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RESEARCH ARTICLE

Susceptibility of rice insect pests and their natural enemies to commonly used insecticides

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Abstract: Insect pests of rice are a major constraint in paddy cultivation in Sri Lanka. Control of insect pests heavily depends on the use of insecticides, for which the major threat has been the development of resistance. It is important to understand the present status of insecticide resistance in rice insects to identify the most suitable insecticides which are most effective against pests and least effective agaist their natural enemies.

Insecticide resistance and the underlying resistance mechanisms were studied in five rice insect pests (brown planthopper *Nilaparvata lugens*; green leafhopper *Nephotettix virescens*; paddy bug *Leptocorisa oratorius*; white planthopper *Cofana spectra* and white-back planthopper *Sogatella furcifera*), and four of their predators (lady-bird beetle *Micraspis discolor*; ground beetle *Ophionea indica*; mired bug *Cytorhinus lividipennis* and spider *Tetragnatha* sp.). Insects were collected from the rice fields at Batalagoda, Kurunegala district (intermediate zone) and Angunakolapalassa, Hambanthota district (dry zone) of Sri Lanka from 2001 to 2003. They were subjected to insecticide bioassays with dimethoate, chlorpyrifos, permethrin, carbosulfan and fenobucarb by topical application. Log-probit mortality lines and LD_{50}/LD_{90} values were obtained. To determine the resistance/susceptibility against malathion, an organophosphate which has been heavily used in mosquito control programmes, and against DDT, an organochlorine which was the major insecticide used from early 1950s to mid 1970s, bioassays were conducted with malathion and DDT as well.

N. lugens collected from both sites showed high resistance to permethrin. *L. oratorius* population at Angunakolapalassa was resistant to carbosulfan compared to Batalagoda population. *L. oratorius, M. discolor* and *Tetragnatha* sp. populations at Angunakolapalassa were susceptible to permethrin compared to Batalagoda populations and *Tetragnatha* sp. population at Angunakolapalassa was susceptible to chlorpyrifos. Others from both study areas showed similar resistance levels. In general, most of the species tested from Batalagoda and Angunakolapalassa had lower tolerance for malathion while some species showed higher tolerance for DDT.

key words: Insecticide resistance, natural enemies, rice insect pests, Sri Lanka.

INTRODUCTION

Rice is the staple food in Sri Lanka accounting for about 45% of the per capita calories and 40% of the per capita protein consumption. Due to the introduction of high yielding varieties and use of fertilizers, the rice production in Sri Lanka increased substantially over the last forty years inspite of various biotic and abiotic stresses which reduce the yield significantly. Apart from the natural factors such as droughts and floods, the highest yield loss is due to insects and weeds.

Use of insecticides has become the most popular and the most efficient method of insect pest control. Although insecticides pollute the environment, they serve as an important tool in the management of insect pests especially during pest outbreaks. Once insecticides are sprayed, both pests and their natural enemies which play a major role in biological control of pest insects are affected. However, farmers prefer to use insecticides to control insect pests because of their high efficacy. Most farmers tend to use dosages which are higher than the recommended dosages, for better results. Continuous use and indiscriminate use of insecticides often cause insecticide resistance in pest populations resulting in pest resurgence.

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The present study was carried out to investigate and compare the insecticide cross-resistance spectra of five rice insect pests and some of their predators from two different rice growing areas of Sri Lanka, to some of the recommended insecticides.

METHODS AND MATERIALS

Study sites: The major study site was an irrigated rice field at the Rice Research and Development Institute, Batalagoda, Kurunegala district, located in the Intermediate zone of Sri Lanka, $7^0 30'$ N and $80^0 20'$E, 100 m above the sea level (Figure1).

Studies were carried out from 2001 to 2003 and covered four consecutive rice cultivation cycles, including two *Maha* (October to March) and two *Yala* (April-September) seasons. Limited studies were carried out in the rice fields at the Regional Agricultural Research and Development Center, Angunakolapelessa, Hambanthota district, located in the Dry zone of Sri Lanka, $6^0 28'$ N, $81^0 2'$ E, 30 m above the sea level (Figure 1). Studies at Angunakolapalassa were carried out in 2002.

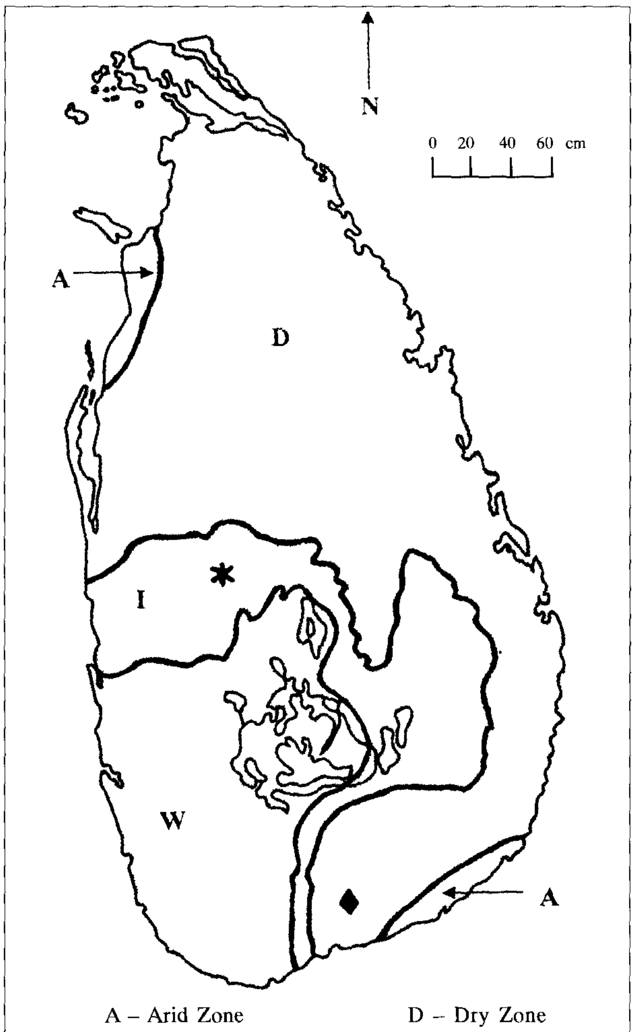

Figure 1: Study sites: Batalagoda (*) and Angunakolapelęssa (♦)

Insects: Five species of adult rice insect pests namely *Nilaparvata lugens* (brown planthopper, Homoptera: Delphacidae), *Leptocorisa oratorius* (paddy bug, Hemiptera: Alydidae), *Nephotettix virescens* (green leafhopper, Homoptera: Cicadellidae), *Sogatella furcifera* (white-back planthopper, Homoptera: Delphacidae) and *Cofana spectra* (white planthopper, Homoptera: Cicadellidae); and four species of predators namely *Micraspis discolor* (ladybird beetle, Coleoptera: Coccinellidae), *Ophionea indica* (ground beetle, Coleoptera: Carabidae), *Tetragnatha* sp. (spider sp., Aranea: Tetragnathidae) and *Cytorhinus lividipennis* (mired bug, Hemiptera: Miridae) were collected from the rice fields using sweep-net and blower-vac methods.

Chemicals and equipment: Chemicals were purchased from Sigma, USA, unless otherwise stated. DDT (97.5% pure) and paraoxon (98% pure) were from Greyhound, UK; malathion (97.5% pure) and chlorpyrifos (98% pure) were a gift from Cheminova, Denmark; propoxur (98.5% pure) from Bayer, Germany and permethrin (98% pure) from Aventis, UK; Dimethoate (96% pure), fenobucarb ((97.5% pure) and carbosulfan (90.81%) were a gift from AgroEvo (Ceylon), Sri Lanka.

Insecticide bioassays: Bioassays were carried out using the topical application method. A drop of the insecticide solution was applied topically on to the thoracic region of the insect using a microapplicator (Burkard, England) with a drop size ranging from 0.25μL to 1μL. Insecticide stock solutions were prepared in acetone. Serial dilutions were made with acetone. A drop of the insecticide solution was applied on to the dorsal surface of the thoracic tergites of each insect[1-3]. As recommended by the International Rice Research Institute (IRRI), 0.25μL was applied for planthoppers and leafhoppers. 0.5μL was used for the ground beetle and ladybird beetle. 1μL was applied for paddy bug and spider[1-3]. Control experiments were done using acetone alone. Five to twenty five insects were used per assay and at least four replicates were used for each dosage. Treated insects were transferred into a clean vial containing food materials. Mortalities were recorded after a 24 h period. Data were analysed only when the mortalities of control experiments were < 20% . Actual mortalities were obtained from the observed mortalities using Abbott's formula[4].

Mortalities (probability scale) obtained with chlorpyrifos, dimethoate, carbosulfan, fenobucarb and permethrin were plotted against dosages (log scale) and

LD_{50}/ LD_{90} values were obtained by regression analysis. All test insect species were treated with a fixed dosage of 3.5 µg/mg (biomass) DDT and 4.4 µg/mg (biomass) malathion[5]. Only dosages resulting in LD_{50} and LD_{90} with the Batalagoda species and the fixed DDT and malathion dosages, were tested against Angunakolapalassa species.

Table 1: LD_{50}* and LD_{90}** (µg/g insect body weight) values of insecticides for the insect pest populations at Batalagoda as determined by the topical application method

Species	Dimethoate		Chlorpyrifos		Carbosulfan		BPMC		Permethrin	
	LD_{50}	LD_{90}	LD_{50}	LD_{90}	LD_{50}	LD_{90}	LD_{50}	LD_{90}	LD_{50}	LD_{90}
N. lugens	10.00	3954.29	0.63	227.60	0.13	23.50	3.75	80.13	66.25	23,812.40
	N=500, n=7		N=500, n=6		N=650, n=4		N=650, n=3		N=500, n=5	
	X^2= 13.50	**p<0.05**	X^2= 73.53	p>0.05	X^2= 29.49	p>0.05	X^2= 41.13	p>0.05	X^2= 7.32	**p<0.05**
S. furcifera	7.43	368.21	16.43	722.86	1.07	67.86	2.14	461.79	0.36	147.50
	N=500, n=4		N=500, n=4		N=500, n=3		N=500, n=2		N=500, n=3	
	X^2= 9.27	p>0.05	X^2= 6.28	**p<0.05**	X^2= 42.40	p>0.05	X^2= 31.85	p>0.05	X^2= 6.60	**p<0.05**
N. virescens	1.88	165.13	5.13	224.13	0.25	5.08	0.25	44.75	0.63	34. 38
	N=500, n=4		N=500, n=4		N=500, n=3		N=500, n=2		N=500, n=3	
	X^2= 20.58	p>0.05	X^2= 8.35	**p<0.05**	X^2= 72.17	p>0.05	X^2= 69.54	p>0.05	X^2= 19.29	p>0.05
C. spectra	0.17	6.79	0.29	2. 90	0.02	1.71	0.14	14.35	0.02	0.31
	N=500, n=3		N=500, n=3		N=500, n=2		N=500, n=3		N=500, n=3	
	X^2= 40.12	p>0.05	X^2= 34.34	p>0.05	X^2= 1.88	**p<0.05**	X^2= 42.76	p>0.05	X^2= 11.51	p>0.05
L. oratorius	0.15	2708.74	0.17	161.20	0.03	3.86	0.09	89.51	0.11	3.86
	N=500, n=3		N=500, n=3		N=500, n=3		N=500, n=3		N=500, n=4	
	X^2= 13.26	p>0.05	X^2= 12.44	p>0.05	X^2= 34.71	p>0.05	X^2= 35.86	p>0.05	X^2= 20.21	p>0.05

N = total number tested n = degree of freedom *= Lethal dosage which kills 50% of the population

**= Lethal dosage which kills 90% of the population

Table 2: LD_{50}* and LD_{90}** (µg/g insect body weight) values of insecticides for the predator populations at Batalagoda as determined by the topical application method.

Species	Dimethoate		Chorpyrifos		Carbosulfan		BPMC		Permethrin	
	LD_{50}	LD_{90}	LD_{50}	LD_{90}	LD_{50}	LD_{90}	LD_{50}	LD_{90}	LD_{50}	LD_{90}
C. lividipennis	0.63	462.50	0.63	5.63	0.63	46.88	1.25	277.50	0.63	8.75
	N=250, n=3		N=250, n=3		N=250, n=2		N=250, n=2		N=250, n=3	
	X^2= 30.11	p>0.05	X^2= 5.65	**p<0.05**	X^2= 1.15	**p<0.05**	X^2= 58.50	p>0.05	X^2= 5.57	**p<0.05**
M. discolor	0.90	16.80	10.10	919.10	1.20	72.30	4.60	98.20	0.20	8.40
	N=350, n=3		N=350, n=3		N=350, n=2		N=350, n=3		N=350, n=3	
	X^2= 52.02	p>0.05	X^2= 21.83	p>0.05	X^2= 37.12	p>0.05	X^2= 53.25	p>0.05	X^2= 30.25	p>0.05
O. indica	0.10	3.50	0.10	15.00	1.10	698.80	18.90	237.40	0.10	30.80
	N=350, n=3		N=350, n=4		N=350, n=2		N=350, n=3		N=350, n=3	
	X^2= 4.75	**p<0.05**	X^2= 64.93	p>0.05	X^2= 76.96	p>0.05	X^2= 39.36	p>0.05	X^2= 7.46	**p<0.05**
Tetragnatha sp.	2.67	2355.33	1.11	22.44	62.89	6551.22	2.67	2693.56	0.22	10.11
	N=200, n=3		N=200, n=2		N=200, n=4		N=200, n=3		N=200, n=3	
	X^2= 21.75	p>0.05	X^2= 6.998	p>0.05	X^2= 43.24	p>0.05	X^2= 6.46	**p<0.05**	X^2= 11.45	p>0.05

N = total number tested n = degree of freedom *= Lethal dosage which kills 50% of the population

**= Lethal dosage which kills 90% of the population

Table 3: Percentage mortalities obtained for Angunakolapalassa insect populations (n = 100)

Species	Percentage mortalities									
	Dimethoate		Chlorpyrifos		Permethrin		Carbosulfan		BPMC	
	A	B	A	B	A	B	A	B	A	B
N lugens	48	90.9	46.86	77.78	37.38	65.81	51.22	86.11	57.5	95.35
S furcifera	56.67	84.44	44.4	75.56	55.56	89.09	50	90	59.52	83.33
L oratorious	45	81.67	54.55	87.22	96.67	100	20	60	100	100
C lividipennis	47.37	68.1	57.45	93.75	50	90	60	88	55.56	86
M discolor	53.33	88	53.85	76.9	96.67	100	27.27	92	40	88.46
O indica	50	98.33	68.33	85	48.33	68.33	45	85	51.67	100
Tetragnatha sp	55	85	95	100	100	100	80	95	5	60

A – for the dosage, which gave 50% mortality of the same species at Batalagoda

B - for the dosage, which gave 90% mortality of the same species at Batalagoda.

Table 4: Percentage mortalities for the fixed dosages of 3,500 μg/g DDT and 4,400 μg/g malathion

Species	Percentage mortality for 3500 μg/g DDT		Percentage mortality for 4400 μg/g malathion	
	Batalagoda	Angunakolapalassa	Batalagoda	Angunakolapalassa
N lugens	80	81.43	85	95
N virescens	75	NT	100	NT
L oratorius	50	38.33	100	90
C spectra	87.1	NT	96.47	NT
S furcifera	56	93.33	90	96.67
C lividipennis	100	92.23	90	100
M discolor	55.4	14.29	96.34	97.14
O indica	40.9	62.22	65	74
Tetragnatha sp	100	64.29	95	65.71

NT= not tested

As the number of *N. virescens* and *C. spectra* were very low at Angunakolapalassa, the insecticide bioassays could not be carried out for these two species.

RESULTS

Log-probit mortality lines for dimethoate, chlorpyrifos, permethrin, carbosulfan and fenobucarb for the Batalagoda rice insect pest and predatory populations were plotted and LD_{50} and LD_{90} values estimated (Tables 1 and 2). Fitness of the log-probit mortality curves is given by the χ^2 values and indicates whether the population showed a homogeneous ($p<0.05$) or a heterogeneous ($p>0.05$) response to the insecticide. These pest and predatory species from Angunakolapalassa were exposed to the insecticide dosages that resulted in LD_{50} and LD_{90} of the same insecticides from Batalagoda and the percentage mortalities obtained are given in Table 3.

Percentage mortalities of both Batalagoda and Angunakolapalassa populations, when exposed to the fixed dosages of malathion and DDT, are given in Table 4.

Table 5: Susceptible/ resistance status of rice insect pests and their predators to seven insecticides

Insecticide	Pest species								Predatory species									
	N. lugens		*S. furcifera*		*N. virescens*		*C. spectra*		*L. oratorius*		*C. lividipennis*		*M. discolor*		*O. indica*		*Tetragnatha* sp.	
	Bat	Ang	Bat	Ang	Bat	Ang	Bat	Ang	Bat	Ang	Bat	Ang	Bat	Ang	Bat	Ang	Bat	Ang
Dimethoate (OP)	RR	RR	RR	RR	RR	NT	R	NT	S	S	R	R	R	R	S	S	R	R
Chlorpyrifos (OP)	RR	RR	RR	RR	RR	NT	R	NT	S	S	R	R	R	R	S	S	R	S
Carbosulfan (Carb)	S	S	S	S	S	NT	S	NT	S	R	S	R	R	RR	R	S	RR	S
BPMC (Carb)	RR	RR	S	S	S	NT	S	NT	S	S	S	R	R	R	RR	R	R	RR
Permethrin (Pyr)	RR	RR	R	R	R	NT	S	NT	R	S	R	R	R	S	R	R	R	S
Malathion (OP)	S	S	S	S	S	NT	S	NT	S	S	S	S	S	S	S	S	S	S
DDT (OGCL)	S	S	R	S	S	NT	S	NT	R	R	S	S	R	RR	RR	R	S	R

OP = organophosphate; Carb = carbomate; Pyr = pyrethroid; OGCL = organochlorine; RR = highly resistant; R = resistant; S = susceptible; NT = not tested; Bat = Batalagoda; Ang = Angunakolapalassa

According to all the bioassay results, comparative resistance/ susceptibility spectra were obtained for all the species and are presented in Table 5.

DISCUSSION

Large-scale use of synthetic insecticides was first started in Sri Lanka in 1945 with the introduction of organochlorines[6]. DDT and γ-BHC dust were heavily used in paddy cultivation until the mid 1970s[7]. DDT was introduced in 1957 and later it was replaced by malathion in 1975[8]. Usage of organophosphates such as malathion and fenitrothion in agriculture was legally restricted because of their important role in malaria control. Organochlorines were followed by carbamates, which was the major group of insecticides used in agriculture until the introduction of pyrethroids in 1994. In the health sector, malathion was the major insecticide of choice until the introduction of pyrethroids in early 1990s. However, it is still used in certain areas of the country in mosquito control activities.

At Batalagoda only paddy is cultivated while at Angunakolapalassa, pulses and cereals are also cultivated in addition to paddy. Insecticides, which are used to manage insect pests at both these sites, have been selected according to the pesticide recommendations of the Department of Agriculture. Most widely used insecticides are carbosulfan, fenobucarb, carbofuran, dimethoate, chlorpyrifos and these insecticides have been used for more than three decades.

Insecticide resistance can be assessed by a variety of bioassay methods i.e. direct application of insecticide droplets on to the insect body (topical application), insecticide impregnated paper method, spraying methods, leaf dip method, mixing insecticides with food etc. In the present study topical application method was used since it has been successfully used for rice insect pests, i.e. for the brown planthopper *N. lugens*[9-15], the green leafhopper *Nephotettix cincticeps*[1,11,16], the white-backed planthopper *S. furcifera*[12,15] and for the small brown planthopper *Laodelphax striatellus*[11]. Other than rice insect pests, topical application method has been successfully used for other insects such as aphids and bruchid pests[5,17-20].

Validity of the present results can be tested against available published data for some of these species. For carbosulfan susceptible populations of *N. lugens* and *S.furcifera* LD_{50} values have been reported as 5µg/g and 3.5µg/g[15].During the present study Batalagoda populations of *N. lugens*, *S. furcifera* had LD_{50} values 0.13µg/g and 1.07µg/g respectively. Susceptible populations of *N. lugens*, *S. furcifera* and *N. virescens* have shown LD_{50} values of 0.02 µg/g, 2.9 µg/g and 2.77 µg/g respectively for the insecticide fenobucarb[11,14]

These populations from Batalagoda had fenobucarb LD_{50} values of 3.75 µg/g, 2.14 µg/g and 0.25 µg/g respectively. This comparison indicates that Batalagoda *N. lugens* population is highly resistant to fenobucarb while *S. furcifera* and *N. virescens* populations are susceptible. Present results show that the chlorpyrifos LD_{50} values ranged from 0.17 to 16.43µg/g for rice pest species and from 0.10 to 10.10µg/g for predatory species at Batalagoda. It has been reported that, for some of the vegetable insect pests, chlorpyrifos LD_{50} values ranged from 0.0013 to 5.3µg/g[20]. Therefore, it appears that rice insects are more tolerant to chlorpyrifos than vegetable insects.

In general, *N. lugens* shows high resistance to several insecticides tested and there is no marked difference in resistance between the pest insect species and the predatory insects (Table 5). Apart from the concerns over the environment, DDT was abandoned in Sri Lanka due to the high resistance developed by insect populations. It appears that the overall resistance shown to DDT is decreasing in rice insects. It is therefore mportant to determine the underlying resistance mechanisms to these cross-resistance spectra.

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