min) were introduced (Khan *et al.*, 2021). Controls comprised distilled water cavity blocks with 10 egg masses (free of pesticides). Three replicate cavity blocks were retained for each treatment (pesticide × dosage). The cavity blocks were placed in a large Petri dish filled with distilled water (diam. 20 cm, 9 cavity blocks per Petri dish) and the lid was closed. For 7 days, the Petri dishes were incubated in a BOD incubator at 27°C and 90% RH. The hatching of J2 in the suspension was determined under a stereomicroscope at the end of the

Table 1. Details of pesticides used in the study with their application dosage.

Pesticides	Trade name	Manufacturer	Recommended dose	Application doses	
				Pots (per kg soil)	Field (per hectare)
Carbofuran	Furadam™ 10 G	Goel chemicals, India	2 kg a.s. ha^{-1}	25 mg	2 kg a.s.
Carbosulfan	Marshal™ 20 EC	FMC, India	2 l a.s. ha^{-1}	25 µl	–
Chlorpyriphos	Lethal™ 20 EC	Insecticides India Ltd., India	2 l a.s. ha^{-1}	25 µl	–
Dimethoate	Rogorin™ 30 EC	Insecticides India Ltd., India	1.5 l a.s. ha^{-1}	70 µl	–
Emamectin benzoate	EM-1™ 5 SG	Dhanuka Agrotech Ltd., India	100 g a.s. ha^{-1}	2.5 mg	–
Fluensulfone	Nimitz™ 2G	ADAMA, India	0.5 kg a.s. ha^{-1}	65 mg	0.5 kg a.s.
Fluopyram	Vellum Prime™ 34 SC	Bayer Crop Science, India	0.5 l a.s. ha^{-1}	20 µl	0.5 l a.s.
Imidacloprid	Victor™ 30 EC	Insecticides India Ltd., India	0.5 l a.s. ha^{-1}	70 µl	–
Malathion	Tusk™ 50 EC	Shivalik Crop Science, India	3 l a.s. ha^{-1}	15 µl	–
Monocrotophos	Monocil™ 36 SL	Insecticides India Ltd., India	1.7 l a.s. ha^{-1}	60 µl	–
Phorate	Thimet™ 10 G	Insecticides India Ltd., India	2 kg a.s. ha^{-1}	25 mg	–
Thiamethoxam	Cruiser™ 350 FS	Syngenta, India	0.7 kg a.s. ha^{-1}	70 mg	–

incubation. The percentage of hatching was calculated by using the formula:

$$\text{Hatching (\%)} = (A/B) \times 100,$$

where A is the number of hatched J2 after pesticide treatment, and B is the number of J2 hatched in the control wells (without pesticide).

To determine the efficacy of the pesticides on the percent mortality of J2, 50 ± 4 freshly hatched J2 (collected within 48 h) standardised in 500 µl of distilled water, were transferred to a 24-well plate (2 ml capacity). Thereafter, 500 µl of the pesticides at 50, 100 and 200 ppm were added to the plates individually. The plates were lightly shaken for uniform mixing of pesticides and covered with the lid, leaving a gap for aeration. Juveniles kept in the distilled water without pesticides served as control. Three replicates for each treatment including the untreated control were maintained. The plates were kept at 27°C and 90% RH under a BOD incubator. Inactive J2 (dead nematodes) were counted after 24, 48 and 72 h. The mortality was calculated by using the formula:

$$\text{Mortality (\%)} = (D/N) \times 100,$$

where D is the number of dead nematodes and N is the total number of J2. Both, the hatching and mortality of juveniles *in vitro* tests were repeated two times for more accuracy.

Pot experiment. To verify the effectiveness of pesticides evaluated *in vitro*, the six most effective nematicides were chosen for pot experiments. The pot trials were done successively for two cropping seasons (2017 and 2018). Rice seedlings, 1-month-old 'PS-5', were uprooted from the nursery pots and planted (1 seedling per pot) into 15 cm diam. clay pots filled with 1 kg sandy loam soil and farmyard manure (4:1), sterilised in a vertical autoclave at 121°C and 103 kPa (15 psi) for 15-20 min. The nematodes (1,000 J2) were inoculated in 2-3 holes made around the seedling using a pencil or fine rod 2 days after transplanting. The nematicides were applied through soil application by diluting the doses (listed in Table 1) in 200 ml water at 20 days after planting, based on our previous optimisation study (Haque & Khan, 2022). For treatments, two sets of pots were maintained, viz. infested (1,000 J2) and non-infested. The pots without nematode and nematicides were used as a non-infested control, whereas the pots containing *M. graminicola* but no nematicide were used as an infested control. Three pots (replicates) were kept for every treatment in both years of study and grouped in a randomised block pattern in a poly house. The ambient temperature, 25-35°C, and relative humidity, 90-95%, were maintained in the poly house during both years of experiments. To prevent nematicide loss from the pots, water (200 ml pot^{-1}) was carefully delivered without overflooding. Watering was done every 24 h until the crop was harvested. At harvest, 4 months after planting, the plants were uprooted carefully to achieve maximum root recovery and washed carefully in a gentle stream of water. Thereafter, the above-ground plant parts were cut to determine plant height and fresh weight (data not presented), dry shoot weight and grain yield per plant. The shoot and panicles were dried for 2 weeks in sun and threshed manually to determine the grain

yield. The population of *M. graminicola* from soil was also determined at harvest and used to calculate the reproduction factor.

Number of galls and egg mass. Roots were carefully washed under a moderate water stream after harvest and dyed with acid fuchsin, as described by Byrd *et al.* (1983) and the number of galls and egg masses were calculated.

Reproduction factor. Cobb's decanting and sieving method with slight modification, followed by Baermann's funnel technique, was used to calculate the final soil population (Pf) of *M. graminicola* at harvest (data not presented) and the data were used to calculate the reproduction factor (Rf) using the formula:

$$\text{Rf} = \frac{\text{final nematode population (Pf)}}{\text{the initial inoculum population (Pi)}}$$

Field trials. Validation trials were conducted at a naturally infested field with rice root-knot nematode, *M. graminicola* at the Iglas block, Aligarh (27°43'N77°55'E), Uttar Pradesh, India to check the effectiveness of the three most effective nematicides, carbofuran, fluensulfone and fluopyram, for two successive cropping seasons (2018 and 2019). In the field, other nematodes, viz. *Radopholus*, *Hoplolaimus*, *Cephalenchus*, *Polenchus*, *Rotylenchus*, *Rotylenchulus*, *Hirschmanniella*, *Ditylenchus*, *Xiphinema*, *Trichodorus* and some free-living nematodes (lacking stylet and fast-moving) were also present and their cumulative population in the suspension was 141 ± 28 nematodes kg^{-1} soil. The first-year experiment included two fields, first approximately 180 m^2 (30 × 6 m) infested naturally with *M. graminicola* (Pi = 1120 ± 84 J2 kg^{-1} soil) and the second 180 m^2 (15 × 12 m) that had low infestation (110 ± 26 J2 kg^{-1}). The second-year experiment included another two fields, one of approximately 195 m^2 (13 × 15 m), with high level of *M. graminicola* infestation (Pi = 1236 ± 104 J2 kg^{-1} soil) and the second 180 m^2 (15 × 12 m) that had low infestation (140 ± 32 J2 kg^{-1}). In the fields with low-infestation, the *M. graminicola* populations were insufficient to achieve the treatment threshold level over the trial period. Both the fields were in the same location, although they were not adjacent but had nearly similar chemical and physical characteristics. In each field, a total of 12 micro-plots (5 × 2 m) were set out. A ridge was made to separate the connected plots in order to control water movement.

Fields were prepared by ploughing 4 to 5 times followed by harrowing and laddering. Well-rotten compost was applied (10 tones ha^{-1}) during field preparation. Further, the well-ploughed fields were covered with water and thoroughly puddled using a helical blade puddler. Micro-plots of 5 × 2 m size were prepared and fertilisers, nitrogen (60 kg ha^{-1}), P_2O_5 (30 kg ha^{-1}) and K_2O (30 kg ha^{-1}) were applied. Rice seedlings, 'PS-5', grown in clay pots for 30 days, were transferred onto the treatment micro-plots (5 × 2 m) in water-saturated conditions with spacing of 20 × 20 cm (total 250 plants per micro-plots). The nematicides were applied only once at 20 days after transplanting. For soil application, a known dose of nematicides mentioned in Table 1 was suspended in 5 l of water and applied to the whole surface of the soil. In total, 4 treatments (3 nematicides + 1 control) were kept for both years in each high infested and low infested field, and three micro-plots (replicate) were kept for every treatment in a completely randomised block pattern. Manual weeding was carried out and watering was done at the time of transplanting, panicle initiation, booting, heading and flowering stages. At harvest (120 days after transplanting), ten plants were carefully picked from a micro-plot in 'W' pattern to assess the tillering and grain yield per plant. The populations of *M. graminicola* in the soil were estimated using Cobb's decanting and sieving method (set of 200 and 500 mesh sieve) followed by Baermann funnel technique (Southey, 1986). The nematode juveniles were isolated from 1 kg composite soil collected from individual micro-plot and processed separately. The samples were left for 72 h in the funnel and the nematode juveniles were collected at 24 h intervals. The number of J2 in the suspensions was quantified and counted in a counting dish using a stereo-microscope. The soil population of nematode was calculated per kg soil. The reproduction factor was also assessed at harvest applying the formula mentioned above.

Statistical analysis. All data were processed by ANOVA (analysis of variance) using SPSS 11.0 for Windows-11. The differences between the data from the two repeated *in vitro* studies and two years of pot experiments were non-significant at $P \leq 0.05$; henceforth, the data were pooled (6 replicates, 3 replicates per experiment), separately. However, the differences in the data on field experiments in two years of study were not uniform, hence data from both years are presented. The data of *in vitro* studies (hatching and mortality of juveniles) were analysed using a two-factor ANOVA, with the pesticides as one factor and doses as the second, and F-values were also calculated to recognise significant treatments ($P \leq 0.05$). The data on the number of galls, egg masses, reproduction factor and soil population of *M. graminicola* were analysed using a single-factor ANOVA. Due to the possible influence

of applied nematicides, the same infested field was not reused because the efficacy may be impacted in the re-evaluation. In the field experiments, the observations from ten plants from a micro-plot were averaged and denoted as a single replicate. The data from the field experiment (three replicates micro plots per year) were analysed using a two-factor ANOVA, with the nematode infestation as one factor and nematicide applications as the second factor to recognise significant treatments ($P \leq 0.05$). All figures were labelled with various letters to differentiate the significant treatments according to Tukey's test ($P \leq 0.05$). The percent variations were calculated using the following formula and utilised to further explain the findings:

Percent variation = [(Treatment/Control) × 100] – 100

RESULTS

***In vitro* effectiveness of pesticides on hatching and mortality.** *In vitro* screening of twelve non-fumigant pesticides exhibited substantial variability on the hatching and mortality of *M. graminicola* J2. Among the pesticides, carbofuran, carbosulfan, chlorpyriphos, fluensulfone, fluopyram and phorate caused the greatest decrease in the hatching (Table 2). In general, the pesticides with significant inhibition in hatching also caused the highest mortality (Table 2). Among the pesticides, carbofuran (100 ppm), carbosulfan (100 ppm), chlorpyriphos (200 ppm), fluensulfone (200 ppm), fluopyram (50 ppm) and phorate (100 ppm) caused 100% mortality of *M. graminicola* J2 (Table 2). Overall, the effectiveness of pesticides on the hatching of *M. graminicola* J2 at 100 ppm was in the order: fluopyram > carbofuran > fluensulfone > phorate > carbosulfan > chlorpyriphos > emamectin > malathion > imidacloprid > monocrotophos > thiamethoxam> dimethoate. Similarly, the order of mortality of *M. graminicola* J2 at 100 ppm was: fluopyram > carbofuran > phorate > fluensulfone > carbosulfan > chlorpyriphos > emamectin > thiamethoxam > monocrotophos > malathion > imidacloprid > dimethoate (Table 2).

Pot experiments. Root-knot severity (number of galls and egg masses). *Meloidogyne graminicola*-infested plants were reduced in height and had chlorotic leaves; hook-shaped terminal galls were present on the roots, resulting in 43 galls and 36.5 egg masses per plant (Fig. 1). However, treatments with nematicides suppressed the root-knot severity, which varied statistically with the nematicides. The soil application of fluopyram was shown to be the most effective nematicide in decreasing the number of galls (70%) and egg masses (76%; Fig. 1). Carbofuran was next in effectiveness and decreased the galling by 65% and egg masses by 68% ($P \leq 0.05$; Fig. 1), followed by fluensulfone, which decreased the galling (59%) and egg mass (61%). Treatment with chlorpyriphos, phorate and carbosulfan also suppressed the root-knot severity, but they were 1-9% less effective than the rest of the nematicides (Fig. 1). Overall, the order of effectiveness was fluopyram > carbofuran > fluensulfone > phorate > carbosulfan > chlorpyriphos (Fig. 1).

Effects on shoot-weight and grain yield. Treatment with all six nematicides in non-infested pots had no significant effect on the dry shoot weight and grain yield; however, when applied in infested pots, it greatly increased the dry shoot weight (Fig. 2A) and grain yield (Fig. 2B). In infested control plants, *M. graminicola* infection resulted in a significant reduction in rice dry shoot weight (32%; Fig. 2B) and grain yield (36%; Fig. 2B). However, treatments with the nematicides reduced the suppressive effect of the nematode but varied with the treatment (Fig. 2A, B). In the nematode-infested soil, treatments of fluopyram and carbofuran resulted in a significant (18-41%) increase in dry shoot weight (Fig. 2A) and grain production over the control (Fig. 2B). The percent increase in these variables was greater with fluopyram (35-41%) followed by carbofuran (18-31%) and fluensulfone (16-26%; Fig. 2A, B). The effect of phorate on the dry shoot weight was statistically equal to fluensulfone ($P \leq 0.05$). In terms of increasing dry shoot weight, the carbosulfan treatment was 14% less effective than the fluopyram treatment. The differences in grain yield between carbosulfan and chlorpyriphos treatments were also significant ($P \leq 0.05$; Fig. 2B).

Reproduction factor of *Meloidogyne graminicola*. In the infested control pots, the nematode density at harvest (Pf) was significantly higher than the initial population (Pi) (Rf = 4.4; Fig. 3). In the other pots, the Rf was influenced by the nematicide application, which significantly reduced (50-83%) the density in infested pots over untreated infested control ($P \leq 0.05$; Fig. 3). The soil application of fluopyram showed maximum suppression of the *M. graminicola* density (Rf < 1), followed by carbofuran and fluensulfone (Fig. 3).

Field experiment. Root-knot severity. Root-knot symptoms were not seen in the rice plants cultivated in low-infested micro-plots in both years of study. In the highly infested micro-plots, however, plants showed severe galling (64 galls plant^{-1}) with 52 egg masses plant^{-1} in the first year and 76 galls plant^{-1} and 63 egg masses plant^{-1} in the

second year (Fig. 4). Fluopyram treatment considerably reduced the number of galls (57-53%) and egg mass (70-64%) in both years of study (Fig. 4). The soil application with fluensulfone and carbofuran also significantly reduced the root-knot severity over untreated control ($P \leq 0.05$; Fig. 4). However, suppression in the egg mass production by fluensulfone was significantly less than fluopyram (Fig. 4). Overall fluopyram was the most effective treatment followed by carbofuran and fluensulfone, respectively (Fig. 4).

Tillering and grain yield. In low-infested micro-plots, applications of all three nematicides did not have any significant effect on the number of tillers (Fig. 5A) and rice grain production (Fig. 5B) in the first year over non-treated control. However, in the second year, tillering and grain production were marginally reduced in carbofuran and fluensulfone treated low-infested micro-plots ($P \leq 0.05$). In the micro-plots highly infested with *M. graminicola*, tillering and grain production were significantly reduced by 40-47% over low-infested control micro-plots in two years of study. However, fluopyram, fluensulfone and carbofuran application increased the tillering (41-63%; Fig. 5A) and grain yield (59-75%; Fig. 5B) over non-treated highly infested control micro-plots. The soil application of fluopyram resulted in the greatest increase in tillering (63-65%) and grain yield (71-75%) in two years. The fluensulfone treatment also increased the number of tillers in the first year (Fig. 5A) and grain yield in the second year (Fig. 5B); however, it was statistically equivalent to the carbofuran treatment ($P \leq 0.05$).

Soil population and reproduction factor of *Meloidogyne graminicola*. The soil population of *M. graminicola* in the highly infested control micro-plots (non-treated) increased considerably (35-37%) compared to their initial population at harvest (Pi) during two years of study.

Table 2. Effect of the different concentrations of pesticides on the percentage hatching and mortality of second-stage juveniles of *Meloidogyne graminicola in vitro*.

Treatments	Hatching of juvenile (%)			LSD $P \leq 0.05$	Mortality of juveniles (%)			LSD $P \leq 0.05$
	50 ppm	100 ppm	200 ppm		50 ppm	100 ppm	200 ppm	
Control	$96^{A\,a}$	$96^{A\,a}$	$95^{A\,a}$	9.57	$1.45^{A\,h}$	$1.50^{A\,h}$	$1.45^{A\,g}$	1.51
Carbofuran	$27^{A\,g}$	$23^{B\,g}$	$18^{C\,g}$	2.27	$95^{A\,a}$	$100^{A\,a}$	$100^{A\,a}$	9.83
Carbosulfan	$32^{A\,f}$	$29^{B\,f}$	$25^{C\,f}$	2.87	$93^{A\,b}$	$90^{A\,b}$	$98^{A\,a}$	9.53
Chlorpyriphos	$39^{A\,e}$	$34^{B\,e}$	$30^{C\,e}$	3.43	$88^{A\,c}$	$90^{A\,b}$	$94^{A\,a}$	9.07
Dimethoate	$81^{A\,b}$	$79^{A\,b}$	$78^{A\,b}$	7.93	$19^{B\,g}$	$20^{A\,g}$	$22^{A\,f}$	2.03
Emamectin	$45^{A\,d}$	$43^{A\,d}$	$40^{B\,d}$	4.27	$63^{B\,d}$	$65^{A\,c}$	$70^{A\,b}$	6.60
Fluensulfone	$24^{A\,g}$	$20^{B\,g}$	$18^{B\,g}$	2.07	$70^{C\,c}$	$95^{B\,a}$	$100^{B\,a}$	8.67
Fluopyram	$13^{A\,h}$	$10^{B\,h}$	$07^{C\,h}$	1.00	$100^{A\,a}$	$100^{A\,a}$	$100^{A\,a}$	10.0
Imidacloprid	$74^{A\,c}$	$71^{A\,c}$	$69^{A\,c}$	7.13	$22^{B\,f}$	$24^{A\,f}$	$26^{A\,e}$	2.40
Monocrotophos	$78^{A\,b}$	$72^{A\,c}$	$72^{A\,c}$	7.47	$34^{A\,e}$	$36^{A\,e}$	$37^{A\,d}$	3.57
Malathion	$73^{A\,c}$	$70^{A\,c}$	$68^{A\,c}$	7.03	$25^{B\,f}$	$28^{A\,f}$	$30^{A\,e}$	2.77
Phorate	$30^{A\,f}$	$27^{B\,f}$	$23^{C\,f}$	2.67	$94^{A\,b}$	$100^{A\,a}$	$100^{A\,a}$	9.80
Thiamethoxam	$74^{X\,c}$	$74^{A\,c}$	$70^{A\,c}$	7.20	$40^{B\,e}$	$45^{A\,d}$	$49^{A\,c}$	4.47
LSD $P \leq 0.05$	5.27	4.98	4.75	–	5.76	6.12	6.27	–
F values ($P \leq 0.05$)								
Pesticides (df = 11)	17.5	17.4	17.1	–	15.2	15.6	15.3	–
Dose (df = 2)	6.2	5.8	NS	–	11.7	9.2	5.9	–
Pesticide × dose (df = 12)	14.3	10.7	NS	–	5.1	4.6	NS	–

Data are means of six replicates. Means in the same row followed by different letters (Uppercase), between different pesticide concentrations, are significantly different at $P \leq 0.05$. Means in the same column followed by different letter (Lowercase), between different pesticides, are significantly different at $P \leq 0.05$ according to Tukey's test. *F*-values are significant otherwise not significant (NS) at $P \leq 0.05$. LSD = least significant difference.

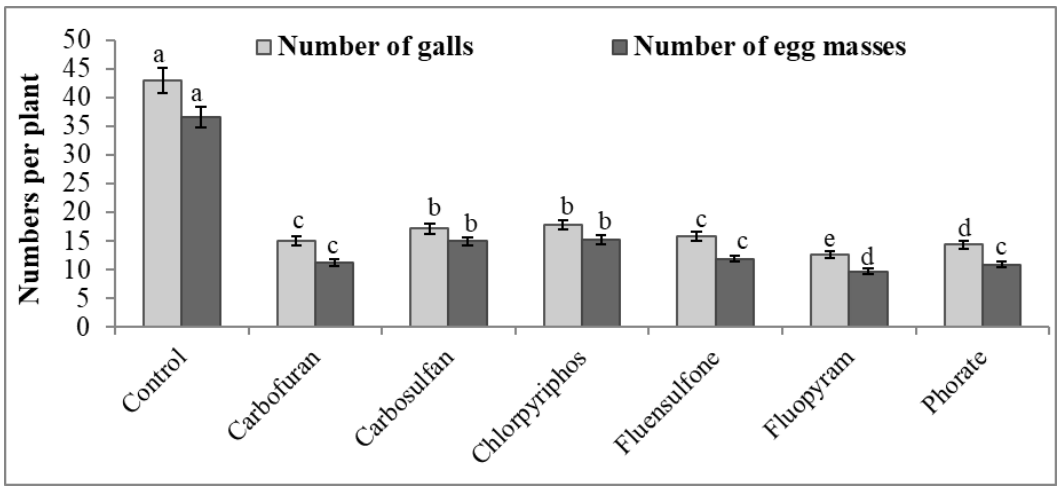

Fig. 1. Effect of nematicides on the number of galls and egg masses of *Meloidogyne graminicola* on rice 'PS-5' grown in pots. According to Tukey's test, bars with different letters are significantly different at $P \leq 0.05$. The error bars denote the standard deviation.

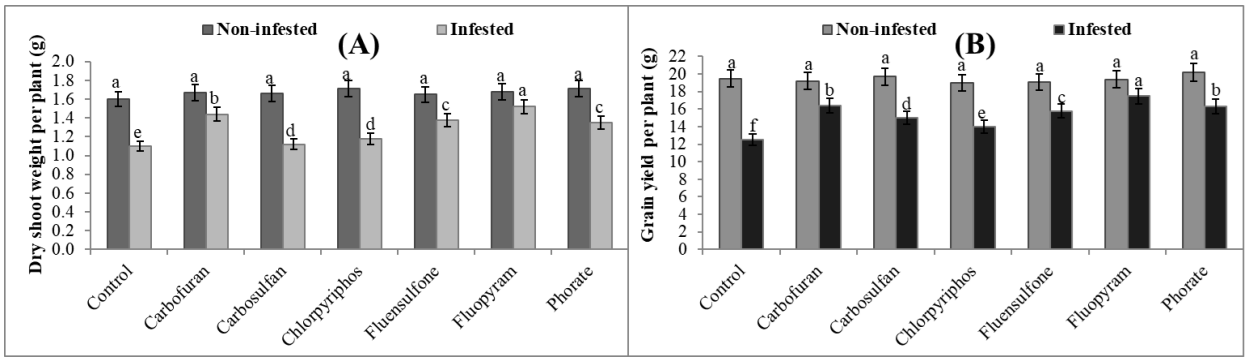

Fig. 2. Effect of nematicides on dry shoot weight (A) and grain yield (B) of rice 'PS-5' grown in pots with soil infested or non-infested with *Meloidogyne graminicola*. According to Tukey's test, bars with different letters are significantly different at $P \leq 0.05$. The error bars denote the standard deviation.

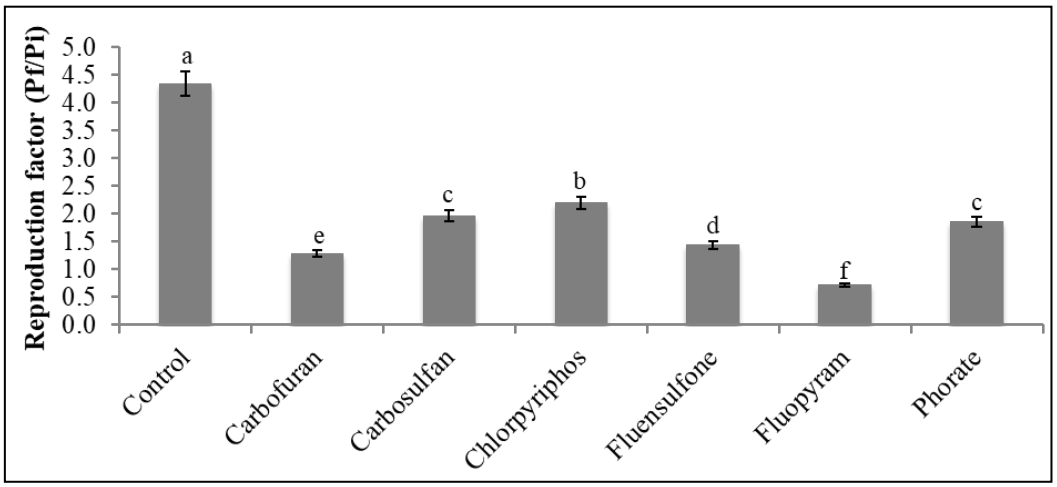

Fig. 3. Effect of nematicides on the reproduction factor of *Meloidogyne graminicola* on rice 'PS-5' grown in pots. According to Tukey's test, bars with different letters are significantly different at $P \leq 0.05$. The error bars denote the standard deviation.

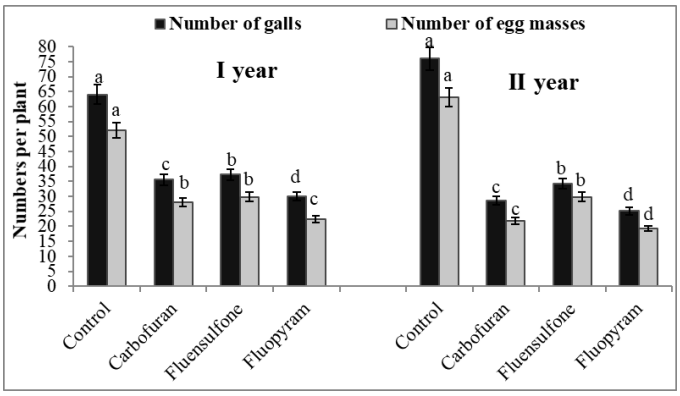

Fig. 4. Effect of carbofuran, fluensulfone and fluopyram on the number of galls and egg masses of *Meloidogyne graminicola* on rice 'PS-5' grown under field conditions for two consecutive years. According to Tukey's test, bars with different letters are significantly different at $P \leq 0.05$. The error bars denote the standard deviation.

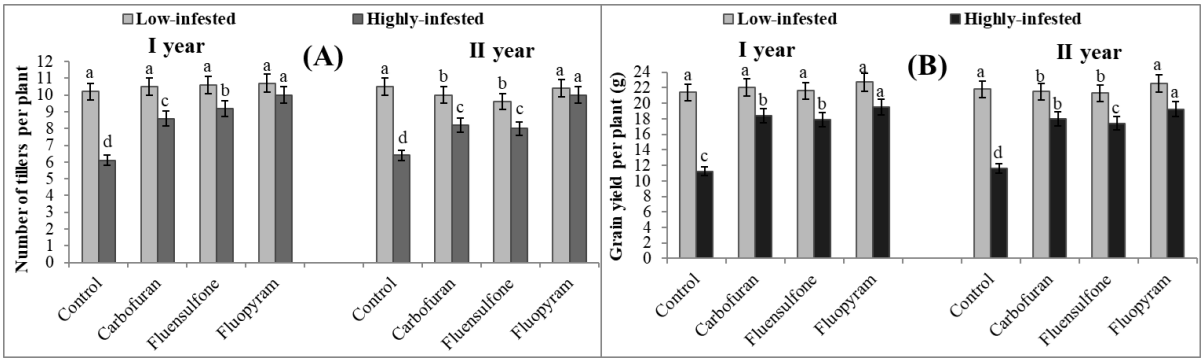

Fig. 5. Effect of carbofuran, fluensulfone and fluopyram on the number of tillers per plant (A) and grain yield (B) of rice 'PS-5' grown in the soil highly infested or low-infested with *Meloidogyne graminicola* under field conditions. According to Tukey's test, bars with different letters are significantly different at $P \leq 0.05$. The error bars denote the standard deviation.

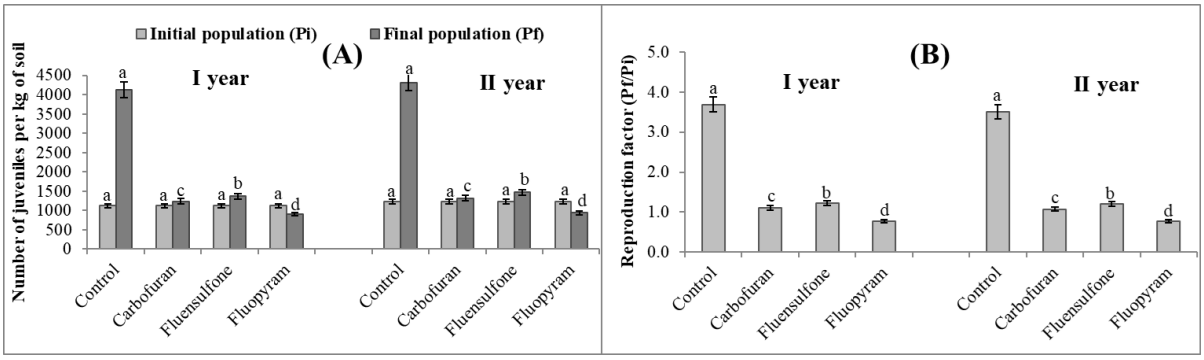

Fig. 6. Effect of carbofuran, fluensulfone and fluopyram on the soil density of nematodes (A) and reproduction factor (B) of *Meloidogyne graminicola* on rice 'PS-5' grown under field conditions. According to Tukey's test, bars with different letters are significantly different at $P \leq 0.05$. The error bars denote the standard deviation.

However, the rate of increase in the nematode population in micro-plots treated with nematicides was noticeably slower than the non-treated control micro-plots (Fig. 6A). In highly infested micro-plots, fluopyram resulted in the highest decrease in the nematode population (75-79%), followed by carbofuran (67-71%) and fluensulfone (65-70%; Fig. 6A).

The Rf in the highly infested control micro-plots noticeably increased over time with 3.5 in the first

and 3.7 in the second year at harvest (Fig. 6B). However, in the nematicide treated micro-plots, the Rf was significantly decreased compared with the non-treated control plots (Fig. 6B). In highly infested micro-plots, treatment with fluopyram exhibited the highest suppression in the Rf of *M. graminicola* with < 1 (0.76 and 0.72), followed by carbofuran (1.11 and 1.07) and fluensulfone (1.22 and 1.19), respectively, in the first and second years (Fig. 6B).

DISCUSSION

The present study evaluated the effectiveness of non-fumigant pesticides against *M. graminicola* at three levels, *in vitro*, pot and field conditions. Screening of pesticides *in vitro*, demonstrated the substantial variability to inhibit hatching and to cause mortality of *M. graminicola* J2. Among the twelve pesticides tested, fluopyram, carbofuran, carbosulfan, chlorpyriphos, fluensulfone and phorate were demonstrated to be most effective against *M. graminicola*, and cause 100% mortality of J2. Carbofuran, phorate, carbosulfan and chlorpyriphos belong to organophosphate/carbamate pesticides and their nematicidal activity is well established (Padgham *et al.*, 2004; Khan *et al.*, 2014). Fluopyram, which is basically a fungicide from the succinate dehydrogenase group and acts *via* Complex II of the mitochondrial respiratory chain, thus selectively inhibiting the nematode's generation of cellular energy (Storelli *et al.*, 2020), exhibited very good nematicidal activity against *M. graminicola*. Fluensulfone, a recently registered nematicide belonging to a new chemical group fluoroalkenyle (thioether), known to cause irreversible paralysis to the nematode (Kearn *et al.*, 2014), also proved effective against rice-root-knot nematode in terms of inhibiting hatching and mortality of J2.

The six non-fumigant chemicals evaluated for their efficiency against *M. graminicola* in pot conditions exhibited varying effects in terms of gall control and shoot weight promotion. Soil application of fluopyram lead to a greater nematode control than carbofuran and fluensulfone and subsequently improved plant yield, which was statistically on par with the former treatments. Recent investigations in strawberries also showed that fluopyram was the most effective nematicide to reduce the populations of sting nematode, while fluensulfone exhibited limited efficacy (Watson & Desaeger, 2019). Phorate was next in effectiveness followed by carbosulfan and chlorpyriphos. Soriano & Reversat (2003) also observed greater effectiveness of phorate in reducing *M. graminicola* infestation than carbosulfan or chlorpyriphos. The nematicide, phorate is a contact pesticide with some systemic capabilities (Johnson, 1985), and it has proven to be extremely efficient against a wide range of soil nematodes when applied to the soil (Khan *et al.*, 2012). Other examined nematicides, particularly carbosulfan and chlorpyriphos, decreased nematode infection in the plant as well (Khan *et al.*, 2014), although their overall effectiveness was considerably lower than that of other tested nematicides.

In naturally infested farmers' fields, the three best-performing nematicides are fluopyram, carbofuran and fluensulfone. They significantly decreased the number of galls, egg masses and soil population of *M. graminicola*. The highest suppression was recorded after soil application of fluopyram. Fluopyram was most effective in the management of rice root-knot nematode and significantly enhanced grain production. In fact, after the discovery of its nematicidal property, fluopyram has been widely studied against the majority of agriculturally important genera of plant-parasitic nematodes, especially root-knot nematodes, *Meloidogyne* spp. (Faske & Hurd, 2015; Khanala & Desaeger, 2020), reniform nematode, *Rotylenchulus reniformis* (Faske & Hurd, 2015), stem nematode, *Ditylenchus dipsaci* (Storelli *et al.*, 2020), soybean cyst nematode, *Heterodera glycines* (Beeman & Tylka, 2018) and sting nematode, *Belonolaimus longicaudatus* (Watson & Desaeger, 2019) but the effectiveness of fluopyram against *M. graminicola* has not been fully explored.

In our study, soil application of fluopyram proved to be most efficacious, because the soil treatment would have directly killed the J2 or inhibited hatching, as evidenced in *in vitro* studies, and prevented them from invading the roots. To the best of our knowledge, the results of all previous studies on fluopyram are based on either other nematode species or as a treatment combined with other pesticides (Zhou *et al.*, 2018; Xiang *et al.*, 2020). Similarly, the nematicidal effect of fluensulfone has been carried out on various crops against several plant-parasitic nematodes (Morris *et al.*, 2016; Oka & Saroya, 2019; Khanal & Desaeger, 2020) and a few studies have also investigated the effect of fluensulfone on *M. graminicola* as a component of integrated management (Pooja *et al.*, 2021) but detailed understanding of nematicidal activity against *M. graminicola* is limited.

In this study, the effect of fluopyram against *M. graminicola* was described with a series of experiments *in vitro*, pot and field experiments. *Meloidogyne graminicola* was successfully

controlled by soil application of fluopyram at 20 days after planting, which greatly improved rice tillering and grain yield. Fluopyram was also relatively more effective than carbofuran and fluensulfone. Carbofuran is one of the most frequently used nematicide against *M. graminicola* in rice (Padgham *et al.*, 2004; Prasad *et al.*, 2010). Hence, fluopyram may provide an alternative chemical for control of rice root-knot nematode in the scenario of carbofuran being banned. This finding could also be used to device suitable integrated management practices to safeguard rice from the *M. graminicola*. However, multi-locational field trials are required to confirm its performance before this chemical management module can be recommended for commercial use.

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Z. Haque and M.R. Khan. Нематицидное действие флуопирама и флуенсульфона на *Meloidogyne graminicola*.

Резюме. Оценивали сравнительную эффективность двенадцати нефумигационных пестицидов (нематицидов) против рисовой нематоды *Meloidogyne graminicola*. Применение этих нематицидов *in vitro* в концентрациях 50, 100 и 200 млн$^{-1}$ (ppm) показало существенную вариабельность их действия в смертности личинок *M. graminicola* и подавлении их вылупления. Шесть наиболее перспективных нематицидов были отобраны для испытаний на растениях риса 'Pusa Sugandha-5' при культивировании в контейнерах. При таком выращивании риса в почве, зараженной *M. graminicola*, наблюдается 32-36% снижение сухого веса корней и выхода зерна. Отмечено, что обработка почвы нематицидами снижала вредное действие нематод, но показывала существенную вариабельность между обработками. Среди всех испытанных нематицидов применение флуопирама оказалось наиболее эффективным, приводя к сокращению числа галлов (76%) и коэффициента воспроизводства нематод на 83%. В результате наблюдали увеличение на 35-41% веса проростков риса с повышением выхода зерна. Следующим по эффективности оказался карбофуран, за которым в порядке убывания эффективности следовали флуенсульфон, форат, карбосульфан и хлорпирифос. Двухлетний полевой эксперимент также подтвердил эффективность флуопирама, карбофурана и флуенсульфона. Таким образом, впервые продемонстрирован нематицидный потенциал флуопирама и флуенсульфона против *M. graminicola* в серии экспериментов *in vitro*, а также в экспериментах на растениях, выращиваемых в контейнерах и в поле.