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Novel acaricide molecules for the management of rice leaf mite, *Oligonychus oryzae* (Hirst) (Acari: Tetranychidae), an emerging pest of rice in Kerala

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Abstract
Rice leaf mite, *Oligonychus oryzae* (Hirst) is now emerging as a major pest of rice in Kerala. Pot culture experiment was conducted to evaluate the efficacy of four new acaricide molecules *viz*., fenazaquin 10 EC, spiromesifen 240 SC, fenpyroximate 5SC and propargite 57S,two botanicals *viz*., azadirachtin 0.005 per cent and neem oil 2 per centas well as wettable sulphur 80 WP along with a standard check, dicofol 18.5 EC against the mite. All the four novel acaricides significantly reduced mite population on par with that of dicofol. Fenazaquin recorded 96-97 per cent mean reduction in mite population by 14 days of spray treatment followed by spiromesifen (93-96%), propargite (93-96%) and fenpyroximate (93-95%). Efficacy of wettable sulphur (89-95%), was also found to be on par with the novel molecules. However, the efficacy of botanicals was inferior to dicofol.

Keywords: *Oligonychus oryzae*; fenazaquin 10 EC; spiromesifen 240 SC; fenpyroximate 5SC

Introduction
Of the several factors that influence the yield levels of paddy, insect and non-insect pests play a major role which demands frequent interventions. In the recent years, the spider mite, *Oligonychus oryzae* (Hirst) has emerged as a serious pest of rice causing considerable damage in South India. In Kerala, sporadic incidence of the mite was recorded during 2010 from many rice growing tracts of Palakkad district, where intensive cultivation was being practiced. In the subsequent years too, the mite incidence was reported in alarming proportions from the district. The mite colonises the undersurface of leaves in large numbers and desap causing white speckles on the upper surface which eventually turn yellow and dry up. Farmers depend mostly on conventional insecticides and acaricides for managing the mite which often lead to problems such as resurgence and adverse effects on native natural enemy fauna. Increasing concerns over their misuse in rice ecosystems have emphasized on the need for identifying safer, more effective acaricide molecules for management of the mite. However, no study on the same has yet been conducted on *O. oryzae*, an emerging mite pest of rice in Kerala

Methodology
A pot culture experiment was conducted to evaluate the efficacy of four new acaricide molecules *viz*., spiromesifen 240 SC, fenazaquin 10 EC, fenpyroximate 5 SC and propargite 57 SC and two botanicals, neem oil 2 per cent and azadirachtin 0.005% along with wettable sulphur 80WP, a standard check, dicofol 18.5 EC and an untreated control, against *O. oryzae*on rice, using the variety Jyothi. The experiment was carried out at College of Horticulture, Vellanikkara during October– November, 2014. The crop was raised in the pots and the experiment was laid out in Completely Randomized Block Design with six replicates per treatment. Mites were released on 45 days old plants by stapling mite infested rice leaf bits of 4×1 cm^2size with an average count of 30 mites per bit, at the rate of one bit per plant on the top leaf.
Treatments (Table 1) were imposed three weeks after the release of mites. Spray solution was prepared by thorough mixing of measured quantity of the insecticide and required amount of water to form a uniform emulsion. The treatments were applied using a hand operated high volume sprayer.

Population counts of eggs and active stages of *O. oryzae* were recorded from 4×1cm^2 area each from three leaves per plant. The population counts were recorded one day before spraying and 1st, 3rd, 7th, 10th and 14th day after spraying. To confirm the results, the experiment was repeated in the existing pot culture. As the population of mite was found to be negligible in all treatments except control following the first round of spray itself, a second release of the mite was made, and two weeks after the release, the treatments were imposed. Observations were recorded in a similar manner. Population difference on one, three, seven, ten and fourteen days after treatment were tested by one way ANOVA. The result obtained was subjected to DMRT (Duncan's Multiple Range Test). The mean per cent reduction in population of mites over untreated control was also worked fourteen days after treatment application.

Table 1: Treatments evaluated against *Oligonychus oryzae* on rice

Sl. No	Treatments	Dose (ml/gm) per litre
1	Fenazaquin 10 EC@ 125g ai/ha	2.5 ml
2	Spiromesifen 240 SC@ 100g ai/ha	0.8ml
3	Fenpyroximate 5 SC@ 30g a.i. ha^{-1}	2.0ml
4	Propargite 57 EC @ 600g a.i. ha^{-1}	2.0 ml
5	Neem oil 2%	20 ml
6	Azadirachtin 0.005%	5.0 ml
7	Wettable sulphur 80 WP @600g a.i. ha^{-1}	3.0gm
8	Standard Check (Dicofol 18.5 EC @ 250g ai/ha^{-1})	2.5 ml
9	Untreated Control	-

Results

Evaluation of new acaricide molecules and selected botanicals against the rice leaf mite *Oligonychus oryzae* in pot culture experiment

A pot culture experiment was conducted to evaluate the efficacy of four new acaricide molecules and two botanicals along with wettable sulphur, standard check and untreated control against the rice leaf mite on rice in the polyhouse at AINPAA, Department of Agricultural Entomology, College of Horticulture, Vellanikkara during October, 2014 to February, 2015. Efficacies of various treatments against *O. oryzae* are presented below.

Efficacy of treatments against *Oligonychus oryzae* on rice after first spray application

The results of the experiment to evaluate the efficacy of different treatments against mite after the first spray are presented in Table 1. The mean mite counts before the application of treatments per 4 cm^2 leaf area ranged from 15.88 to 20.02.

One day after spraying in (Table 1), the lowest mite count was recorded in wettable sulphur (1.14 / 4 cm^2 leaf area) and standard check, dicofol (2.13 /4cm^2 leaf area) which were on par with fenazaquin (2.23 / 4cm^2 leaf area), fenpyroximate (3.74), propargite (3.77) and spiromesifen (4.53). The botanicals *viz*, neem oil and azadirachtin recorded 4.91 and 3.40 mites respectively per 4 cm^2 leaf area.

At three days after application in (Table 1), all the treatments significantly reduced the mite population compared to untreated control. The lowest mite population of 0.33 mites per 4cm^2 leaf area was recorded in the treatment fenazaquin. The treatment spiromesifen andpropargite recorded a mite population of 0.56 and 0.61 per 4 cm^2 leaf areaand were on par with each other. Fenpyroximate recorded an average of 0.78 mites per 4 cm^2 leaf areaand was on par with standard check, dicofol (0.83 per 4 cm^2 leaf area)and wettable sulphur 80 WP (0.94 per 4 cm^2 leaf area). Azadirachtin and neem oil recorded a population of 1.00 and 1.03 mites per 4 cm^2 leaf area respectively. All were superior over untreated control which recorded a mean population of 28.2 mites per 4 cm^2 area.

At seven days after spraying in (Table 1), The lowest mite population of 0.33 per 4 cm^2 leaf area was recorded by fenazaquin. This was followed by spiromesifen (0.79), fenpyroximate (0.99) and propargite (1.53) which were on par with each other as well as standard check, dicofol (1.14 per 4 cm^2 leaf area). All the above treatments were significantly superior to botanicals and wettable sulphur. Wettable sulphur (1.70 per 4 cm^2 leaf area) was on par with the botanical, azadirachtin (1.75 per 4 cm^2 leaf area) but was superior to neem oil (1.93 per 4 cm^2 leaf area) and untreated control which recorded 31.00 mites per 4 cm^{2}leaf area.

Similar trend was observed at ten days after spraying where the chemical molecules continued to record lower mite populations. Fenazaquin recorded the lowest count of 0.68 per 4 cm^2 leaf area followed by spiromesifen (0.74) (Table 1). Both fenazaquin and spiromesifen were found to be superior over standard check dicofol. Fenpyroximate (0.87/ 4 cm^2/leaf area) and propargite (1.10 / 4 cm^2 leaf area) were found to be on par with standard check dicofol (1.29 / 4 cm^2 leaf area) (Table 1). Wettable sulphur (1.56/ 4 cm^2 leaf area) was also statistically on par with standard check dicofol. The botanicals azadirachtin (2.23) and neem oil (2.70) were found to be on par with each other and superior over untreated control (29.20) (Table 1).

At fourteen days after spraying, all the new molecules recorded lower mite population. The lowest mean mite population was recorded by fenazaquin 10 EC (0.36) which was on par with spiromesifen (0.46), fenpyroximate (0.58) and propargite (0.65).Wettable sulphur (0.64) and standard check dicofol (0.75) were also on par with new molecules. Among the botanicals,azadirachtin recorded lower mite population (1.77) as compared to neem oil (2.02). All the treatments recorded significant reduction in mite population as compared to untreated control.

Fenazaquin recorded the highest reduction in mite counts of 97.10 per cent followed by spiromesifen (96.60), propargite (96.03), fenpyroximate (95.07), wettable sulphur (95.46) and standard check, dicofol (94.64) (Table 1). Among botanicals, highest reduction in mite population was recorded by azadirachtin (87.32 %) in comparison to neem oil (85.53%) (Table 1).

Table 1: Effect of various treatments on population of *Oligonychus oryzae* on rice in First spray application

Sl. No	Treatments	Mean no. of mites / 4 cm^2 leaf area						Mean (%) reduction over control
		1DBT	1DAS	3DAS	7DAS	10DAS	14DAS	
1	T1- Fenazaquin 10 EC	15.88 (4.04)^{b}	2.23 (1.64)^{cd}	0.33 (0.91)^{e}	0.33 (0.90)^{e}	0.68 (1.08)^{e}	0.36 (0.91)^{c}	97.1
2	T2-Spiromesifen 240 SC	16.77 (4.16)^{b}	4.53 (2.21)^{b}	0.56 (1.03)^{de}	0.79 (1.14)^{d}	0.74 (1.09)^{e}	0.46 (0.97)^{c}	96.6

3	T3-Fenpyroximate 5SC	15.55 (4.00)^{b}	3.74 (2.04)^{b}	0.78 (1.12)^{bcd}	0.99 (1.22)^{d}	0.87 (1.17)^{de}	0.58 (1.01)^{c}	95.07
4	T4- Propargite 57 EC	16.84 (4.16)^{b}	3.77 (2.05)^{b}	0.61 (1.05)^{cde}	1.53 (1.14)^{d}	1.10 (1.25)^{cde}	0.65 (1.06)^{c}	96.03
5	T5-Azadiractin 0.005%	15.82 (4.03)^{b}	3.40 (1.96)^{bc}	1.00 (1.22)^{bc}	1.75 (1.48)^{bc}	2.23 (1.65)^{b}	1.77 (1.50)^{b}	87.32
6	T6- Neem oil 2%	15.63 (4.01)^{b}	4.91 (2.28)^{b}	1.03 (1.23)^{b}	1.93 (1.55)^{b}	2.70 (1.77)^{b}	2.02 (1.60)^{b}	85.53
7	T7-Wettable sulphur 80 WP	15.67 (4.01)^{b}	1.14 (1.28)^{e}	0.94 (1.19)^{bcd}	1.70 (1.44)^{bc}	1.56 (1.43)^{c}	0.64 (1.06)^{c}	95.46
8	T8- Standard check (Dicofol18.5 EC)	15.92 (4.04)^{b}	2.13 (1.58)^{de}	0.83 (1.15)^{bcd}	1.14 (1.27)^{cd}	1.29 (1.33)^{cd}	0.75 (1.10)^{c}	94.64
9	Control	20.02 (5.00)^{a}	27.90 (5.33)^{a}	28.20 (5.35)^{a}	31.00 (5.60)^{a}	29.20 (5.45)^{a}	28.00 (5.33)^{a}	
	Cd value (p=0.05)		0.37	0.15	0.22	0.2	0.21	

DBT = Day Before Treatment; DAT = Days After Treatment; Means followed by same letters do not differ significantly by DMRT (p = 0.05); Mean reduction- Mean reduction over untreated control; †- Values in the parenthesis are square root transformed values

Efficacy of treatments against *Oligonychus oryzae* on rice after second spray application

The mean count of mites before the second spray application of the treatments per 4 cm^2 leaf area ranged from 14.35 to 20.78. The results of the pot culture experiment to evaluate the efficacy of different treatments against mite after the second spray are presented in Table 2.

At one day after spraying, the new acaricide molecule fenazaquin showed a maximum reduction in mite population of 1.80/ 4cm^2 leaf area and was on par with standard check, dicofol (1.40/ 4 cm^2 leaf area) (Table 2). This was followed by the treatments propargite (2.33/ 4 cm^2 leaf area), fenpyroximate (2.84/ 4 cm^2 leaf area), spiromesifen (3.41/ 4 cm^2 leaf area) and wettable sulphur (3.70/ 4 cm^2 leaf area) which were statistically on par with each other (Table 2). The botanicals, Azadirachtin and neem oil recorded 3.79 and 4.03 mites / 4 cm^2 leaf area and were inferior to the standard check, dicofol. At seven days after spraying, the lowest mite population of 0.31 per 4 cm^2 leaf area was recorded by fenazaquin followed by spiromesifen (0.70/ 4 cm^2 leaf area),both being on par with each other (Table 2). These treatments were significantly superior to with fenpyroximate (0.64) and propargite (0.72 per 4 cm^2 leaf area) which were statistically on par with each other. Wettable sulphur (1.75/ 4 cm^2 leaf area) and azadirachtin(1.76) were on par with standard check, dicofol (1.71 per 4 cm^2 leaf area) (Table 2). Neem oil showed a mean mite population of 3.11 per 4 cm^2 leaf area and was superior over untreated control which recorded 33.6 mites per 4 cm^2 leaf area.

Similar trend was observed at ten days after spraying where the chemical molecules continued to record lower mite populations withfenazaquin recording the lowest count of 0.33 per 4 cm^2 leaf area. It was found to be on par with spiromesifen (0.52 / 4 cm^2 leaf area) and propargite (0.72 / 4 cm^2 leaf area). The treatments fenpyroximate (1.03/ 4 cm^2 leaf area), wettable sulphur (1.15/ 4 cm^2 leaf area), neem oil 2 per cent (1.25/ 4 cm^{2}leaf area) and standard check, dicofol (1.19 / 4 cm^{2}leaf area) were on par with each other. The botanical azadirachtin recorded a mite population of 1.52 per 4 cm^{2}leaf area and was superior over untreated control (29.44 per 4 cm^{2}leaf area) (Table 2).

At fourteen days after spraying, the lowest mean mite population was recorded by fenazaquin (0.31) which was on par with spiromesifen (0.30), fenpyroximate (0.54), propargite (0.56), wettable sulphur (0.48) and standard check dicofol (0.73). Among the botanicals, azadirachtin recorded lower mite population (1.24) as compared to neem oil (1.51) (Table 2). All the treatments recorded significant reduction in mite population as compared to untreated control.

After the second spray application, fenazaquin recorded the highest reduction in mite count of 96.34 per cent followed by spiromesifen (93.43) propargite 57 EC (93.75), fenpyroximate (93.44), standard check dicofol (92.16) and wettable sulphur (89.51). In case of botanicals, highest reduction in mite population was recorded in azadirachtin with 87.96 percent in comparison to neem oil with 85.43 percent (Table 2).

Table 2: Effect of various treatments on population of *Oligonychus oryzae* on rice in Second spray application

Sl No	Treatments		Mean no. of mites / cm^2 leaf area					Mean reduction (%)
		1DBS	1DAS	3DAS	7DAS	10DAS	14DAS	
1	T1- Fenazaquin 10 EC	15.41 (3.98)^{bc}	1.80 (1.46)^{c}	0.17 (0.80)^{d}	0.31 (0.89)^{e}	0.33 (0.90)^{e}	0.31 (0.90)^{c}	96.34
2	T2-Spiromesifen 240 SC	16.13 (4.06)^{bc}	3.41 (1.90)^{bc}	0.41 (0.93)^{d}	0.70 (1.05)^{de}	0.52 (0.99)^{de}	0.30 (0.91)^{c}	93.43
3	T3-Fenpyroximate 5SC	14.35 (3.85)^{bc}	2.84 (1.86)^{bc}	0.25 (0.87)^{d}	0.64 (1.06)^{d}	1.03 (1.10)^{bcd}	0.54 (0.97)^{c}	93.44
4	T4- Propargite 57 EC	14.43 (3.85)^{bc}	2.33 (1.68)^{bc}	0.47 (0.98)^{d}	0.72 (1.10)^{d}	0.72 (1.09)^{cde}	0.56 (1.00)^{c}	93.75
5	T5-Azadiractin 0.005%	15.65 (4.01)^{bc}	3.79 (2.00)^{b}	1.45 (1.39)^{b}	1.76 (1.49)^{c}	1.52 (1.41)^{b}	1.24 (1.32)^{b}	87.96
6	T6- Neem oil 2%	14.88 (3.91)^{bc}	4.03 (2.07)^{b}	1.65 (1.45)^{b}	3.11 (1.89)^{b}	1.25 (1.31)^{bc}	1.51 (1.40)^{b}	85.43
7	T7-Wettable sulphur 80 WP	16.35 (4.1)^{b}	3.70 (1.94)^{bc}	1.22 (1.31)^{bc}	1.75 (1.5)^{c}	1.15 (1.28)^{bc}	0.48 (1.02)^{c}	89.51
8	T8- Standard check (Dicofol 18.5 EC)	13.91 (3.79)^{c}	1.40 (1.44)^{c}	0.88 (1.16)^{c}	1.71 (1.48)^{c}	1.19 (1.29)^{bc}	0.73 (1.06)^{c}	92.16
9	T9- Control	20.78	27.19	29.12	33.6	29.44	31.65	

		(5.84)[a]	(5.26)[a]	(5.44)[a]	(5.83)[a]	(5.67)[a]	(5.60)[a]	
	C d value (p = 0.05)	NS	0.5	0.19	0.17	0.24	0.22	

DBT = Day Before Treatment; DAT = Days After Treatment; Means followed by same letters do not differ significantly by DMRT (p = 0.05); Mean reduction- Mean reduction over untreated control; †- Values in the parenthesis are square root transformed values

Discussion

Evaluation of the efficacy of new acaricide molecules and botanicals against the Rice leaf mite *Oligonychus oryzae* on rice.

Pot culture study was conducted to evaluate the efficacy of four new acaricide molecules, two botanicals and wettable sulphur, along with standard check and an untreated control against the rice leaf mite on rice in the poly house at College of Horticulture, Vellanikkara during December – March, 2015.

The results of the experiment showed that all the four novel acaricide molecules tested namely fenazaquin, spiromesifen, fenpyroximate and propargite were effective in reducing the population of *O. oryzae* after first and second spray applications (Fig 1 and 2). Efficacy of these molecules in reducing the mite population was pronounced from 3rd day after spray application onwards. In the study, wettable sulphur also showed good efficacy against *O. oryzae* and by 14 days after treatment application its efficacy was on par with novel acaricide molecules. However the botanicals tested were found to be inferior to all other treatments though significantly reduced mite population over untreated control.

Fenazaquin constantly recorded lower mite count up to fourteen days. At 14 DAS, it recorded the highest reduction in mite population over untreated control after each spray. Fenazaquin is an acaricide which belongs to quinazoline class of chemicals which inhibits mitochondrial electron transport (MET) at complex 1. It has high efficacy against eggs and motile stages of tetranychid mites (Marcic *et al*. 2011) [4]. In the field experiment conducted in the rice fields of Tamil Nadu during 2005 and 2006, to evaluate the efficacy of pesticides against *O. oryzae*, fenazaquin was found to be the most effective treatment against mite eggs (Radhakrishnan and Ramaraju, 2009) [7]. A significant reduction in the mite population of motile stages was also reported in the study. Fenazaquin 10 EC was also reported to cause 90.52 percent mortality of adult mites of *T. macfarlanei* (Patil, 2005) [5]. Study conducted to determine the relative toxicity of different acaricides against *O. coffeae* on tea revealed that fenazaquin was the most toxic compound against the eggs of *O. coffeae* (Roy *et al*. 2011) [9].

Spiromesifen, a tetraonic acid derivative acts as inhibitor of acetyl coA carboxylase, a key enzyme in fatty acid biosynthesis. It is highly toxic to eggs and immature stages of spider mites, while it acts more slowly against adult females causing reduction in fertility and fecundity (Marcic *et al*. 2011) [4]. A complete suppression of population of *T. urticae* could be achieved in ten days time using spiromesifen under field condition (Sato *et al*. 2011) [10]. Spiromesifen significantly reduced mite population from one day after spray application and was found to be on par with fenazaquin recording a higher reduction in mite count over untreated control. In a field experiment at Tamil Nadu, spiromesifen was reported to bring down *O. oryzae* population by 87.3 per cent. Spiromesifen has been reported to show better efficacy than dicofol against phytophagous mites (Kavitha *et al*. 2006) [2].

Fenpyroximate is a mitochondrial electron transport inhibitor with similar mode of action as fenazaquin. It causes rapid knockdown effect against larva, nymph and adult, mainly by contact and ingestion. In the present study, fenpyroximate significantly reduced mite population from 1 DAS till 14 DAS. Its efficacy was on par with other acaricidal molecules fenazaquin and spiromesifen with significant reduction in mite population over untreated control. When fenpyroximate was evaluated against *O. oryzae* in rice field of Tamil Nadu, 87.2 per cent reduction in mite population was recorded (AINPAA, 2013) [1]. Fenpyroximate recorded 99.89 per cent reduction in the mite population of *T. urticae* on chrysanthemum in polyhouse (Reddy *et al*. 2014) [8]. In the present study fenpyroximate recorded a very high reduction of 95.07 per cent after first spray and 93.44 per cent after the second spray. This substantial reduction in population may be because the experiment was carried out in controlled condition in polyhouse.

Propargite is a sulphate ester compound which is a potent inhibitor of mitochondrial ATP ase effective against synthesis of energy molecules. It is effective against all stages of mite. In the present study its efficacy was on par with other novel molecules namely fenazaquin, spiromesifen and fenpyroximate showing 96.03 per cent and 93.15 per cent reduction in mite population over untreated control. However in the rice field of Tamil Nadu only 79.53 per cent reduction in the mite population was recorded by propargite (AINPAA, 2013) [1]. As the present study was carried out in the polyhouse where controlled condition prevails the molecule might have shown better performance than expected in the open field.

Wettable sulphur though recorded lowest mite population at 1DAS during first spray, its efficacy was significantly inferior to novel acaricide molecule at 3 and 7 DAS. However at 14 DAS its efficacy was found to be on par with novel molecules. When Fenazaquin and spiromesifen was tested along with the conventional acaricides, wettable sulphur for efficacy against *T. urticae*, fenazaquin recorded the minimum mite population at two, four and seven days after treatment which was on par with sulphur. Though the population of mites two days after treatment was significantly high in spiromesifen compared to other treatments, it was found to be on par with them at four and seven days after treatment (AINPAA, 2013) [1].

In the pot culture study, considerable reduction in mite population was found in the botanicals azadirachtin (87.32 % and 87.96 %) and neems oil (85.53 % and 85.43 %) after the first and second spray application respectively. Patil and Nandihalli (2009) [6] reported that neem oil 2 per cent exhibited maximum acaricidal action against red spider mite in brinjal. When different botanicals were tested in the poly house against *T. urticae* on rose, neem oil 2 per cent was found to be the most effective treatment (Kumar, 2007) [3]. However the efficacy of both the botanicals was found to be inferior to novel molecules, wettable sulphur as well as standard check, dicofol. Field trials conducted in Tamil Nadu to study the efficacy of different acaricides against *O. oryzae* also showed that novel molecules were superior to azadirachtin (Radhakrishnan and Ramaraju, 2009) [7].

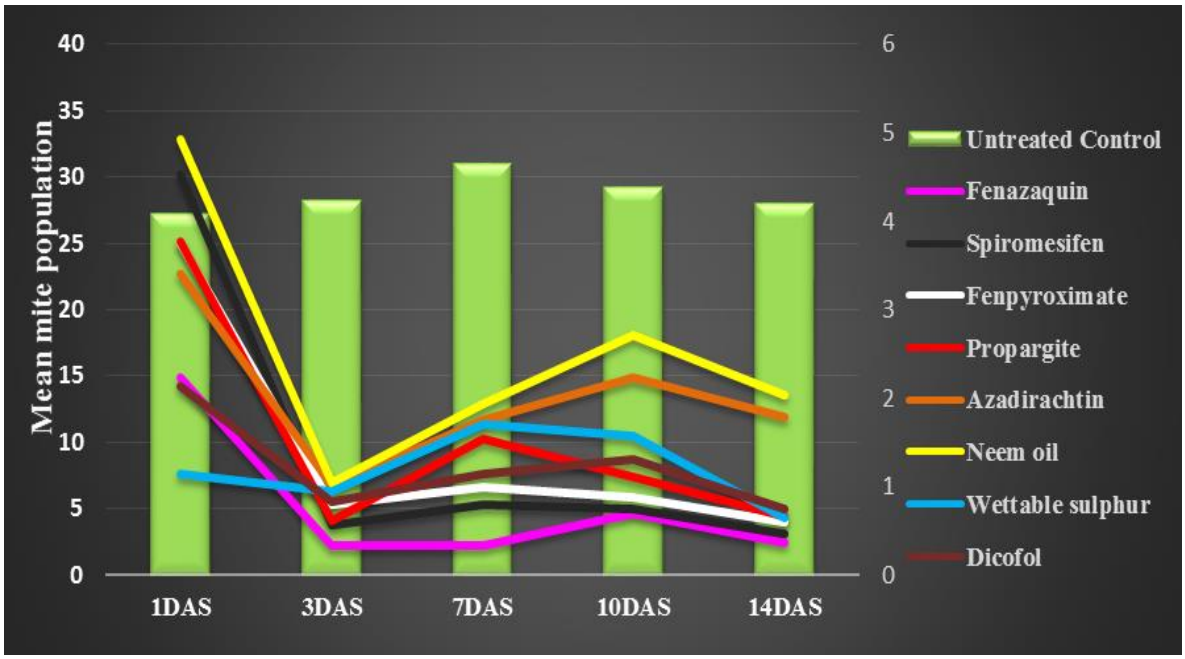

Fig 1: Efficacy of various treatments on population of *Oligonychus oryzae* on rice after first spray Days after spraying

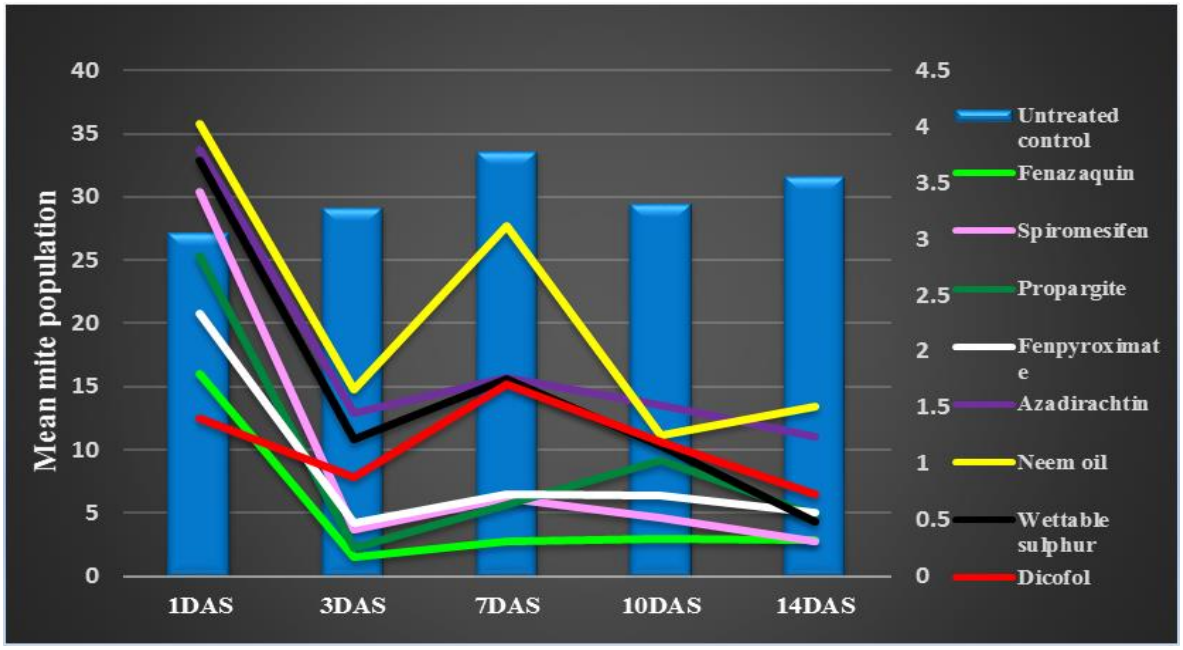

Fig 2: Efficacy of various treatments on population of *Oligonychus oryzae* on rice after second spray

Conclusion

In the pot culture experiment, all the four novel acaricide molecules tested namely fenazaquin (0.36, 0.31), spiromesifen (0.46, 0.30), fenpyroximate (0.58, 0.54) and propargite (0.65, 0.56) succeeded in reducing the population of *O. oryzae* significantly after two spray applications. Efficacy of these molecules in reducing the mite population was pronounced from 3rd day after spray application. Wettable sulphur (0.75, 0.48) also showed high efficacy against *O. oryzae* 14 days after treatment application, its efficacy was on par with novel acaricide molecules. The botanicals tested significantly reduced mite population over untreated control, though were found to be inferior to all other treatments.

The study identified a number of novel acaricide molecules namely, fenazaquin, spiromesifen, fenpyroximate and propargite for the effective management of rice leaf mite. Further, these molecules, due to their unique target specific mode of action can be considered as safe alternatives to conventional acaricides for use in rice ecosystem.

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