



Variation in the susceptibility to insecticides in rice leaf folder, *Cnaphalocrocis medinalis* (Guenee) populations from Kerala, India

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Abstract

The rice leaf folder, *Cnaphalocrocis medinalis*, is a major pest causing substantial yield losses in key rice-producing states of India, including Kerala. Despite widespread insecticide use, there is a lack of comprehensive data on the susceptibility of *C. medinalis* populations to commonly used insecticides in the region. This study evaluates the susceptibility status of *C. medinalis* to ten commonly used insecticides across five major rice-growing agroecological units in Kerala: AEU 23 (Palakkad Eastern Plains), AEU 4 (Kuttanad), AEU 3 (Onattukara Sandy Plains), AEU 6 (Kole Lands), and AEU 20 (Wayanad Central Plateau). Bioassay results indicated that the population from Wayanad remained susceptible to all tested insecticides, while other field populations exhibited high resistance to quinalphos (133.24-fold to 611.37-fold) and lambda-cyhalothrin (170.73-fold to 763.66-fold). Moderate to high resistance was observed against carbosulfan (25.40-fold to 347.96-fold), chlorantraniliprole (71.75-fold to 1089.63-fold), and flubendiamide (67.91-fold to 1572.64-fold). Correlogram analysis highlighted acephate as a viable option for management, as it showed no cross-resistance with other insecticides. Cartap and emamectin benzoate were detected as potential alternatives for managing diamide-resistant populations. The high resistance to quinalphos and carbosulfan, and their correlation with multiple insecticides, indicate the need to temporarily discontinue their use for *C. medinalis* management in Kerala. This study provides the first comprehensive assessment of insecticide resistance in *C. medinalis* populations in Kerala and establishes crucial LC₅₀ benchmarks for future resistance monitoring and management strategies.

Keywords: *Cnaphalocrocis medinalis*, cross-resistance, insecticide resistance

Introduction

In India, the rice leaf folder, *Cnaphalocrocis medinalis* (Guenee) (Lepidoptera: Crambidae) has been responsible for major outbreaks, leading to substantial crop damage in states such as Uttar Pradesh, Haryana, and Tamil Nadu (Verma et al., 1979; Khuswaha and Singh, 1985; Balasubramani et al., 2000). In Kerala, these pests are most active during the *Mundakan* season (second crop), with their populations thriving year-round (Samui et al., 2007). In 2016, rice fields in Alathur, Kollenkode, Trithala, Malampuzha, and Nemmara blocks of Kerala suffered yield losses of up to 85% (ICAR-IIRR, 2017) due to infestation of rice leaf folder. Farmers in Kerala rely heavily on insecticides to manage infestations (Devi, 2007); however, this extensive use creates selection pressure, accelerating the development of resistance. Recent

failures in controlling rice leaf folders have raised concerns among farmers, researchers, and policymakers. Despite the widespread use of insecticides, there is a lack of data on the susceptibility of *C. medinalis* to these chemicals in Kerala, and no documented cases of resistance have been reported. To address this gap, the present study examined the susceptibility of *C. medinalis* populations from five key rice-growing agroecological units (AEUs) in Kerala to ten insecticides recommended for managing rice leaf folder (KAU, 2024; DPPQS, 2025).

Materials and methods

Insects

Five field populations of *C. medinalis* were gathered from five major rice-growing AEUs of Kerala: AEU 23

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(10.86966°N, 76.2959°E) - Palakkad Eastern Plains, AEU 4 (9.519773°N, 76.359784°E) - Kuttanad, AEU 3 (9.101141°N, 76.554231°E) - Onattukara Sandy Plains, AEU 6 (10.567219°N, 76.131089°E) - Kole Lands and AEU 20 (11.849598°N, 76.043735°E) - Wayanad Central Plateau from 2023 to 2025 and assigned codes as PKD, KUD, ONT, TCR and WYD, respectively. More than 100–150 adults were collected from each site. A modified version of the rearing method proposed by Waldbauer and Marciano (1979) was employed to mass rear *C. medinalis* populations. The third instar larvae (L3) of the first (F1) generation were used for the susceptibility bioassay.

Chemicals

Commercial formulations of acephate 75% SP (Rallis), quinalphos 25% EC (Syngenta), carbosulfan 25% EC (FMC), lambda-cyhalothrin 5% EC (Syngenta), fipronil 5% SC (Bayer), cartap hydrochloride 50% SP (Dhanuka), chlorantraniliprole 18.5% SC (FMC), flubendiamide 20% WG (Rallis), tetraniliprole 18.18 % SC (Bayer) and emamectin benzoate 5% SG (Syngenta) were used for toxicological bioassay.

Toxicological bioassay

Insecticide susceptibility of each population of *C. medinalis* to selected insecticides was assessed using the seedling dip method (Zheng et al., 2011). Stock solutions for each of the ten insecticides were prepared and serial dilutions were carried out using distilled water to create six different concentrations of each insecticide with four biological replicates for each concentration (20 larvae per concentration). The control group was exposed to distilled water instead of an insecticide solution. Four-week-old TN-1 seedlings were dipped in insecticide solutions and air-dried until no visible residual water remained. The insecticide-treated rice seedlings were cut into 5 cm lengths and placed in the petri dishes (9 cm) containing hydrated tissue paper. L3 larvae were then randomly transferred to each dish using a fine, wet brush. The bioassay was conducted in controlled conditions at $28 \pm 1^\circ\text{C}$ and relative humidity of 70-80% and mortalities were checked 48 h later to determine the impact

of acephate, quinalphos, carbosulfan, lambda-cyhalothrin, fipronil and cartap and 72 h later for chlorantraniliprole, flubendiamide, tetraniliprole and emamectin benzoate.

Statistical analysis

Mortality data corrected using Abbott's formula, were subjected to probit analysis (Finney, 1971) to determine LC_{50} values and 95% fiducial limits (FL) using Polo Plus 2.0 software. The resistance ratio (RR) was determined by dividing the LC_{50} of the resistant (field) population by the LC_{50} of the susceptible (field) population. The degree of resistance was classified as demonstrated by Sun et al. (2023): $RR < 3.0$ -fold was grouped as susceptible, $3.0 \leq RR < 5.0$ -fold as decreased sensitivity, $5.0 \leq RR < 10.0$ -fold as a low resistance level, $10.0 \leq RR < 100.0$ -fold as a moderate resistance level, and $RR \geq 100.0$ -fold as a high resistance level. A correlogram analysis between LC_{50} values was performed with the Pearson method using GRAPES version 1.1.0 software (Gopinath et al., 2021), with statistical significance set at $p < 0.05$.

Results and discussion

The susceptibility of *C. medinalis* field populations from major rice-growing regions in Kerala to ten selected insecticides (2 organophosphates, 1 carbamate, 1 synthetic pyrethroid, 1 phenyl pyrazole, 1 neriectoxin analogue, 3 diamides, and 1 avermectin) are presented in Tables 1-5. The WYD population exhibited the lowest LC_{50} values across all tested insecticides (Table 1) and was used as the susceptibility baseline for calculating RR. The low LC_{50} values in WYD may be attributed to the absence of insecticide exposure, as there was no history or current use of insecticides in the area where the GI-tagged traditional aromatic rice cultivar, *Jeerakasala*, was grown.

Susceptibility variation in *Cnaphalocrocis medinalis* populations from Palakkad Eastern Plains (PKD)

The susceptibility variation in *C. medinalis* populations from PKD revealed significant resistance across multiple insecticide groups (Table 2). Among organophosphates,

Table 1. Susceptibility of *Cnaphalocrocis medinalis* from Wayanad Central Plateau (WYD)

Chemical group	Insecticides studied	LC_{50} (95%FL) ppm
Organophosphates	Acephate 75% SP	7.39 (4.06 - 10.50)
	Quinalphos 25% EC	10.22 (6.96 - 14.36)
Carbamates	Carbosulfan 25% EC	0.98 (0.71 - 1.51)
Synthetic pyrethroids	Lambda-cyhalothrin 5% EC	3.07 (1.82 - 8.79)
Phenyl pyrazoles	Fipronil 5% SG	0.61 (0.36 - 0.90)
Neriectoxin analogues	Cartap HCl 50% SP	26.53 (18.13 - 44.03)
Diamides	Chlorantraniliprole 18.5% SC	0.08 (0.06 - 0.13)
	Flubendiamide 20% WG	0.34 (0.22 - 0.48)
	Tetraniliprole 18.18% SC	0.21 (0.05 - 0.37)
Avermectins	Emamectin benzoate 5% SG	0.30 (0.17 - 0.91)

LC_{50} = Concentration (ppm) calculated to give 50 per cent mortality; FL – Fiducial limits

quinalphos exhibited the highest LC_{50} (3316.2 ppm) and RR (324.42-fold), while acephate showed a lower LC_{50} (379.76 ppm) with moderate resistance (51.40-fold). Likewise, the carbamate insecticide carbosulfan (LC_{50} 255.89 ppm) and the synthetic pyrethroid lambda-cyhalothrin (LC_{50} 1084.36 ppm) exhibited high resistance levels, with 261.03-fold and 353.26-fold, respectively. In contrast, the phenyl pyrazole insecticide fipronil (LC_{50} 26.11 ppm) and the nereistoxin analogue cartap (LC_{50} 633.87 ppm) exhibited moderate resistance, with 42.99-fold and 23.90-fold resistance, respectively. Notably, diamides demonstrated the highest resistance with flubendiamide (LC_{50} 457.03 ppm), showing an RR of 1328.60-fold, followed by chlorantraniliprole (LC_{50} 80.93 ppm) exhibiting a RR of 965.80-fold, while the population showed low resistance to the diamide tetraniliprole (6.25-fold) with an LC_{50} of 1.33 ppm. In the avermectin group, emamectin benzoate exhibited an LC_{50} of 24.03 ppm with a moderate RR of 81.09. Overall, the results indicate alarming resistance levels to multiple insecticide groups, particularly among diamides, synthetic pyrethroids, and organophosphates, highlighting the potential challenges in effective pest management.

Similar to our findings, resistance in *C. medinalis* has been reported against diamides (Zheng et al., 2011; Zhang et al., 2014), avermectins (Shiyu et al., 2016), nereistoxin analogues (Sun et al., 2023), and organophosphates (Nayak et al., 2017a). Our results align with previous findings on insecticide resistance in lepidopteran pests from Palakkad district, Kerala. The high resistance to quinalphos (324.42-fold) observed in *C. medinalis* in PKD parallels the 208.41-fold resistance reported in *Helicoverpa armigera* (Thomas, 2024b), underscoring the growing challenge of organophosphate resistance. Similarly, the high resistance to lambda-cyhalothrin (353.26-fold) in our study is consistent with the 173.08-fold resistance reported in *H. armigera* (Thomas, 2024b) in Palakkad. Furthermore, significant resistance to diamides, including chlorantraniliprole (965.80-fold) and flubendiamide (1328.60-fold), mirrors the alarming resistance levels in *Leucinodes orbonalis* from Palakkad (Thomas, 2024a). These findings highlight the escalating

resistance among lepidopteran pests in the region and emphasize the urgent need for effective resistance management interventions. Our observation of moderate resistance to emamectin benzoate (81.09-fold) aligns with the findings of Sun et al. (2023), who reported low to moderate resistance (1.9–40.3-fold) in *C. medinalis* field populations in China. Similar resistance patterns have also been documented in other lepidopteran pests, including *H. armigera* (Sene et al., 2020), *Spodoptera exigua* (Aldini et al., 2021), and *Plutella xylostella* (Oplopoiou et al., 2024).

Susceptibility variation in Cnaphalocrocis medinalis populations from Kuttanad (KUD)

The *C. medinalis* population from KUD exhibited varying degrees of resistance across different insecticide groups, with several insecticides showing high resistance levels (Table 3). Among organophosphates, both quinalphos (354.60-fold) and acephate (172.53-fold) displayed high resistance, unlike other study populations. The carbamate insecticide carbosulfan with an RR of 327.27 (LC_{50} = 320.83 ppm), the synthetic pyrethroid, lambda-cyhalothrin with an RR of 612.11 (LC_{50} = 1878.89 ppm) and the phenyl pyrazole fipronil with an RR of 116.16 (LC_{50} = 70.55 ppm) also demonstrated high resistance. The nereistoxin analogue cartap, recorded the lowest resistance among the tested insecticides (RR = 21.99, LC_{50} = 583.42 ppm). Diamides exhibited a wide range of resistance, with flubendiamide showing the highest RR in this group (933.25, LC_{50} = 321.02 ppm), followed by chlorantraniliprole (RR = 603.65, LC_{50} = 50.58 ppm). However, tetraniliprole had the lowest resistance in this category (RR = 22.37, LC_{50} = 4.75 ppm). In the avermectin group, emamectin benzoate displayed moderate resistance, with an RR of 52.69 (LC_{50} = 15.62 ppm). Overall, the findings highlight the alarming resistance levels in quinalphos, carbosulfan, lambda-cyhalothrin, chlorantraniliprole and flubendiamide, while cartap and tetraniliprole remain comparatively effective options.

The detection of resistance to acephate in this study marks the first documented case of acephate resistance in *C. medinalis* from India. This high resistance (172.53-fold) in

Table 2. Susceptibility variation in *Cnaphalocrocis medinalis* populations from PKD population

Chemical group	Insecticides studied	LC_{50} (95%FL) ppm	RR
Organophosphates	Acephate 75% SP	379.76 (248.54 - 540.61)	51.40
	Quinalphos 25% EC	3316.2 (1938.9 - 13262)	324.42
Carbamates	Carbosulfan 25% EC	255.89 (147.82 - 412.79)	261.03
Synthetic pyrethroids	Lambda-cyhalothrin 5% EC	1084.36 (775.82 - 1795.32)	353.26
Phenyl pyrazoles	Fipronil 5% SG	26.11 (18.24 - 37.51)	42.99
Nereistoxin analogues	Cartap HCl 50% SP	633.87 (443.97 - 839.42)	23.90
Diamides	Chlorantraniliprole 18.5% SC	80.93 (50.41 - 256.75)	965.80
	Flubendiamide 20% WG	457.03 (248.32 - 3307.22)	1328.60
	Tetraniliprole 18.18% SC	1.33 (0.83 - 2.00)	6.25
	Emamectin benzoate 5% SG	24.03 (15.09 - 37.53)	81.09

LC_{50} = Concentration (ppm) calculated to give 50 per cent mortality; FL – Fiducial limits; RR – Resistance Ratio

the KUD population compared to other study populations is likely a consequence of the overuse of acephate, as Devi (2007) reported that its application in Kuttanad exceeded Kerala Agricultural University's recommended limit by 83.38%. Additionally, with 16% of total pesticide sprays specifically targeting leaf folder infestations, repeated exposure to acephate has likely intensified selection pressure in this region. Our findings on quinalphos resistance (354.60-fold) align with previous reports of high resistance frequency (53.34% to 80.00%) to chlorpyrifos and (43.34% to 61.34%) to profenophos in *C. medinalis* populations from Tamil Nadu (Nayak et al., 2017b). Moreover, this study presents the first global report of resistance to lambda-cyhalothrin (612.11-fold) in *C. medinalis*, which is consistent with the findings of Pattappu et al. (2018), who documented lambda-cyhalothrin RRs of 926-fold, 250-fold, and 108-fold in *S. litura* populations from Thiruvananthapuram, Alappuzha and Pathanamthitta districts of Kerala, respectively. Similarly, the significant resistance levels observed for chlorantraniliprole (603.65-fold) in KUD correspond with previous reports of 1196.3-fold (Cho et al., 2018) and 2157-fold (Kang et al., 2017) resistance to chlorantraniliprole in *P. xylostella* populations from Korea.

Susceptibility variation in Cnaphalocrocis medinalis populations from Onattukara Sandy Plains (ONT)

Compared to other study populations, the *C. medinalis* population from ONT exhibited lower resistance to several insecticides, with some remaining feasible for effective control (Table 4). Among organophosphates, quinalphos showed high resistance (RR = 133.24, LC_{50} = 1361.95 ppm), whereas acephate exhibited very low resistance (RR = 5.07, LC_{50} = 37.47 ppm), making it a more effective option in this region. Carbosulfan (LC_{50} = 224.90 ppm, RR = 25.40) and the phenyl pyrazole fipronil (LC_{50} = 7.09 ppm, RR = 11.67) showed moderate resistance, whereas lambda-cyhalothrin (LC_{50} 524.07 ppm) exhibited high resistance (170.73-fold). Among nereistoxin analogues and diamides, the populations showed susceptibility to cartap (RR = 2.28, LC_{50} = 60.47 ppm) and tetraniliprole (RR = 2.15, LC_{50} = 0.46 ppm), suggesting that they remain valuable in managing *C.*

medinalis in this region. In contrast, chlorantraniliprole (RR = 71.75, LC_{50} = 6.01 ppm) and flubendiamide (RR = 67.91, LC_{50} = 23.36 ppm) exhibited moderate resistance, limiting their efficacy. In the avermectin group, emamectin benzoate showed moderate resistance (RR = 52.69, LC_{50} = 7.40 ppm). Overall, the ONT population exhibited greater susceptibility than other populations, particularly acephate, cartap, and tetraniliprole, which remain useful insecticidal options.

Our findings on quinalphos resistance (133.24-fold) in the ONT population align with previous reports from Tamil Nadu, where *C. medinalis* exhibited a resistance frequency of 0 to 21.6% (Anandan and Regupathy, 2002). Additionally, a gradual decline in *C. medinalis* susceptibility to chlorantraniliprole was observed across three regions in China from 2011 to 2013 compared to baseline data from 2010, demonstrating the progressive nature of resistance (Zhang et al., 2014). More recently, Sun et al. (2023) reported a 113.7-fold increase in resistance to chlorantraniliprole in *C. medinalis* in China, which aligns with the moderate resistance (71.75-fold) observed in our study. However, due to the absence of baseline data in Kerala, we could not assess the progression of resistance over time as was done in China. Furthermore, our findings on chlorantraniliprole (71.75-fold) and flubendiamide resistance (67.91-fold) correspond with Thomas (2024a), who reported resistance in *L. orbonalis* from Kerala, including 18.27-fold resistance to chlorantraniliprole and 47.59-fold resistance to flubendiamide in the Kollam population.

Susceptibility variation in Cnaphalocrocis medinalis populations from Kole lands (TCR)

The *C. medinalis* population from TCR exhibited high resistance across multiple insecticide classes, particularly against organophosphates, carbamates, synthetic pyrethroids, and diamides (Table 5). Among organophosphates, quinalphos showed the highest resistance (RR = 611.37, LC_{50} = 6249.5 ppm), whereas acephate had a lower resistance (RR = 28.48, LC_{50} = 210.47 ppm), suggesting its potential for effective use in this region. Carbosulfan (LC_{50} 341.11 ppm), lambda-cyhalothrin (LC_{50} 2344.07 ppm) and the phenyl

Table 3. Susceptibility variation in *Cnaphalocrocis medinalis* populations from KUD population

Chemical group	Insecticides studied	LC_{50} (95%FL) ppm	RR
Organophosphates	Acephate 75% SP	1274.85 (944.54 - 1865.9)	172.53
	Quinalphos 25% EC	3624.7 (2476.5 - 5524.17)	354.60
Carbamates	Carbosulfan 25% EC	320.83 (199.5 - 530.7)	327.27
Synthetic pyrethroids	Lambda-cyhalothrin 5% EC	1878.89 (1290.7 - 2915.5)	612.11
Phenyl pyrazoles	Fipronil 5% SG	70.55 (41.03 - 285.02)	116.16
Nereistoxin analogues	Cartap HCl 50% SP	583.42 (427.35 - 739.4)	21.99
Diamides	Chlorantraniliprole 18.5% SC	50.58 (32.8 - 95.09)	603.65
	Flubendiamide 20% WG	321.02 (205.19 - 719.86)	933.25
	Tetraniliprole 18.18% SC	4.75 (2.78 - 6.86)	22.37
Avermectins	Emamectin benzoate 5% SG	15.62 (7.15 - 25.11)	52.69

LC_{50} = Concentration (ppm) calculated to give 50 per cent mortality; FL – Fiducial limits; RR – Resistance Ratio

Table 4. Susceptibility variation in *Cnaphalocrocis medinalis* populations from ONT population

Chemical group	Insecticides studied	LC ₅₀ (95%FL) ppm	RR
Organophosphates	Acephate 75% SP	37.47 (22.72 - 50.99)	5.07
	Quinalphos 25% EC	1361.95 (784.92 - 3493.80)	133.24
Carbamates	Carbosulfan 25% EC	24.90 (15.75 - 34.04)	25.40
Synthetic pyrethroids	Lambda-cyhalothrin 5% EC	524.07(280.78 - 1017.02)	170.73
Phenyl pyrazoles	Fipronil 5% SG	7.09(3.36 - 10.75)	11.67
Neriestoxin analogues	Cartap HCl 50% SP	60.47 (25.21 - 91.25)	2.28
Diamides	Chlorantraniliprole 18.5% SC	6.01 (2.79 - 8.84)	71.75
	Flubendiamide 20% WG	23.36 (5.71 - 42.64)	67.91
	Tetraniliprole 18.18% SC	0.46 (0.07 - 0.86)	2.15
Avermectins	Emamectin benzoate 5% SG	7.40 (2.99 - 11.94)	52.69

LC₅₀ = Concentration (ppm) calculated to give 50 per cent mortality; FL – Fiducial limits; RR – Resistance Ratio

pyrazole, fipronil (LC₅₀ 94.05 ppm), displayed high resistance of 347.96-fold, 763.66-fold and 154.83-fold, respectively, limiting its effectiveness for managing *C. medinalis* in this population. The nereistoxin analogue cartap (RR = 14.85, LC₅₀ = 393.87 ppm) and the avermectin group, emamectin benzoate (RR = 34.94, LC₅₀ = 10.36 ppm) exhibited relatively lower resistance, making these a possible alternative. However, the diamide group presented the highest resistance levels in the TCR population, with chlorantraniliprole (RR = 1089.63, LC₅₀ = 91.30 ppm) and flubendiamide (RR = 1572.64, LC₅₀ = 540.97 ppm) showing extreme resistance, indicating a significant decline in their effectiveness. In contrast, tetraniliprole displayed comparatively lower resistance (RR = 32.37, LC₅₀ = 6.88 ppm), suggesting its potential utility in resistance management strategies. Overall, the TCR population demonstrated severe resistance to quinalphos, lambda-cyhalothrin, chlorantraniliprole, flubendiamide and fipronil, whereas acephate, cartap, tetraniliprole, and emamectin benzoate showed comparatively lower resistance, making them relatively better options for rotation in insecticide resistance management programs.

Quinalphos is recommended for managing the rice leaf folder in Kerala (KAU, 2024) and is widely used across all AEUs of the state. This extensive application may have contributed to the high resistance observed in study populations. Pattappu et al. (2018) documented significant resistance in *S.litura* populations from vegetable crops in Kerala, with RRs of

320-fold, 840-fold, and 1965-fold against chlorpyrifos and 59-fold, 255-fold, and 605-fold against quinalphos in Alappuzha, Pathanamthitta, and Thiruvananthapuram districts, respectively. Similarly, the TCR population exhibited extremely high resistance to quinalphos (RR = 611.37), reinforcing the alarming trend of resistance development in the region. To our knowledge, this study is the first to report a resistance to carbosulfan in *C. medinalis*. However, resistance to carbosulfan has been documented in other lepidopteran pests like *H. armigera* (Faheem et al., 2013) and *S. litura* (Tong et al., 2013). The LC₅₀ value of 94.05 ppm for fipronil in the TCR population is close to the LC₉₀ value (125 ppm) reported in Thailand (Supawan and Chongrattanameteekul, 2017), suggesting a critical resistance concern. This finding is consistent with previous research by Li et al. (2007), who reported fipronil resistance in *Chilo suppressalis* populations in China. The high resistance to chlorantraniliprole (RR = 1089.63) observed in this study is consistent with previous reports, such as Huang et al. (2021), who documented a 2020.1-fold resistance in *C. suppressalis* populations, and Guo et al. (2014), who reported 2128-fold resistance in *P. xylostella* in China. Our study, conducted in key rice-growing regions of Kerala, is the first to document flubendiamide resistance in *C. medinalis* populations in India. Flubendiamide resistance has been reported in other lepidopteran pests like *C. suppressalis* (Yao et al. 2017), *Tuta absoluta* (Zang et al., 2022), *H. armigera* (Abbade-Neto et al. 2022) etc.

Table 5. Susceptibility variation in *Cnaphalocrocis medinalis* populations from TCR population

Chemical group	Insecticides studied	LC ₅₀ (95%FL) ppm	RR
Organophosphates	Acephate 75% SP	210.47 (116.25 - 399.54)	28.48
	Quinalphos 25% EC	6249.5 (4006.9 - 14728)	611.37
Carbamates	Carbosulfan 25% EC	341.11 (193.90 - 659.50)	347.96
Synthetic pyrethroids	Lambda-cyhalothrin 5% EC	2344.07(1490.07 - 4651.43)	763.66
Phenyl pyrazoles	Fipronil 5% SG	94.05 (47.30 - 1315.80)	154.83
Neriestoxin analogues	Cartap HCl 50% SP	393.87 (233.30 - 802.66)	14.85
Diamides	Chlorantraniliprole 18.5% SC	91.30 (56.17 - 173.41)	1089.63
	Flubendiamide 20% WG	540.97 (360.86 - 961.64)	1572.64
	Tetraniliprole 18.18% SC	6.88 (3.41 - 30.21)	32.37
Avermectins	Emamectin benzoate 5% SG	10.36 (4.12 - 17.72)	34.94

LC₅₀ = Concentration (ppm) calculated to give 50 percent mortality; FL – Fiducial limits; RR – Resistance Ratio

*Correlogram analysis between the LC_{50} values of the tested insecticides in the field populations of *Cnaphalocrocis medinalis**

The correlogram of the analysis done to assess resistance patterns across various insecticides based on LC_{50} values is depicted in Fig. 1. Acephate has a non-significant correlation with other insecticides, indicating that resistance to acephate is unlikely to confer resistance to other compounds. Notably, acephate remains a practical option for insecticide rotation, as it does not exhibit cross-resistance with the compounds analyzed. In contrast, quinalphos, another organophosphate, showed significant correlations with carbosulfan, lambda-

cyhalothrin, fipronil, chlorantraniliprole, flubendiamide, and tetraniliprole, while its correlation with cartap hydrochloride and emamectin benzoate was not significant. The carbamate insecticide carbosulfan was significantly associated with all tested insecticides except acephate, tetraniliprole, and emamectin benzoate. Given the high resistance ratio of quinalphos and carbosulfan across all study populations and their significant correlation with most tested insecticides, avoiding their use for managing rice leaf folders in Kerala is advisable. Moreover, the significant correlation between quinalphos and carbosulfan suggests the likelihood of cross-resistance between these insecticides, which can be attributed

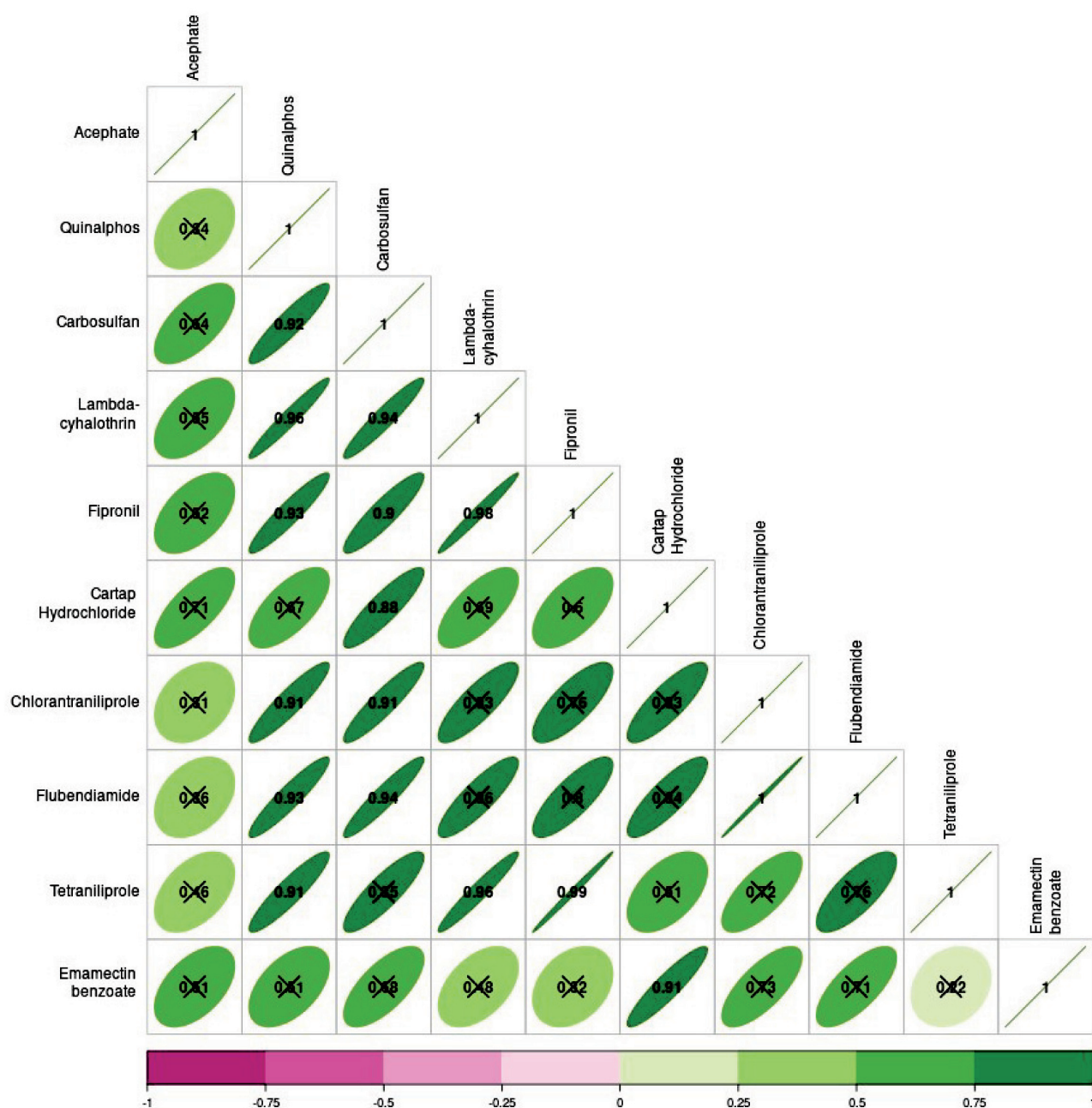


Figure 1. Correlogram between the LC_{50} values of tested insecticides in the field populations of *Cnaphalocrocis medinalis* (Significance at 0.05 ; non significant correlations are crossed in the plot)

to their shared mode of action on acetylcholinesterase enzyme (IRAC, 2025). Our findings are consistent with the pairwise comparisons of LC_{50} values for organophosphate and carbamate insecticides in *S. litura* populations, where a potential cross-resistance mechanism was identified (Saleem et al., 2008).

The nereistoxin analogue cartap hydrochloride showed a significant correlation only with carbosulfan and emamectin benzoate. Our study did not identify cross-resistance between cartap and lambda-cyhalothrin, unlike the cross-resistance between the synthetic pyrethroids fenvalerate and cartap reported by Ninsin (2015) in *P. xylostella*. However, our findings align with Pereira et al. (2023), who observed no cross-resistance of cartap to diamides in *T. absoluta*. Correlogram analysis in this study revealed that the diamide insecticides chlorantraniliprole and flubendiamide were strongly correlated and significantly associated with quinalphos and carbosulfan. Similarly, cross-resistance between chlorantraniliprole and flubendiamide has been reported in *P. xylostella* (Wang et al., 2013) and *S. frugiperda* (Bolzan et al., 2019). Moreover, the analysis suggests that quinalphos and carbosulfan should be avoided in diamide-resistant populations due to their significant correlation with resistance. In contrast, cartap and emamectin benzoate remain workable options for rotation as they do not exhibit a significant correlation with diamide resistance. However, tetraniliprole, another diamide, was not significantly correlated to chlorantraniliprole and flubendiamide resistance. Additionally, while the KUD and TCR populations showed moderate resistance, the PKD and ONT populations remained susceptible to the diamide tetraniliprole. Since tetraniliprole does not exhibit cross-resistance with other diamide insecticides, it presents a promising option for rotation in managing diamide-resistant populations. This agrees with the findings of Mbuji et al. (2024) in *H. armigera* suggesting tetraniliprole as a substitute for other insecticides. Emamectin benzoate, an avermectin insecticide, exhibited a significant correlation only with cartap hydrochloride, contrasting with the cross-resistance reported between emamectin benzoate and other insecticides, such as lambda-cyhalothrin and chlorantraniliprole, in *S. frugiperda* populations in Brazil (Muraro et al., 2021). Its lack of significant correlation with most other insecticides suggests that emamectin benzoate remains a promising candidate for inclusion in resistance management strategies for *C. medinalis*.

The synthetic pyrethroid lambda-cyhalothrin (sodium channel modulator) demonstrated significant correlations with quinalphos (acetylcholinesterase inhibitor), carbosulfan

(acetylcholinesterase inhibitor), fipronil (GABA-gated chloride channel blocker), and tetraniliprole (ryanodine receptor modulator). Since these insecticides have different modes of action, it is likely that non-specific resistance mechanisms, such as enhanced detoxification, may be contributing to cross-resistance among these insecticides (Siqueira et al. 2000). Prolonged exposure to insecticides can enhance detoxification enzyme activity, potentially influencing variations in *C. medinalis* susceptibility to the tested insecticides (Karunaratne and Surendran, 2022). The monooxygenase system consists of multiple isoenzymes, and if a particular insecticide is selected by specific isoenzymes capable of metabolizing different insecticides, cross-resistance may occur (Ishaaya and Casida, 1980). In this study, correlogram analysis revealed significant correlations between insecticides with distinct modes of action, indicating a potential shared metabolic resistance mechanism. This finding underscores the need for further biochemical investigations to understand the underlying resistance mechanisms.

Conclusion

This study provides the first comprehensive assessment of insecticide resistance in *C. medinalis* populations across Kerala, offering critical insights into resistance patterns. Among the tested populations, WYD exhibited the lowest LC_{50} values and remained susceptible to all insecticides, whereas the other three populations (PKD, KUD and TCR) demonstrated high resistance to quinalphos, carbosulfan, lambda-cyhalothrin, chlorantraniliprole, and flubendiamide, highlighting a concerning resistance scenario. Notably, high resistance to acephate was observed only in the KUD population, while fipronil resistance was high in KUD and TCR. Based on our findings, acephate remains a feasible option for integrated resistance management, as it does not exhibit cross-resistance with other tested insecticides. Cartap and emamectin benzoate remain effective alternatives for populations resistant to diamides, given their lack of significant correlation with diamide resistance. Conversely, the widespread resistance to quinalphos and carbosulfan, coupled with their strong correlations with multiple insecticides, suggests that their use should be avoided in Kerala for *C. medinalis* management. The study underscores the necessity of continuous resistance monitoring to inform sustainable insecticide management and safeguard rice production. The LC_{50} values established here serve as a crucial reference for tracking future shifts in susceptibility. To mitigate resistance development, rotation strategies should avoid consecutive application of insecticides with strong positive correlations, thereby delaying resistance escalation.

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References

- Abbade-Neto, D., Amado, D., Pereira, R.M., Basso, M., Spineli-Abbade-Neto, D., Amado, D., Pereira, R.M., Basso, M., Spineli-Silva, S., Gonçalves, T.M., Correa, A.S. and Omoto, C. 2022. First report of *Helicoverpa armigera* (Lepidoptera: Noctuidae) resistance to flubendiamide in Brazil: Genetic basis and mechanisms of the resistance. *Agronomy*, 12:1664. <https://doi.org/10.3390/agronomy12071664>
- Aldini, G. M., Wijonarko, A., Witjaksono, de Putter, H., Hengsdijk, H. and Trisyono, Y.A. 2021. Insecticide Resistance in *Spodoptera exigua* (Lepidoptera: Noctuidae) Populations in Shallot Areas of Java, Indonesia. *J. Econ. Entomol.* 20: 1-7 <https://doi.org/10.1093/jee/toab183>
- Anandan, G. K. and Regupathy, A. 2002. Insecticide resistance in rice leaffolder *Cnaphalocrocis medinalis* (Guenee) in Tamil Nadu. *Pesticide Res. J.* 14(1): 169-173.
- Balasubramani, V., Sridharan, S., and Sadakathulla, S. 2000. Effect of shade on leaf folder incidence in hybrid rice. *Insect Environ.* 6(1): 15-16.
- Bolzan, A., Padovez, F. E., Nascimento, A. R., Kaiser, I. S., Lira, E. C., Amaral, F. S., Kanno, R. H., Malaquias, J. B. and Omoto, C. 2019. Selection and characterization of the inheritance of resistance of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to chlorantraniliprole and cross-resistance to other diamide insecticides. *Pest Manag. Sci.* 75: 2682-2689. <https://doi.org/10.1002/ps.5376>
- Cho, S. R., Kyung, Y., Shin, S., Kang, W. J., Jung, D. H., Lee, S. J., Park, G. H., Kim, S. I., Cho, S. W., Kim, H. K., Koo, H. N. and Kim, G. H. 2018. Susceptibility of field populations of *Plutellaxylostella* and *Spodoptera exigua* to four diamide insecticides. *Korean J. Appl. Entomol.* 57(1):43-50. <https://doi.org/10.5656/KSAE.2018.02.0.009>
- Devi, I. P. 2007. *Pesticide use in the rice bowl of Kerala: Health costs and policy options*. SANDEE Working Paper No. 20-07, South Asian Network for Development and Environmental Economics (SANDEE), Kathmandu, Nepal, 47 p.
- DPPQS [Directorate of Plant Protection, Quarantine and Storage], 2025. [Online]. Available: https://www.ppqs.gov.in/sites/default/files/mup_insecticide_30.11.2024.pdf [Accessed 3 February 2025].
- Faheem, U., Nazir, T., Saleem, M. A., Yasin, M. and Bakhsh, M. 2013. Status of insecticide resistance in *Helicoverpa armigera* (Hübner) in southern Punjab, Pakistan. *Sarhad J. Agric.* 29 (4):563-572.
- Finney, D.J. 1971. *Probit Analysis* (3rd ed.). Cambridge University Press, Cambridge, 333 p.
- Gopinath, P.P., Prasad, R., Joseph, B., Adarsh, V.S., 2021. grapesAgri1: Collection of Shiny Apps for Data Analysis in Agriculture. *J. Open Source Software* 6(63): 34–37. <https://doi.org/10.21105/joss.03437>
- Guo, L., Liang, P., Zhou, X. and Gao, X. 2014. Novel mutations and mutation combinations of ryanodine receptor in a chlorantraniliprole resistant population of *Plutellaxylostella* (L.). *Sci. Rep.* 4: 6924. <https://doi.org/10.1038/srep06924>
- Huang, J. M., Sun, H., He, L. F., Liu, C., Ge, W. C., Ni, H., Gao, C. F. and Wu, S. F. 2021. Double ryanodine receptor mutations confer higher diamide resistance in rice stem borer, *Chilo suppressalis*. *Pest Manag. Sci.* 77(11): 4971-4979. <https://doi.org/10.1002/ps.6539>
- ICAR-IIIR (ICAR-Indian Institute of Rice Research) 2017. *AICRIP Progress Report 2016*. Entomology and Pathology, ICAR- Indian Institute of Rice Research, Rajendranagar, Hyderabad. 2: 310p.
- IRAC [Insecticide Resistance Action Committee], 2025. IRAC home page [Online]. Available: <https://irac-online.org/mode-of-action/> [Accessed on 03 February 2025].
- Ishaaya, I. and Casida, J. E. 1980. Properties and toxicological significance of esterases hydrolyzing permethrin and cypermethrin in *Trichoplusiani* larval gut and integument. *Pestic.Biochem. Physiol.* 14(2): 178–184. [https://doi.org/10.1016/0048-3575\(80\)90109-1](https://doi.org/10.1016/0048-3575(80)90109-1)
- Kang, W. J., Koo, H. N., Jeong, D. H., Kim, H. K., Kim, J. and Kim, G. H. 2017. Functional and genetic characteristics of chlorantraniliprole resistance in the diamondback moth, *Plutellaxylostella* (Lepidoptera: Plutellidae). *Entomol. Res.* 47:394-403. <http://dx.doi.org/10.1111/1748-5967.12258>
- Karunaratne S. and Surendran S. N. 2022. Mosquito control: A review on the past, present and future strategies. *J. Natl. Sci. Found. Sri Lanka.* 50: 277. <https://doi.org/10.4038/jnsfsr.v50i0.11244>
- KAU [Kerala Agricultural University], 2024. Package of Practices Recommendations: Crops 2024 (16th ed.). Kerala Agricultural University, Thrissur, 435 p.
- Khuswaha, K.S. and Singh, R. 1985. Leaf folder outbreaks in Haryana, India. *Rev. Appl. Entomol.* 73(4): 2332.
- Li, X., Huang, Q., Yuan, J. and Tang, Z. 2007. Fipronil resistance mechanisms in the rice stem borer, *Chilo suppressalis* Walker. *Pestic. Biochem. Physiol.* 89(3):169-174. <http://dx.doi.org/10.1016/j.pestbp.2007.06.002>
- Mbuji, A. L., Xue, Z., Guo, M., Li, M., Lv, S. and Zhang, L. 2024. Resistance and fitness costs of *Helicoverpa armigera* after selection with the tetraniliprole newly developed diamide insecticide. *Crop Prot.* 179: 106622. <https://doi.org/10.1016/j.cropro.2024.106622>
- Muraro, D. S., Neto D. O. A., Kanno, R. H., Kaiser, I. S., Bernardi, O. and Omoto, C. 2021. Inheritance patterns, cross-resistance and synergism in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) resistant to emamectin benzoate. *Pest Manag. Sci.* 77(11): 5049-5057. <https://doi.org/10.1002/ps.6545>
- Nayak, S. B., Manoharan, T., and Soundararajan, R. P. 2017a. Monitoring insecticide resistance to leaffolder, *Cnaphalocrocis medinalis* (Guenee) (Lepidoptera: Pyralidae) in rice growing regions of Tamil Nadu. *J. Entomol. Zool. Stud.* 5(6): 23-26.
- Nayak, S.B., Manoharan, T. and Soundararajan, R.P. 2017b. Assessment of baseline toxicity of newer insecticides for rice

- leaffolder *Cnaphalocrocis medinalis* (Guenee) (Lepidoptera: Pyralidae). *J. Ent. Res.* 41: 19-23.
- Ninsin, K. D. 2015. Cross-resistance assessment in cartap- and esfenvalerate-selected strains of the diamondback moth, *Plutellaxylostella* (L.) (Lepidoptera: Plutellidae). *West Afr. J. Appl. Ecol.* 23(2): 1-6.
- Opolopoiou, M., Elias, J., Slater, R., Bass, C. and Zimmer, C. T. 2024. Characterization of emamectin benzoate resistance in the diamondback moth, *Plutellaxylostella* (Lepidoptera: Plutellidae). *Pest Manag. Sci.* 80(2): 498-507. <https://doi.org/10.1002/ps.7778>
- Pattappu, S., Mathew, B. J., Josephraj Kumar, A. and Paul, A. 2018. Synergist induced susceptibility of tobacco caterpillar, *Spodoptera litura* (Fabricius) from Kerala, India exposed to conventional insecticides. *Phytoparasitica*, 46:97-104. <https://link.springer.com/article/10.1007/s12600-018-0641-2>
- Pereira, D. L., Silva, P. A., Langa, T. P., De Oliveira, M., Ribeiro, L. M. and Siqueira, H. A. 2023. Recent assessment and characterization of *Tuta absoluta* resistance to cartap hydrochloride. *Pestic. Biochem. Physiol.* 193: 105420. <https://doi.org/10.1016/j.pestbp.2023.105420>
- Saleem, M. A., Ahmad, M., Ahmad, M., Aslam, M. and Sayyed, A. H. 2008. Resistance to selected organochlorine, organophosphate, carbamate and pyrethroid, in *Spodoptera litura* (Lepidoptera: Noctuidae) from Pakistan. *J. Econ. Entomol.* 101(5):1667-1675.
- Samui, R. P., Chattopadhyay, N., Sabale, J. P., Kartikeyan, K. and Balachandran, P. V. 2007. Weather-based forewarning of leaffolder attack on kharif rice and operational crop protection at Pattambi, Kerala. *Mausam*, 58(4): 525-536.
- Sene, S. O., Tendeng, E., Diate, M., Sylla, S., Labou, B., Diallo A. W. and Diarra, K. 2020. Insecticide resistance in field populations of the tomato fruitworm, *Helicoverpa armigera*, from Senegal. *Int. J. Biol. Chem. Sci.* 14(1): 181-191. <http://ajol.info/index.php/ijbcs>
- Shiyu, W., Yuije, T., Miaomiao, R., Bo, H. and Jianya, S. 2016. The establishment of insecticide susceptibility baselines and resistance monitoring for rice leaffolder, *Cnaphalocrocis medinalis* (Guenee). *J. Nanjing Agric. Uty.* 39 (3): 402-407.
- Siqueira, H. A. A., Guedes, R. N. C. and Picanço, M. C. 2000. Cartap resistance and synergism in populations of *Tuta absoluta* (Lepidoptera: Gelechiidae). *J. Appl. Entomol.* 124: 233-238. <https://doi.org/10.1046/j.1439-0418.2000.00470.x>.
- Sun, Y., Liu, T., Ling, Y., Wang, L., Ni, H., Guo, D., Dong, B. B., Huang, Q., Long, L. P., Zhang, S., Wu, S. F. and Gao, C. F. 2023. Insecticide resistance monitoring of *Cnaphalocrocis medinalis* (Lepidoptera: Pyralidae) and its mechanism to chlorantraniliprole. *Pest Manag. Sci.* 79: 3290-3299. <https://doi.org/10.1002/ps.7512>
- Supawan, J. and Chongrattanameteekul, W. 2017. Influence of humidity, rainfall, and fipronil toxicity on rice leaffolder (*Cnaphalocrocis medinalis*). *ScienceAsia*, 43: 83-87. doi: 10.2306/scienceasia1513-1874.2017.43.082
- Thomas, A. 2024a. Mechanisms of resistance in Brinjal Shoot and fruit borer, *Leucinodes orbonalis* (Guenee) (Lepidoptera: Crambidae) to diamide insecticides. PhD. (Ag) thesis, Kerala Agricultural University, Thrissur, 159p.
- Thomas, H. 2024b. Detection and biochemical characterization of insecticide resistance in *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae). M.Sc (Ag) thesis, Kerala Agricultural University, Thrissur, 120p.
- Tong, H., Su, Q., Zhou, X. and Bai, L. 2013. Field resistance of *Spodoptera litura* (Lepidoptera: Noctuidae) to organophosphates, pyrethroids, carbamates, and four newer chemistry insecticides in Hunan, China. *J. Pest Sci.* 86(3): 599-609. <https://doi.org/10.1007/s10340-013-0505-y>
- Verma, S. K., Pathak, P. K., Singh, B. N. and Lal, M. N. 1979. Leaf folder outbreak in tarai and hill regions of Uttar Pradesh, India. *Int. Rice Res. Newsl.* 4: 3.
- Waldbauer, G. P. and Marciano, A. P. 1979. Rice leaf folder: Mass rearing and a proposal for screening for varietal resistance in the greenhouse. IRRI Research Paper Series No. 27. International Rice Research Institute, Philippines, 20p.
- Wang, X. L., Khakame, S. K., Ye, C., Yang, Y. H. and Wu, Y. D. 2013. Characterisation of field-evolved resistance to chlorantraniliprole in the diamondback moth, *Plutellaxylostella*, from China. *Pest Manag. Sci.* 69(5): 661-665. <https://doi.org/10.1002/ps.3422>
- Yao, R., Zhao, D. D., Zhang, S., Zhou, L. Q., Wang, X., Gao, C. F. and Wu, S. F. 2017. Monitoring and mechanisms of insecticide resistance in *Chilo suppressalis* (Lepidoptera: Crambidae), with special reference to diamides. *Pest Manag. Sci.* 73(6): 1169-1178. <https://doi.org/10.1002/ps.4439>
- Zang, L. S., Akhtar, Z. R., Ali, A., Tariq, K. and Campos, M. R. 2022. Flubendiamide resistance and its mode of inheritance in tomato pinworm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Insects* 13(11): 1023. <https://doi.org/10.3390/insects13111023>
- Zhang, S., Ren, X., Wang, Y. and Su, J. 2014. Resistance in *Cnaphalocrocis medinalis* (Lepidoptera: Pyralidae) to new chemistry insecticides. *J. Econ. Entomol.* 107(2): 815-820. <https://doi.org/10.1603/ec13506>
- Zheng, X., Ren, X. and Su, J. 2011. Insecticide susceptibility of *Cnaphalocrocis medinalis* (Lepidoptera: Pyralidae) in China. *J. Econ. Entomol.* 104: 653-658. <https://doi.org/10.1603/ec10419>