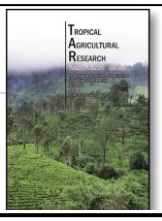
Contents available at: Sri Lanka Journals Online

Tropical Agricultural Research

Journal Home Page: https://tar.sljol.info



Insecticide Resistance, Resistance Mechanisms, and Phylogeny of Three *Myzus persicae* Populations in Cabbage from Three Agroclimatic Zones of Sri Lanka

J. P. Marasinghe[1]*, S.H.P.P. Karunarane[2], S.N. Surendran[3], K.S. Hemachandra[4] and L. Nugaliadde[5]

[1]*Office of the Registrar of Pesticides, Department of Agriculture, Peradeniya*
[2]*Department of Zoology, Faculty of Science, University of Peradeniya, Getambe, Peradeniya*
[3]*Department of Zoology, Faculty of Science, University of Jaffna, Jaffna*
[4]*Department of Agricultural Biology, Faculty of Agriculture, University of Peradeniya, Peradeniya*
[5]*Sri Lanka Institute of Agriculture, Gannoruwa, Peradeniya*

ARTICLE INFO

Article history:
Received: 20 July 2022
Revised version received: 10 October 2022
Accepted: 08 March 2023
Available online: 01 April 2023

Keywords:
Bioassays
Biochemical assays
Haplotypes
Pesticide resistance

Citation:
Marasinghe J. P., Karunarane, S.H.P.P., Surendran, S.N. Hemachandra, K.S. and Nugaliadde, L. (2023). Insecticide Resistance, Resistance Mechanisms, and Phylogeny of Three *Myzus persicae* Populations in Cabbage from Three Agroclimatic Zones of Sri Lanka. Tropical Agricultural Research, 34(2): 80-93.

DOI:
http://doi.org/10.4038/tar.v34i2.8621

Marasinghe, J.
https://orcid.org/0000-0001-8886-4732

ABSTRACT

The aphid, *Myzus persicae* is an important sucking pest and a virus vector in many crops. The management of *M. persicae* is mainly through synthetic pesticides. The development of resistance against common pesticides has resulted in control failures of *M. persicae.* The objective of this study was to examine the status of pesticide resistance and the genetic structure of *M. persicae* populations. Samples were collected from cabbage cultivations in the dry (Anuradhapura), wet (Kandy), and intermediate (Badulla) zones of Sri Lanka during 2017-2019. Bioassays were conducted against five insecticides using the leaf dip method. Biochemical assays examined the activity of insecticide detoxifying enzymes and the insensitivity of the organophosphate/carbamate target site, acetylcholinesterases (AChEs) in the Badulla and Kandy populations. A 636 bp mtCOI gene sequence was used to infer the phylogeny. The resistance spectrum of the populations was low to moderate; 17-25% resistance to thiamethoxam, 11-15% to acetamiprid, 20-28 % to carbosulfan, 20-41 % to etofenprox, and 4-9 % to profenofos. Elevated activity of esterases (specific activities 0.91-1.11 µmol/min/mg protein) and glutathione S-transferases (1.27-1.82 µmol/min/mg protein) were observed for increased metabolism of pesticides. Monooxygenase-based detoxification was noticeable in neonicotinoid resistance. Insensitive AChEs contribute to organophosphate and carbamate resistance. Phylogenetic analysis revealed that 2 haplotypes of the studied mtCOI gene sequence are present in the three populations tested, indicating that environmental and biological pressure, including pesticide selection pressure, may be contributing to the emergence of novel genotypes of *M. persicae*.

* Corresponding author- jeevanimarasinghe@gmail.com

INTRODUCTION

Aphids are harmful pests in many crops. They suck sap from plant parts and weaken the plants while reducing the quality and quantity of fruits. Under heavy infestations, the plants may die. Due to heavy secretions of the sugary exudate called honeydew, the sooty mold grows on leaves, resulting in a reduction of photosynthesis (Perring *et al*., 2018). Aphids can transmit certain plant viruses, causing the heaviest damage with deformation of leaves, buds, and flowers, stunting of plants, and producing plant galls (Capinera, 2020). The green peach aphid, *Myzus persicae* (Sulzer), is considered to be the most economically important aphid species, causing damage to over 40 different plant families, and spreading viruses to over 30 plant families, including Crucifers and Solanaceous crops (van Emden and Harrington, 2007). It is a cosmopolitan species, and the economic importance of *M. persicae* as a plant virus vector in different crops in Sri Lanka has been emphasized (Shivanathan, 1977). Its distribution is through various means, including passive movement through plant material, wind, and storms (Capinera, 2020). *Myzus persicae* was first described as *Aphis persicae.* It is of variable colour with different shades of green, pink, or red and about 1.2–2.1 mm body length (Blackman and Eastop, 2007). It has a heteroecious life cycle that occurs obligatorily on two host plants (Margaritopoulos *et al*., 2002) and the life cycle depends on the climate and availability of the primary host (Blackman, 1974). In mild climates, a complete life cycle can take 10–12 days with over 20 generations per year. When the climate is warmer, population outbreaks occur through rapid multiplication through continuous parthenogenesis, which allows for shorter generation times.

Aphids are controlled using a variety of methods, including the use of insecticides. In Sri Lanka, organophosphates, carbamates, and neonicotinoids have been recommended since the 1990s for aphid control (DOA, 2009; DOA, 2015). However, a variety of factors, such as the life cycle, rapid multiplication, host range, and insecticide resistance development, cause problems in the successful management of this pest. It has developed resistance to most classes of insecticides, including carbamates, pyrethroids, organophosphates, and neonicotinoids, making most of the insecticides ineffective globally (Fuentes-Contreras et al., 2013; Bass et al., 2014; Umina et al., 2014).

Repeated and continuous application of insecticides has led to the rapid development of multiple mechanisms of resistance to most classes of insecticides in M. persicae (Bass et al., 2014). Insecticide resistance is a heritable trait and a global issue. Knowledge of the resistance status of a particular insect pest is vital for evaluating the validity of existing control measures and devising new strategies. In insects, there can be several underlying mechanisms for resistance development. The two major mechanisms are target site mutation (insensitive target sites) and enhanced detoxification of insecticides by enzymes (Karunaratne, 1998; WHO, 1998). In M. persicae about 7 biochemical and molecular resistance mechanisms have been described (Bass et al., 2014). Aphids develop various adaptive mechanisms under intense selective pressures (Simon and Peccoud, 2018). As such, virulent biotypes and resistant genotypes can be evolved. Myzus persicae is also known to have an exceptional ability to adapt to new host plants (Blackman, 1987). The adaptation of M. persicae to tobacco has led to the development of subspecies with increased resistance to neonicotinoids, causing control failure.

This study aimed to evaluate the susceptibility/ resistance status of *M. persicae* collected from cabbage to selected insecticides, to understand the underlying resistance mechanisms, and to understand the population genetics of the individuals from 3 locations in wet (annual rainfall > 2500 mm), dry (annual rainfall < 1750 mm) and intermediate (annual rainfall 1750- 2500 mm) zones in Sri Lanka.

MATERIALS AND METHODS

Aphid samples were collected from cabbage cultivation in Gannoruwa, district of Kandy, Galenbindunuwewa, district of Anuradhapura, and Welimada, district of

Badulla during 2017 -2019. Aphids in cabbage leaves were packed loosely in rigid foam boxes and transported to the Entomology Laboratory of the Horticulture Research and Development Institute, Gannoruwa, for identification using published keys (Blackman and Eastop, 2000). They were reared on potted cabbage in fine mesh aluminum cages of 80 x 40 x 60 cm^3 at room temperature. Some of the aphids were preserved in 70% ethyl alcohol and stored at -20^{0}C for biochemical analysis.

Insecticide bioassays

The leaf dip method has been adopted with minor modifications for laboratory insecticide bioassays (IRAC, 2016). Accordingly, an agar solution was prepared with 1% agar powder mixed with distilled water, heated to boiling, and allowed to cool with constant mixing. After about 10 minutes, the warm agar solution was poured into plastic cups to a depth of 1 cm. The center of the plastic lid was cut open and glued with pieces of fine-mesh cloth to ensure adequate ventilation of the cup. Insecticide dilutions were prepared from the test compounds of neonicotinoids (thiamethoxam 25% WG, acetamiprid 20% SP), organophosphate (profenofos 500 g/L SC), carbamate (carbosulfan 200 g/L SC) and pyrethroid (etofenprox 100 g/L EC) using distilled water. Eight to ten concentrations of each insecticide (five replicates with 150 aphids per concentration) that gave mortalities between 0% to 100% were used for testing each population *i.e.* thiamethoxam 1, 10, 100, 250, 500, 1,000, 2,500, 5,000, 10,000 ppm; acetamiprid 1, 10,100, 250, 500, 1,000, 2,000, 5,000, 10,000 ppm; carbosulfan 10, 100, 300, 500, 1,000, 2,000, 5,000, 10,000 ppm; profenofos 1, 10, 50, 100, 250, 500, 800, 1,000, 2,000, 5,000 ppm and etofenprox 10, 50, 100, 300, 500, 1,000, 2,000, 5,000, 10,000, 15,000 ppm. Distilled water without insecticides was used as the untreated control.

Leaf discs were cut from healthy, untreated cabbage leaves and completely immersed in individual test liquids for 10 seconds with gentle agitation, then placed on paper tissues to dry the surfaces. Once the agar was set in the cups, the leaf discs were laid individually on the agar in each labelled cup with the abaxial surface facing upwards. A small drop of distilled water was placed on the surface of the agar before placing the leaves to moisten and stick the leaf to the surface. Thirty apterous adult aphids were transferred to each leaf disc using a paintbrush. Each cup was sealed with a ventilated lid and stored upright in a place not exposed to direct sunlight, at room temperature. Mortality assessments were made after 72 hours. The aphids, which were unable to adjust their posture within 10 seconds once turned on their backs, were presumed dead.

Data analysis

Bioassay data were used when the control mortality was less than 20%. Mortalities were adjusted with control mortalities using Abbott's formula (Abbott, 1987). Adjusted mortalities were transformed into probits using Sigma Plot software (version 10) and plotted against the logarithmic values of insecticide concentrations. The LC_{50}, LC_{90}, and percent mortality for the doses recommended by the Department of Agriculture (DOA, 2015) were estimated by regression analysis. Chi-square values ($\chi 2$) were calculated by evaluating the goodness of fit of linear regression to log-probit transformed data. The software provides the slope and 95% confidence limits for each mortality line.

Biochemical assays

Adult aphids were collected in batches of 10 individuals, totaling up to 50 individuals per population, and subjected to enzymatic assays using a kinetic microplate reader (Bio-Tek, USA). Mass homogenates were prepared separately in 150 µl of distilled water. The crude homogenate was directly used for the acetylcholine esterase assay. The supernatant, was used for esterase, GST, monooxygenase, and protein assays, after centrifugation for 2 minutes at 10,000 g. Distilled water was used for the controls. All the tests were replicated. The assaying methods described in the field and laboratory manuals of the World Health Organization (WHO, 1998) were adopted with slight modifications.

Esterase assay

Ten µl of homogenate was mixed with 200 µl of 1mM p-nitrophenyl acetate (pNPA) working solution in 50 mM sodium phosphate buffer (pH 7.4). At 405 nm, the absorbance of the reaction was measured kinetically for 2 min and converted to moles using an extinction coefficient of 6.53 mM^{-1} (corrected for a path length of 0.6 cm).

Glutathione S-transferase (GST) assay

Ten µl of homogenate was mixed with 200 µl of substrate solution prepared using 10.5 mM reduced glutathione (95 parts GSH in 100 mM phosphate buffer) and 63 mM 1-chloro 2,4-dinitrobenzene (5 parts of CDNB in methanol). The reaction rate was measured at 340 nm for 5 minutes. The absorbance was converted to moles using an extinction coefficient of 5.76 mM^{-1} (corrected for a path length of 0.6 cm).

Monooxygenase assay

Twenty µl of homogenate was mixed with 80 µl potassium phosphate buffer (pH 7.2) + 200 µl 6.3 mM tetramethyl benzidine working solution (0.01 g TMBZ dissolved in 5 ml methanol and then in 15 ml of sodium acetate buffer at pH 5.0) + 25 µl of 3% H_2O_2 solution in a microtitre plate well. The mixture was left for a 2 hour period of incubation at room temperature, and the absorbance was then read as an endpoint assay at 630 nm. The amount of monooxygenase was expressed as equivalent units of cytochrome P^{450} (Brogdon *et al.*, 1997).

Protein assay

Protein assays were conducted to obtain the protein concentrations of the homogenates to determine the specific activities of the enzymes. A BIO-RAD protein determination kit with bovine serum albumin was used as the standard protein. Ten µl of the homogenate was mixed with 300 µl of BIO-RAD working solution (prepared according to the manufacturer's instructions), and after a 5 min incubation period at room temperature, the reaction was read at 570 nm as an endpoint assay.

Acetylcholinesterase (AChE) assay

Two × 25 µl of crude homogenate were placed in two consecutive wells of a microtiter plate, each containing 145 µl of 1% Triton buffer in 100 mM phosphate buffer at pH 7.8 with 10 µl of 10 mM dithiobis 2-nitrobenzoic acid (0.0396g of DTNB in 100 mM phosphate buffer at pH 7.0). An aliquot of 25 µl of 10 mM acetylthiocholine iodide (0.0578g ASCHI in 20 ml distilled water) was added to one of the wells. In comparison, 25 µl of 10mM ASCHI with propoxur (10 ml of 10 mM ASCHI + 20 µl of 0.1 M propoxur in acetone) was added to the other. Acetylcholinesterase activity was measured at 405 nm for 5 min. The results were expressed as the percentage of remaining activity in the inhibited fraction compared to the control (uninhibited) activity.

Population genetics

DNA was extracted from individual aphids by using the DNeasy blood and tissue kit (Qiagen, CA, USA) following the manufacturer's protocol. A portion of the mitochondrial gene *mtCOI* was amplified using the forward primer LCO1490 (5' GGTCAACAAATCATAAAGATATTGG 3') and the reverse primer HCO2198 (5' TAAACTTCAGGGTGACCAAAAAATCA 3') (Herbert *et al.,* 2003; Rebijith *et al*., 2012). For each amplification, PCR reactions were performed in a 25-µl volume that consisted of 1.50 µl of DNA, each primer at 0.8 µM, 200 mM of each deoxyribonucleoside triphosphate (dNTPs), 1.5 mM $MgCl_2$ and 0.625 U *Taq* DNA polymerase in 1xPCR buffer. PCR parameters were; initial denaturation at 94^0C for 4 min followed by 35 cycles of 94^0C for 30 s, 53^0C for 45s, 72^0C for 45s, and a final extension step of 72^0C for 10 min. The prominently amplified PCR products were purified using the QIAquick PCR purification kit (Qiagen, CA, USA) using the manufacturer protocol and were sequenced at a sequencing facility at the Department of Molecular Biology and Biotechnology, Faculty of Science, University of Peradeniya.

The sequences were manually edited in Finch TV (Geospiza, USA) and aligned in Clustal W in MEGA 11.0 software (Tamura *et al*., 2021). The consensus sequence was obtained for each species. The aligned sequences for

coding genes were translated into amino acid sequences by using the invertebrate mitochondrial coding to determine that they were coding and, therefore, unlikely to be nuclear pseudogenes. Once the alignment was completed, sequences were compared with the publicly available sequence data in GenBank using BLAST (http://www.blast.ncbi.nlm.nih.gov/Blast.cgi) and the BOLD interface (http://www.boldsystems.org) to confirm species identification. Phylogenetic relationships among *M. persicae* from India, Bangladesh, and Japan were inferred using the maximum likelihood (ML) method. The substitution model selection was also performed in MEGA (version 11.0) based on the lowest Bayesian information criterion value. The Tamura 3-parameter model for the *mt co1* sequence data set was selected. Bootstrap (Felsenstein, 1985) supports were based on 1,000 resampled datasets using MEGA (version 11). Samples identified as *A. craccivora* during the study and available GenBank sequences of *A. craccivora* were used as outgroups in phylogenetic analyses.

RESULTS & DISCUSSION

Insecticide bioassays

Log-probit mortality curves were obtained for the three tested aphid populations against thiamethoxam, acetamiprid, profenofos, carbosulfan, and etofenprox (Figure 1). For neonicotinoids, the LC_{50}s were in a comparable range with thiamethoxam at 11-24 ppm and acetamiprid at 11-12 ppm (Table 1). For profenofos, the highest LC_{50} of 27 ppm was obtained for the Badulla population. The LC_{50} for carbosulfan ranged between 22-42 ppm. The LC_{50} values for etofenprox were distributed over a larger range for the 3 populations. The maximum and minimum etofenprox LC_{50}s were 89 ppm and 26 ppm respectively, for the Badulla and Anuradhapura populations. Chi-square values ($\chi 2$) which give the goodness of fit of dose-mortality lines for the populations of both neonicotinoids and profenofos, were non-significant. Anuradhapura's population exhibited a heterogeneous response with a significant chi-square value ($P< 0.05$) for carbosulfan toxicity, while the Kandy population had a similar response to etofenprox.

The percentage of mortalities against neonicotinoids at the recommended rates of application was below 90% but above 75% for all the populations, with 10- 25% survival expected for respective aphid populations (Table 2). For profenofos, the estimated percentage of mortalities from probit dose-mortality regression lines indicated very good efficacy above 90%, for all the populations. Published data on the LC_{50} of profenofos using similar methodologies was not available for susceptible populations to calculate resistance factors. The percentage mortalities for carbosulfan were comparable and high for the 3 populations, but they were below 90%. The efficacy of etofenprox, was moderate ranging from 59% to 80% respectively, for the Badulla and Anuradhapura populations. For all the tested insecticides, the lowest efficacy was detected against the Badulla population.

Myzus persicae has been reported to be resistant to all groups of insecticides discussed above (Bass *et al*., 2014; Umina *et al.*, 2014; Fuentes-Contreras *et al*., 2013). It is a widely spread pest in Sri Lanka among many crops, including cabbage, cucurbits, and brinjal. In the present study, the aphids were collected from the cabbage crop. The overall percentage of mortalities for the insecticides demonstrated a moderate to high efficacy against the tested aphid populations. The highest and lowest efficacies were observed against profenofos and etofenprox respectively. Accordingly, the maximum resistance shown against neonicotinoids, profenofos, carbosulfan, and etofenprox were 25%, 9%, 18%, and 41%, respectively.

The cabbage crop in Sri Lanka, is generally treated with Insect Growth Regulator insecticides recommended by the Department of Agriculture, to control the most injurious caterpillar pests. Thus, the use of neonicotinoids, profenofos, carbosulfan, and etofenprox on this crop is considered to be low but not zero. Less exposure to the studied insecticides could be a major reason for having comparatively less resistance to these insecticides. However, *M.*

persicae, which is a polyphagous pest, is supposed to get a pre-exposure to the above insecticides from adjacent other crops from which they migrate from and which might have resulted in a moderate resistance level to some of the studied insecticide groups.

Biochemical assays

Aphid populations from Kandy and Badulla, which were subjected to explore the metabolic resistance, have exhibited comparable levels of enzyme activities (Figure 2). Specific activities of esterases and GSTs were 0.91-1.11 µmol/min/mg and 1.27-

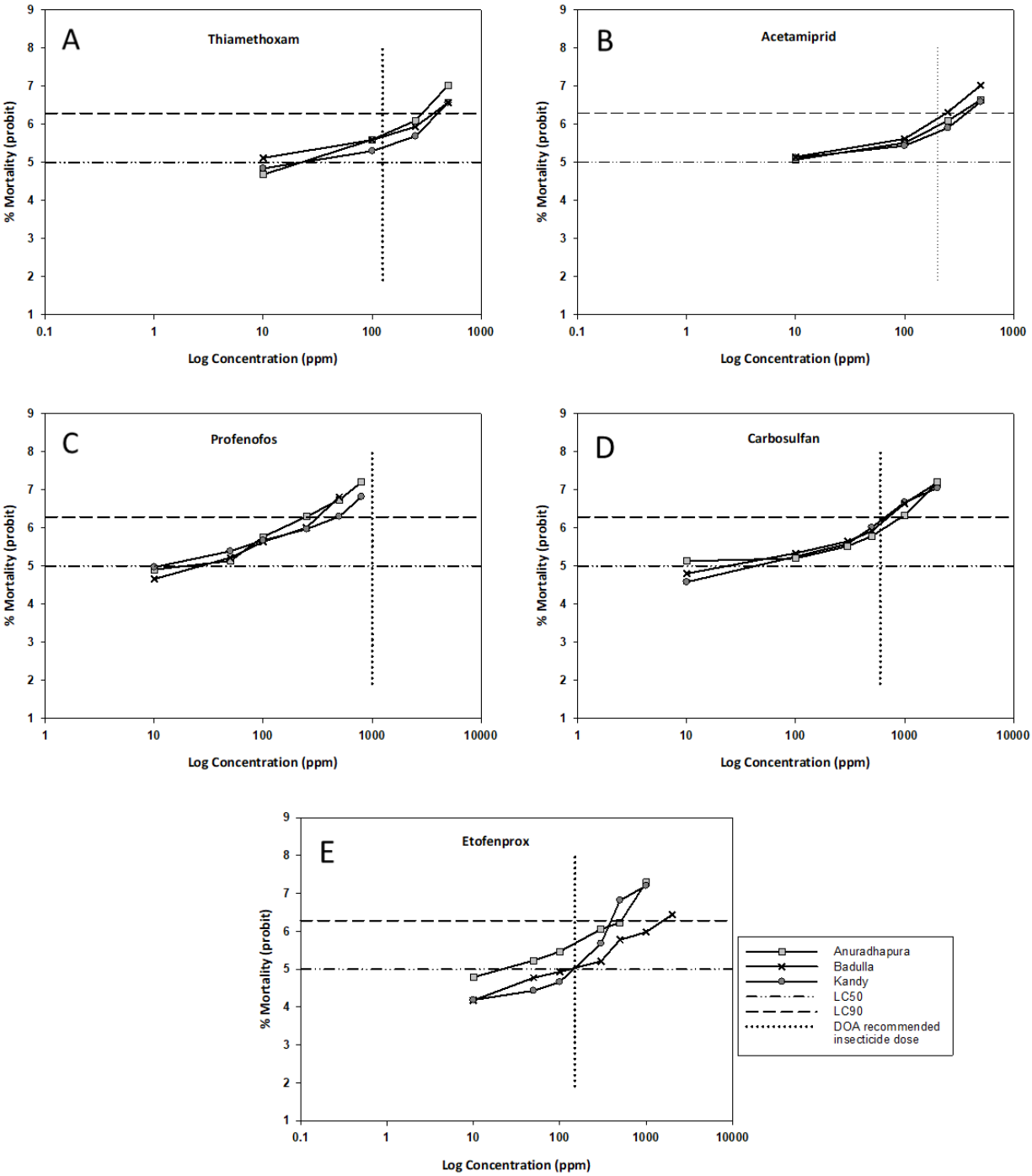


Figure 1. Probit dose –mortality curves for *Myzus persicae* aphid populations collected from Kandy, Bandarawela, and Anuradhapura exposed to thiamethoxam (A), acetamiprid (B), profenofos (C), carbosulfan (D), and etofenprox (E) using leaf-dip bioassays.

Table 1. Mortality data from leaf-dip bioassays for *Myzus persicae* populations exposed to thiamethoxam, acetamiprid, profenofos, carbosulfan, and etofenprox in 2017-2019

Insecticide	Location	Number tested	LC_{50} (ppm)	CI	LC_{90} (ppm)	CI	X^2 (df)	Slope	R^2
Thiamethoxam	Kandy (Gannoruwa)	1500	23	<1-225	634	73->10000	2.5 (3)	0.9	0.81
	Anuradhapura (Galenbindunuwewa)	1500	24	<1-85	237	65->10000	2.4 (3)	1.3	0.92
	Badulla (Welimada)	1500	11	<1-60	488	89->10000	1.8 (3)	0.8	0.88
Acetamiprid	Kandy (Gannoruwa)	1500	11	<1-100	516	35>10000	2.7 (3)	0.8	0.81
	Anuradhapura (Galenbindunuwewa)	1500	12	<1-67	376	70->10000	2.4 (3)	0.9	0.88
	Badulla (Welimada)	1500	11	<1-71	194	<1->10000	3.0 (3)	1.0	0.86
Profenofos	Kandy (Gannoruwa)	1650	15	7-32	410	245-892	1.2 (5)	0.9	0.95
	Anuradhapura (Galenbindunuwewa)	1650	20	6-44	230	123-480	6.6 (5)	1.2	0.93
	Badulla (Welimada)	1650	27	7-50	305	155-1039	1.1 (4)	1.2	0.94
Carbosulfan	Kandy (Gannoruwa)	1350	42	9-83	682	329-1813	4.9 (5)	1.1	0.90
	Anuradhapura (Galenbindunuwewa)	1350	22	<1-124	892	188->10000	11.4*(5)	0.8	0.70
	Badulla (Welimada)	1350	30	2-80	631	237-2987	6.3 (5)	0.9	0.87
Etofenprox	Kandy (Gannoruwa)	1650	69	13-150	460	183-3076	30.9* (5)	1.6	0.85
	Anuradhapura (Galenbindunuwewa)	1650	26	4-56	376	163-1295	4.7 (5)	1.4	0.88
	Badulla (Welimada)	1650	89	69-130	2177	1260-4345	2.8 (6)	1.0	0.96

LC_{50}- lethal concentration which kills 50% of the population; CI- confidence interval; LC_{90}- lethal concentration which kills 90% of the population; $\chi 2$=Chi-square values; R^2-coefficient of determination of goodness of fit; *P<0.05

Table 2. The percentage mortalities estimated for the Department of Agriculture (DOA) recommended dosages of six insecticides against *Myzus persicae* in cabbage collected from locations in Kandy, Bandarawela, and Anuradhapura districts

Insecticide	Mode of action*	DOA recommended dosage per 16 L tank	Percentage mortality (%)		
			Kandy	Anuradhapura	Badulla
Thiamethoxam 25 WG	4A	8 g	75 %	83 %	80 %
Acetamiprid 20 SP	4A	16 g	85 %	83 %	89 %
Profenofos 50 EC	1B	32 ml	91 %	96 %	92 %
Carbosulfan 20 EC	1A	48 ml	80 %	72 %	77 %
Etofenprox 10 EC	3A	24 ml	68 %	80 %	59 %

*As described by the Insecticide Resistance Action Committee: 4A= nicotinic acetylcholine receptor competitive modulators- neonicotinoids; 1B= acetylcholinesterase inhibitors- organophosphates; 1A- acetylcholinesterase inhibitors- carbamates; 3A= sodium channel modulators- pyrethroids

1.82 µmol/min/mg for the respective populations, while monooxygenase estimates were 11.0 and 10.9 equivalent units of cytochrome P450. The esterase levels were comparable with the levels reported for *M. persicae* in a previous study (Figure 2A; Damayanthi and Karunaratne, 2005). The overproduction of certain esterases confers resistance in *M. persicae* against organophosphates, mono methyl carbamates, and to a lesser extent pyrethroids (Devonshire and Moores, 1982; Devonshire, 1983). Although organophosphates can be easily metabolized by esterases, the development of resistance to profenofos was not much evidenced in the aphids, indicating a low involvement of the esterase-mediated resistance

mechanism. Etofenprox, which is a non-ester pyrethroid, has the least chance for esterase inhibition by the hydrolyzing pathway. A study revealed that esterases in *M. persicae* do not detoxify certain dimethyl carbamates (Moores *et al*., 1994) and thus, synergistic studies are required to investigate whether this is a common phenomenon for every dimethyl carbamate, including carbosulfan as well. However, carboxylesterases induced resistance against thiamethoxam in the aphid, *Aphis crassivora in a s*ynergistic bioassay using S, S, S-tributyl phosphorotrithioate (DEF), a known inhibitor of esterases (Abdallah *et al*., 2016). Thus, a partial involvement of esterases in neonicotinoid resistance can be expected in these populations.

The GST-specific activities in the present study are slightly higher than the activities reported before (Figure 2B). High GST levels observed in the studied aphid populations may have a wider general role in giving resistance to all insecticide groups, including pyrethroids, as evidenced in mosquitoes (Che-Mendoza *et al*., 2008). In parallel, both test populations have shown high pyrethroid resistance, as discussed previously. However, the primary mechanism of pyrethroid resistance in *M. persicae* is the insensitive target site mechanism known as *kdr* (knockdown resistance) and super-*kdr* caused by L1014F, M918T, M918L, and L932F mutations in the voltage-gated sodium channel, the target site for pyrethroids and DDT-like organochlorines (Martinez-Torres *et al*., 1999; Eleftherianos *et al*., 2008; Fontaine *et al*., 2011). The kdr mutations have not yet been studied in Sri Lankan aphid populations.

Our study exhibited the presence of enhanced mono-oxygenase estimates in the studied aphid populations, although the extent was lesser than the amounts reported previously (Figure 2C). Synergistic studies show that the primary mechanism of neonicotinoid resistance in a *M. persicae* population was increased detoxification caused by overproduction of the monooxygenase enzyme through CYP6CY3 gene amplification (Puinean *et al*., 2010; Philippou *et al*., 2009). Monooxygenase CYP6CY3 detoxifies nicotine and confers cross-resistance to neonicotinoids (Ramsey *et al.,* 2014; Bass *et al.,* 2014). The moderate resistance detected to neonicotinoids in the two aphid populations studied could have resulted from enhanced oxidases. However, the fact that the involvement of other mechanisms, such as esterase (Abdallah *et al*., 2016), reduced cuticle penetration (Puinean *et al*., 2010) and mutation of gene R811 in the target site nicotinic acetylcholine receptor (nAChR) (Bass *et al*., 2011; Charaabi *et al*., 2018) in neonicotinoid resistance also cannot be overlooked.

The percent remaining activities of acetylcholinesterase in the Badulla and Kandy populations were 51 and 59 respectively (Figure 3). According to the AChE assay, adopted in the present study (WHO, 2016) both populations are heterozygous for resistance to either organophosphates or carbamates. AChE insensitivity to certain dimethyl carbamates, *viz* pirimicarb and triazamate, were found in heterozygous forms of some *M. persicae* populations that were sensitive to elevated esterase activities (Moores *et al*., 1994). Further, a point mutation (S431F) of the acetylcholinesterase enzyme (AChE) has resulted in pirimicarb resistance in *M. persicae* (Andrews *et al.,* 2002; Nabeshima *et al*., 2003).

Myzus persicae is a major polyphagous pest of many crops in Sri Lanka. A large number of

insecticides have been recommended for the control of this pest from time to time. Out of the tested insecticides, etofenprox is the only insecticide that is not recommended against aphids. However, etofenprox is used to control caterpillar pests in cabbage (DOA, 2009). Since *M. persicae* occurs on cabbage simultaneously with caterpillars, there is a potential for them to be exposed to several other non-recommended insecticides, such as etofenprox. Thus, the studied aphids also could have a history of pre-exposure to many groups of insecticides, including etofenprox

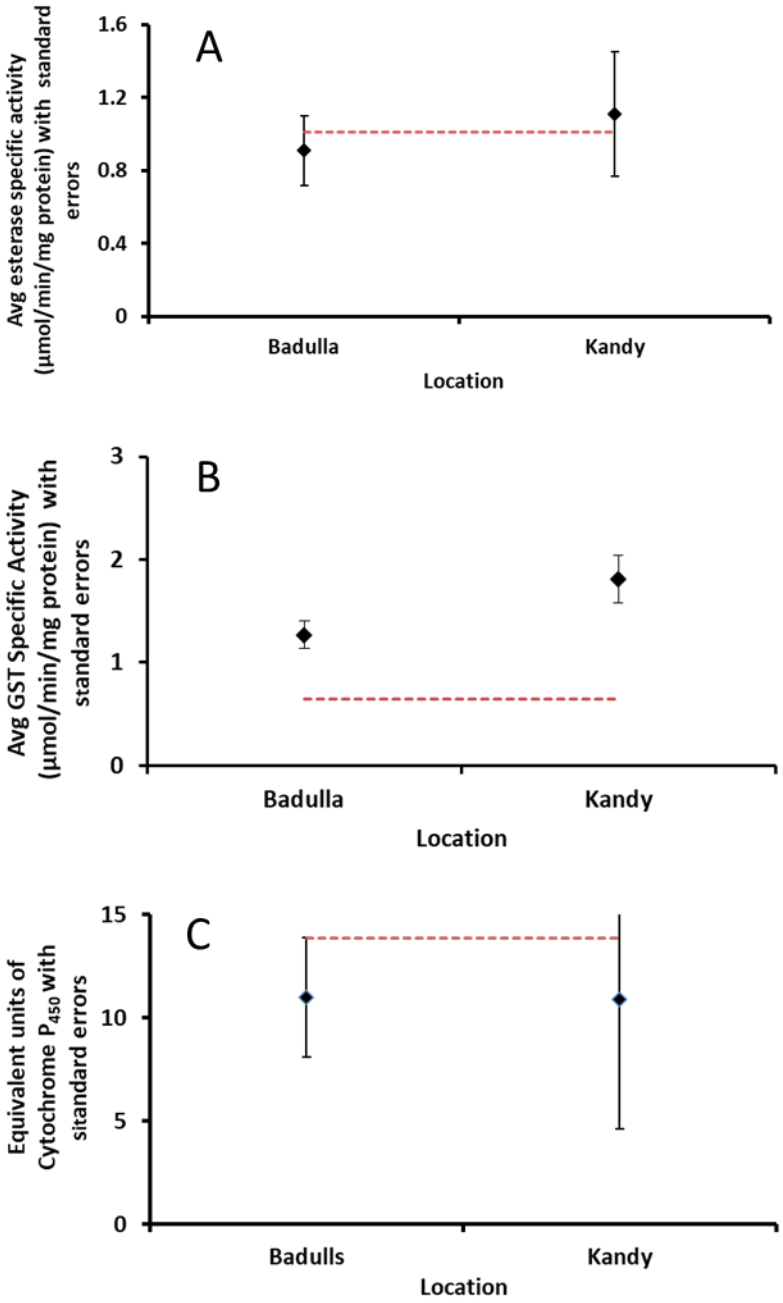


Figure 2. Average activities of insecticide metabolizing enzymes in *Myzus persicae* populations. A- esterase specific activity; B- glutathione-S-transferase (GST) specific activity; C-monooxygenase amounts in equivalent units of cytP_{450}; previously reported levels are also presented in dotted lines for comparison (Damayanthi and Karunaratne, 2005).

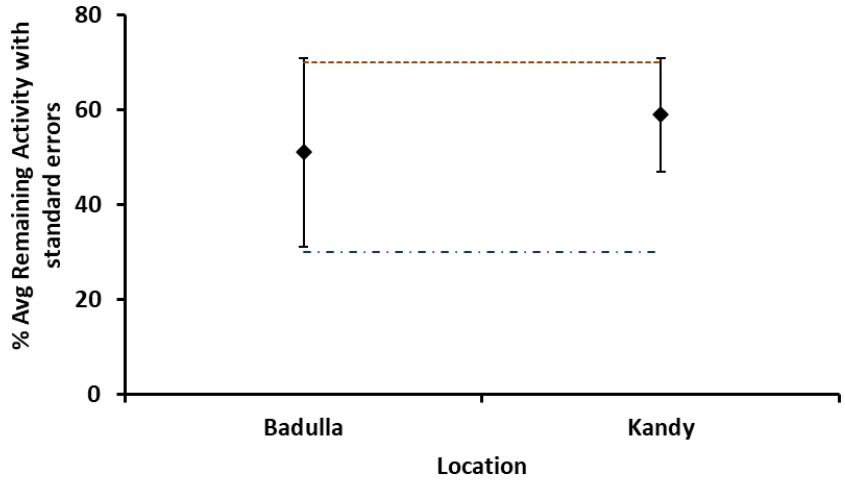


Figure 3. Percentage remaining activities of propoxur inhibited acetylcholinesterases of *Myzus persicae*; two dotted lines show the WHO recommended discriminating remaining activities for mosquitoes: ·····30% remaining activity, -·-·-·70% remaining activity (<30% susceptible; 30–70% heterozygous resistance; >70% homozygous resistance) (WHO, 2016).

Population genetics

The 636 bp mtCOI sequences of 26 Sri Lankan *M. persicae* from the three localities in wet, dry, and intermediate zones were aligned along with GenBank entries for *M. persicae* from India, Bangladesh, and Japan. Figure 4, presents the maximum likelihood tree with corresponding GenBank accession numbers. The out-groups were *Aphis craccivora* in Sri Lanka, Japan, Kenya, and Botswana. The phylogenetic analysis revealed that all 26 mtCOI sequences are from the same clade but have two haplotypes with a 1 bp SNP difference. The two haplotypes are shown in the ML tree with GenBank accession numbers (Table 3). The 636 bp mtCOI sequences of two *A. craccivora* from Kandy were submitted to GenBank (GenBank accession number OP185228.1) and used as outgroups from Sri Lanka in the phylogenetic analysis.

A total of 26 mtCOI sequences of *M. persicae,* representing 11, 11, and 4 individuals respectively from Anuradhapura, Badulla, and Kandy districts, were subjected to population genetic analysis. Translated amino acid sequences revealed that there are no frame shifts or stop codons in all the edited sequences. Among the 26 sequences, 15 were of haplotype 1, H1 (GenBank accession number OP185221.1) with 6, 5, and 4 representing Anuradhapura, Badulla, and Kandy respectively. The other 21 were of Haplotype 2, H2 (GenBank accession number OP185222.1) were from Anuradhapura and Badulla. It is believed that the lesser representation of Kandy individuals might be the reason to identify only H1 from Kandy, in the analysis. Depending on the low variation among the haplotype number, a further diversity analysis was not carried out.

This is the first report of the genotyping of Sri Lankan *M. persicae*, a major pest of cabbage cultivation, using the mtCOI gene. *Myzus persicae* is dispersed in a wider variety of crops and is known to show host race adaptations (Bass *et al*., 2014). Although direct evidence cannot be found on the relationship between insecticide resistance and the development of *M. persicae* biotypes, the popular example of *M. persicae* nicotinae being tolerant to neonicotinoids shows the exceptional ability of *M. persicae* to adapt to new host plants, leading to the formation of host races in some cases (Bass *et al.,* 2014). Therefore, knowledge of existing subspecies/ haplotypes in different host crops is important for establishing future aphid management programs.

Table 3. *Myzus persicae* species collected during 2017-2019; host plant, locality, and the agricultural zone from where the collection was made, haplotypes, and the GenBank accession numbers are also given.

Locality	District	Agro-climatic zone/ Elevation above mean sea level/ Annual rainfall	Host plant	Whitefly genetic group/biotype	Reference GenBank Accession Numbers
Gannoruwa	Kandy	Mid-country Wet Zone 2 (WM2)/ 300- 900 m/ > 2500 mm	Cabbage	*M. persicae* Haplotype 1	OP185221.1
Galenbindu nuwewa	Anuradhapura	Low-country Dry Zone 1 (DL1)/ < 300 m/ < 1750 mm		*M. persicae* Haplotype 1 Haplotype 2	OP185221.1 OP185222.1
Welimada	Badulla	Up-country Intermediate Zone 3 (IU3)/ > 900 m/ 1750- 2500 mm			

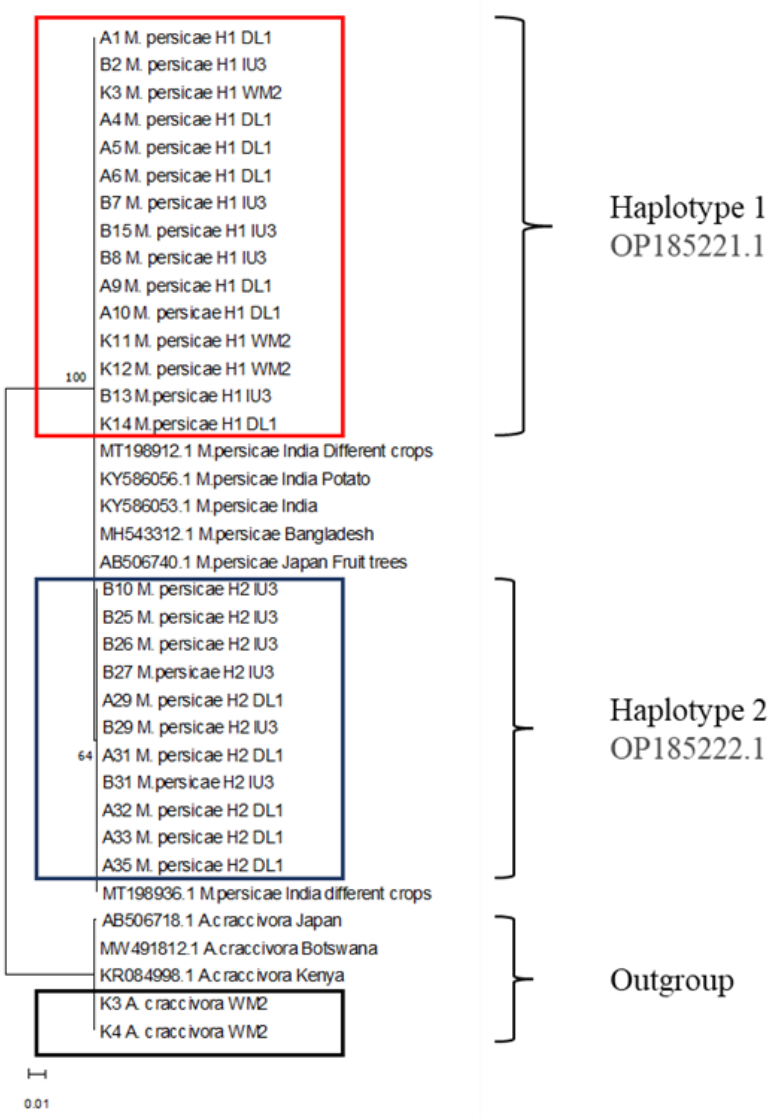


Figure 4: Phylogenetic analysis based on mtCOI sequence which has 636 positions in the data set, was constructed using the maximum likelihood method using the Tamura 3-parameter model, showing bootstrap values >55%. All Sri Lankan samples are boxed and represented by GenBank accession numbers. *Myzus persicae* haplotpes H1 and H2 are collected from the three localities of Anuradhapura, Badulla and Kandy. Samples identified as *Aphis craccivora* that were collected from Kandy district along with other GenBank entries were used as out-groups.

CONCLUSIONS

Myzus persicae populations had moderate resistance against thiamethoxam, acetamiprid, carbosulfan, and etofenprox. More than 90% of the tested aphids were susceptible to profenofos. Both, the increased detoxification and AChE target site insensitivity contribute to the observed resistance. Resistance to pyrethroids should be further investigated to identify the involvement of insensitive target sites caused by *kdr* type mutations. The phylogenetic analysis using the mtCOI gene revealed 2 haplotypes to occur in 2 out of 3 locations. Understanding novel genotypes in *M. persicae* under varying environmental and biological pressure in their host range is extremely important in developing effective management strategies. The outcome of the study can be successfully utilized for developing fact-based aphid control programs, avoiding the indiscriminate use of insecticides. Monitoring the insecticide resistance and phylogeny of *M. persicae* in other crops will elaborate our knowledge on *M. persicae* control in Sri Lanka.

ACKNOWLEDGEMENTS

We are thankful to the Director and the staff of the Horticultural Crop Research and Development Institute, Gannoruwa for providing the laboratory facilities at the Division of Entomology and the Zoology Department of the Faculty of Science, the University of Peradeniya for facilitating the studies. Also, the funding agency Sri Lanka CARP is greatly appreciated.

REFERENCES

Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. *J. econ. Entomol*, *18*(2), 265-267.

Abdallah, I. S., Abou-Yousef, H. M., Fouad, E. A. & El-Hady Kandil, M. A. (2016). The role of detoxifying enzymes in the resistance of the cowpea aphid (*Aphis craccivora* Koch) to thiamethoxam. *Journal of Plant Protection Research,* 56(1), 67-72

Andrews, M. C., Callaghan, A., Bass, C., Williamson, M. S., Field, L. M., & Moores, G. D. (2002). *A single amino acid substitution was found in pirimicarb-insensitive acetylcholinesterase of the peach-potato aphid, Myzus persicae (Sulzer)*. Cholinergic Mechanisms: Function and Dysfunction, St Moritz, Switzerland.

Bass, C., Puinean, A. M., Andrews, M., Cutler, P., Daniels, M., Elias, J., Paul, V. L., Crossthwaite, A. J., Denholm, I., Field, L. M., Foster, S. P., Lind, R., Williamson, M. S. & Slater, R. (2011). Mutation of a nicotinic acetylcholine receptor β subunit is associated with resistance to neonicotinoid insecticides in the aphid *Myzus persicae.* *BMC Neuroscience,e* 12, 51.

Bass, C., Puinean, A.M., Zimme, C., Denholm, I., Field, L. M., Foster, S.P., Gutbrod, O., Nauen, R., Slater, R., & Williamson, M.S., (2014). The evolution of insecticide resistance in the peach potato aphid, *Myzus persicae*. *Insect Biochemistry and Molecular Biology*, 51, 41-51.
https://doi.org/10.1016/j.ibmb.2014.05.003.

Blackman, R.L. (1974). Life-cycle variation of *Myzus persicae* (Sulz.) (Hom., Aphididae) in different parts of the world, in relation to genotype and environment. Bulletin of Entomological Research, 63(4), 595-607.

Blackman, R. L. (1987). Morphological discrimination of a tobacco feeding form from *Myzus* persicae (Sulzer) (Hemiptera: Aphididae), and a key to New World *Myzus* (Necarosiphon) species. *Bulletin of Entomological Research,* 77, 713-730.

Blackman, R.L. & Eastop, V.F. (2000) *Aphids on the World's Crops* (2nd edition). Wiley, Chichester.

Brogdon W.G., McAllister J.C. & Vulule J.M. (1997). Heam peroxidase activity measured in single mosquitoes identifies individuals expressing an elevated oxidase for insecticide resistance. *Journal of American Mosquito Association* 13, 233

Blackman, R.L. and Eastop, V.F. (2007). Aphids on the World's Herbaceous Plants and

Shrubs. The Aphids. John Wiley and Sons with the Natural History Museum, London. 2, 1025–1439.

Capinera, J. (2020). Handbook of vegetable pests. Academic press.

Charaabi, K., Boukhris-bouhachem, S., Makni, M. & Denholm, I. (2018). Occurrence of target-site resistance to neonicotinoids in the aphid *Myzus persicae* in Tunisia, and its status on different host plants. *Pest Management Science*, 74(6), 1297-1301.

Che-Mendoza A., Penilla R.P. & Rodríguez D.A. (2008). Insecticide resistance and glutathione S-transferases in mosquitoes: A review. *African Journal of Biotechnology,* 8(8), 1–12.

Damayanthi, B.T. & Karunaratne, S.H.P.P. (2005). Biochemical characterization of insecticide resistance in insect pests of vegetables and predatory ladybird beetles. Journal of the National Science Foundation of Sri Lanka, 33(2), 115-122.

Devonshire, A.L. & Moores, G.D. (1982). A carboxylesterase with broad substrate specificity causes organophosphorus, carbamate and pyrethroid resistance in peach potato aphids (*Myzus persicae*). *Pesticide Biochemistry and Physiology* 18, 235-246.

Devonshire, A.L., Moores, G.D. & Chiang, C. (1983). The biochemistry of insecticide resistance in the peach-potato aphid, *Myzus persicae* pp. 191-196. In: Miyamoto, J. and Kearney, P.C. (Ed.) Pesticide Chemistry, Human Welfare and the Environment. Proceedings of the 5th International Congress of Pesticide Chemistry. Pergamon Press,

DOA (2009). *Pesticide Recommendations*. Department of Agriculture, Gannoruwa, Peradeniya.

DOA (2015). *Pesticide Recommendations.* Department of Agriculture, Gannoruwa, Peradeniya.

Felsenstein, J. (1985). Confidence limits on phylogenesis: an approach using the bootstrap. *Evolution*, 39, 783- 791.

Fontaine, S., Caddoux, L., Brazier, C., Bertho, C., Bertolla, P., Micoud, A. & Roy, L. (2011). Uncommon associations in target resistance among French populations of *Myzus persicae* from oilseed rape crops. Pest management science, 67(8), 881-885.

Fuentes-Contreras, E., Figueroa, C. C., Silva, A. X., Bacigalupe, L. D., Briones, L. M., Foster, S. P. & Unruh, . R. (2013). Survey of Resistance to Four Insecticides and their Associated Mechanisms in Different Genotypes of the Green Peach Aphid (Hemiptera: Aphididae) From Chile. *Journal of Economic Entomology,* 106(1), 400-407.

Karunaratne, S.H.P.P. (1998). Insecticide resistance in insects: A review. *Ceylon Journal of Science* (*Biological Sciences*) 26, 73–98.

Marasinghe J.P., Hemachandra K.S., Nugaliyadde L. & Karunaratne, S.H.P.P. (2017). Control failure of Sri Lankan whitefly (*Bemisia tabaci* Genn) is due to high resistance development against recommended insecticides. *Journal of the National Science Foundation of Sri Lanka,* 45(1), 23–31.

Margaritopoulos J.T., Kasprowicz L., Malloch G.L. & Fenton B. (2009) Tracking the global dispersal of a cosmopolitan insect pest, the peach potato aphid. BMC Ecol. 9:13. DOI: https://doi: 10.1186/1472-6785-9-13.

Martinez-Torres, D., Foster, S. P., Field, L. M., Devonshire, A. L. & Williamson, M. S. (1999). A sodium channel point mutation is associated with resistance to DDT and pyrethroid insecticides in the peach potato aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). *Insect Molecular Biology*, 8(3), 339- 346. https:/doi.org/10.1046/j.1365-2583.1999.83121.x

Moores, G.D., Devine, G.J. & Devonshire, A. L. (1994). Insecticide-Insensitive Acetylcholinesterase Can Enhance Esterase-Based Resistance in *Myzus persicae* and *Myzus nicotianae*. *Pesticide Biochemistry and Physiology,* 49 (2), 114-120

Nabeshima, T., Kozaki, T., Tomita, T. & Kono, Y. (2003). An amino acid substitution on the second acetylcholinesterase in the

pirimicarb-resistant strains of the peach potato aphid, *Myzus persicae*. *Biochem. Biophys. Res. Commun*., 307, 15-22.

Perring, T.M, Battaglia, D., Walling, L. L., Toma, I. & Fanti, P. (2018). Aphids: Biology, Ecology, and Management Section 2, Chapter 4 pp. 73-110. In: Wakil, W., Brust, G.E. and Perring, T. M. (Ed.) *Sustainable Management of Arthropod Pests of Tomato*, Section 2. Chapter 4, Academic Press, UK.

Philippou, D., Field, L.M. & Moores, G.D. (2009). Metabolic enzyme(s) confer imidacloprid resistance in a clone of *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) from Greece. Pest Manag. Sci. 66, 390-395.

Puinean, A..M., Foster, S.P., Oliphant, L., Denholm, I., Field, L.. M., Millar, N. S., Williamson, M. S. & Bass, C. (2010). Amplification of a Cytochrome P_{450} gene is associated with resistance to neonicotinoid Insecticides in the aphid *Myzus persicae*, *PLoS Genetics* 6(6). doi: 10.1371/journal.pgen.1000999

Simon, J. C., & Peccoud, J. (2018). Rapid evolution of aphid pests in agricultural environments. *Curr. Opin. Insec Sci.,* 26, 17-24. Doi: 10.1016/j.cois.2017.12.009

Sivanahan, P. (1977). Virus diseases of crops in Sri Lanka. *Trop Agric Res ser*, 10, 65-68

Umina, P. A., Edwards, O., Carson, P., Van Rooyen, A., & Anderson, A. (2014). High Levels of Resistance to Carbamate and Pyrethroid Chemicals Widespread in Australian *Myzus persicae* (Hemiptera: Aphididae) Populations. *Journal of Economic Entomology,* 107(4), 1626-1638

Van Emden, H. F., & Harrington, R. (Eds.). (2017). *Aphids as crop pests*. Cabi.

WHO. (1998). *Techniques to detect resistance mechanisms* (field and laboratory manual). WHO/CDS/CPC/MAL/98.6, World Health Organization, Geneva, Switzerland.

WHO. (2016). *Test procedures for insecticide resistance monitoring in malaria vector mosquitoes.* World Health Organization, Genewa, Switzerland.