

Supplementary Materials: Proteomic Evaluation of Insecticidal Action of Phosphine on Green Peach Aphids, *Myzus Persicae*

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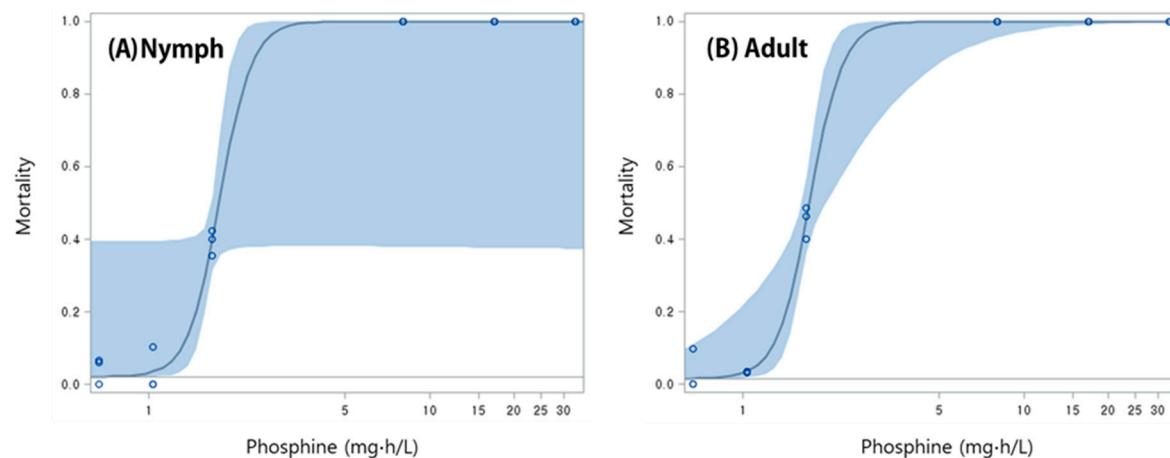


Figure S1. Mortality curve for the treatment of phosphine according to its concentration and fumigation duration towards *Myzus persicae*. (A), nymphs; (B), adults.

Table S1. Primer list using RT-qPCR analysis.

Gene name	Gene symbol	Accession number	Primer sequence (5'-3')
beta-actin	<i>actb</i>	Bass et al. (2011) [17]	F) GGTGTCTCACACACAGTGCC R) CGGCGGTGGTGGAAGCTG
ribosomal protein S2	<i>rps2</i>	KF246568.1	F) TGCCAAAGAACATTGCCTG R) TGCTACTGCACCCTCATTCC
cytochrome P450 (CYP6CY3)	<i>cyp6cy3</i>	HM009309.1	F) TCTTTTCAGGGAGCTGTGCC R) ACGCCGAGTGAAAGAAAGTCA F) GTCCGTCTGATTCCGGTTGA
farnesyl diphosphate synthase 1	<i>fps1</i>	EU334430.1	R) CGTATGGATCGTTGGTCGGT F) AGAGGAATCACACAGGGAGC R) GCGAGGGTGCCTAACCTAA
acetylcholinesterase	<i>ache</i>	KJ561353.1	F) TGACGCGGAGGAACACTTAC R) GTATGACACGGGCACGATGA
voltage gated sodium channel subunit 1	<i>para</i>	FN601405.1	F) TCCC GTTCGATGAAACAGACG R) ACTACTCGTAACCGCTGAC
nicotinic acetylcholine receptor alpha 3 subunit	<i>ni-acr</i>	AJ236786.1	F) R)
ecdysone receptor	<i>ecr</i>	EF174334.1	CAAAGCTGGATTGGGAGATGC R) CGATGGCGGTCAATAAGGCA

Table S2. Effects of phosphine fumigation on the different developmental stages of *Myzus persicae*.

Fumigant	Developmental stage	LCT_{10} (mg * h / L)	LCT_{50} (mg * h / L)	Slope±s.e.m.	df	χ^2
PH ₃	nymph	1.048 (0.189- 1.753)	1.983 (0.770- 2.713)	4.632±0.925	8	1.5
	adult	1.056 (0.112- 1.821)	1.845 (0.478- 2.609)	5.290±1.178	8	2.11

Expression of toxicity values are based on LCT.

Table S3. Proteins differentially expressed in adults of *Myzus persicae* after phosphine treatment at the concentration of 0.5 mg/L for 4 h. CON; control treatment group, PH3; phosphine treatment group. Proteomic analyses of *Myzus persicae* were undertaken using a Nano-LC-ESI-MS/MS technique. Statistical difference was analyzed using *t*-test ($p < 0.05$). Proteins were involved in energy metabolism (especially, electron transport chain), defense, metabolism, and other proteins.

Category	Protein Name	Accession Number	Quantity (Normalized NSAF)/ Fold-change*			Biological function
			CON	PH3	Fold*	
Energy metabolism	NADH dehydrogenase [ubiquinone] flavoprotein 1, mitochondrial-like	XP_022181147.1	$2.12 \times 10^{-4} \pm 2.73 \times 10^{-5}$	$7.11 \times 10^{-4} \pm 6.21 \times 10^{-5}$	3.35	core subunit of the mitochondrial membrane respiratory chain NADH dehydrogenase (Complex I)
	cytochrome b-c1 complex subunit Rieske, mitochondrial	XP_022165229.1	$1.11 \times 10^{-3} \pm 5.03 \times 10^{-5}$	$5.89 \times 10^{-4} \pm 1.00 \times 10^{-4}$	0.53	respiration, ubiquinol-cytochrome c reductase activity
	cytochrome oxidase subunit II, partial (mitochondrion)	AAD42212.1 (+33)	$7.37 \times 10^{-4} \pm 5.09 \times 10^{-5}$	$5.22 \times 10^{-4} \pm 7.50 \times 10^{-5}$	0.70	respiration
	cytochrome c oxidase subunit 6C	XP_022172575.1	0	$7.31 \times 10^{-4} \pm 1.74 \times 10^{-4}$	—**	respiration
	ATP synthase subunit g, mitochondrial-like	XP_022172403.1	$1.26 \times 10^{-3} \pm 1.19 \times 10^{-4}$	$6.86 \times 10^{-4} \pm 1.49 \times 10^{-4}$	0.54	respiration, energy metabolism
Defense	peroxiredoxin 1-like	XP_022181926.1	$2.40 \times 10^{-3} \pm 1.35 \times 10^{-4}$	$4.08 \times 10^{-3} \pm 2.20 \times 10^{-4}$	1.70	defense
	glutathione S-transferase-like	XP_022173704.1	$4.63 \times 10^{-4} \pm 2.02 \times 10^{-4}$	$1.05 \times 10^{-3} \pm 1.46 \times 10^{-4}$	2.26	defense
	lysozyme-like	XP_022168131.1	$4.39 \times 10^{-4} \pm 8.46 \times 10^{-6}$	$1.38 \times 10^{-3} \pm 1.03 \times 10^{-5}$	3.14	defense response to gram-negative and positive bacterium, cytolysis
	probable protein phosphatase 2C 6	XP_022169006.1	$1.02 \times 10^{-4} \pm 2.47 \times 10^{-5}$	$2.07 \times 10^{-4} \pm 2.58 \times 10^{-5}$	2.02	metal ion binding, defense response to fungus, response to wounding
	Cluster of esterase E4-like isoform X1	XP_022165703.1 [4]	0	$7.59 \times 10^{-5} \pm 1.92 \times 10^{-5}$	—	hydrolase, conferring insecticide resistance

	Ion protease homolog, mitochondrial	XP_022168293.1	$2.20 \times 10^{-4} \pm$ 1.78×10^{-5}	$4.63 \times 10^{-5} \pm$ 1.63×10^{-5}	0.21	ATP binding, cellular response to oxidative stress, protein quality control for misfolded or incompletely synthesized proteins
	multidrug resistance-associated protein 4-like	XP_022166664.1	$2.31 \times 10^{-5} \pm$ 9.88×10^{-6}	0	—	ATPase activity, response to drug, transmembrane transport, xenobiotic transport
Metabolism	galactokinase-like	XP_022162633.1 (+1)	$2.75 \times 10^{-4} \pm$ 8.69×10^{-5}	$6.06 \times 10^{-4} \pm$ 9.54×10^{-5}	2.20	glycolytic process from galactose
	glucosidase 2 subunit beta-like	XP_022181844.1	$3.05 \times 10^{-5} \pm$ 2.64×10^{-5}	$3.14 \times 10^{-4} \pm$ 2.60×10^{-5}	10.3	carbohydrate binding, glycogen breakdown
	protein obstructor-E-like	XP_022167308.1	$1.28 \times 10^{-4} \pm$ 5.34×10^{-5}	$4.55 \times 10^{-4} \pm$ 6.46×10^{-5}	3.55	chitin binding, chitin metabolic process
	hydroxysteroid dehydrogenase-like protein 2	XP_022170969.1	$5.71 \times 10^{-5} \pm$ 1.10×10^{-6}	$1.66 \times 10^{-4} \pm$ 3.61×10^{-5}	2.97	oxidoreductase activity
	sugar transporter SWEET1-like	XP_022159995.1 (+1)	0	$3.13 \times 10^{-4} \pm$ 6.12×10^{-5}	—	sugar transmembrane transporter activity, carbohydrate transport
	Carb	AHG60226.1 (+3)	$5.82 \times 10^{-5} \pm$ 2.43×10^{-5}	0	—	amino acid binding, urea cycle, arginine biosynthetic process
Others	protein obstructor-E isoform X1	XP_022167038.1	$4.12 \times 10^{-4} \pm$ 1.14×10^{-4}	$1.49 \times 10^{-3} \pm$ 2.31×10^{-5}	3.61	chitin binding Peritrophin-A domain, interaction with chitin
	innexin inx2	XP_022174218.1	$1.10 \times 10^{-4} \pm$ 3.91×10^{-5}	$3.34 \times 10^{-4} \pm$ 4.16×10^{-5}	3.03	gap junction channel activity, morphogenesis of embryonic epithelium
	involucrin-like	XP_022166998.1	0	$6.26 \times 10^{-5} \pm$ 5.78×10^{-7}	—	integral component of membrane

choline transporter-like protein 1	XP_022173488.1	0	$1.16 \times 10^{-4} \pm 2.51 \times 10^{-5}$	–	choline transmembrane transporter activity, choline transport
methionine aminopeptidase 2	XP_022168312.1	$3.35 \times 10^{-5} \pm 2.90 \times 10^{-5}$	$1.09 \times 10^{-4} \pm 1.01 \times 10^{-6}$	3.25	aminopeptidase activity, releasing of N-terminal amino acids, preferentially methionine from peptides
tubulin beta chain-like	XP_022175330.1	$3.01 \times 10^{-4} \pm 6.75 \times 10^{-5}$	$1.15 \times 10^{-4} \pm 1.70 \times 10^{-6}$	0.38	structural constituent of cytoskeleton
dystonin isoform X1	XP_022169485.1 (+22)	$1.02 \times 10^{-4} \pm 7.86 \times 10^{-6}$	$3.46 \times 10^{-5} \pm 1.04 \times 10^{-6}$	0.34	skin blistering and neurodegeneration
calphotin-like	XP_022179079.1	$1.01 \times 10^{-4} \pm 1.95 \times 10^{-6}$	0	–	calcium ion binding, response to stimulus, visual perception

*Indicates fold-changes in the phosphine-treated *M. persicae* when compared with that in the control (no used PH3). ** indicates not available data for fold changes.

Category and functions are matched with the UniProt database supplied by the UniProt Consortium.

Table S4. Proteins differentially expressed in adults of *Myzus persicae* after phosphine treatment at the concentration of 0.5 mg/L for 4 h. CON; control treatment group, PH3; phosphine treatment group. Proteomic analysis was conducted by a Nano-LC-MS/MS. Statistical difference was analyzed using *t*-test ($p < 0.05$). Proteins are classified in chromatin and histones, transcription, transition and post-translational modification, signaling pathways and other functional roles in cells.

Category	Protein Name	Accession Number	Quantity(Normalized NSAF)/			Biological function		
			Fold-change*					
Chromatin and histone	histone H2A.V	XP_022172768.1	$2.45 \times 10^{-3} \pm 1.03 \times 10^{-4}$	$6.64 \times 10^{-5} \pm 1.15 \times 10^{-4}$	0.032	DNA binding, chromatin silencing, DNA repair		
	histone H2A-like	XP_022162784.1 (+5)	$8.67 \times 10^{-3} \pm 1.74 \times 10^{-4}$	$1.03 \times 10^{-3} \pm 2.15 \times 10^{-4}$	0.19	chromatin silencing		
	histone H2B-like	XP_022162783.1 (+4)	$3.43 \times 10^{-3} \pm 1.09 \times 10^{-4}$	$1.22 \times 10^{-3} \pm 2.11 \times 10^{-4}$	0.35	antibacterial response, protein ubiquitination	humoral	

	histone H3	XP_022162782.1 (+7)	$2.01 \times 10^{-3} \pm 4.00 \times 10^{-4}$	$3.77 \times 10^{-4} \pm 1.87 \times 10^{-4}$	0.19	blood coagulation, regulation of gene silencing by miRNA
	circadian clock-controlled protein-like	XP_022160865.1	$7.31 \times 10^{-4} \pm 1.71 \times 10^{-4}$	$1.26 \times 10^{-4} \pm 5.59 \times 10^{-5}$	0.17	histone deacetylase binding, histone methyltransferase binding, circadian regulation of gene expression, fatty acid metabolic process, gluconeogenesis, negative regulation of protein ubiquitination
	cleavage and polyadenylation specificity factor subunit 5 isoform X1	XP_022180817.1	$5.63 \times 10^{-4} \pm 5.60 \times 10^{-5}$	0	-**	histone deacetylase binding, positive regulation of mRNA polyadenylation
Transcription	U6 snRNA-associated Sm-like protein LSm7	XP_022168331.1	$1.91 \times 10^{-3} \pm 5.31 \times 10^{-4}$	$4.81 \times 10^{-4} \pm 4.43 \times 10^{-5}$	0.25	RNA binding, maturation of SSU-rRNA, mRNA splicing
	myelin expression factor 2	XP_022171055.1 (+1)	$1.75 \times 10^{-4} \pm 2.47 \times 10^{-5}$	$3.45 \times 10^{-5} \pm 5.98 \times 10^{-5}$	0.20	RNA binding, negative regulation of transcription by RNA polymerase II
	hrp65 protein-like isoform X1	XP_022173748.1 (+8)	$2.85 \times 10^{-4} \pm 3.29 \times 10^{-5}$	0	-	RNA binding, mRNA export from nucleu
Translation and post-translational modification	aspartate-tRNA cytoplasmic ligase	XP_022172279.1 (+1)	$8.76 \times 10^{-5} \pm 1.24 \times 10^{-6}$	$1.91 \times 10^{-4} \pm 1.78 \times 10^{-6}$	2.18	ligase activity, tRNA aminoacylation for protein translation

	ubiquitin-conjugating enzyme E2-17 kDa	XP_022174670.1	$5.35 \times 10^{-4} \pm 1.04 \times 10^{-4}$	$1.93 \times 10^{-3} \pm 1.57 \times 10^{-4}$	3.60	protein polyubiquitination, ubiquitin-dependent protein catabolic process
	serine/threonine-protein phosphatase 2A catalytic subunit (+1) alpha isoform	XP_022182715.1	$4.05 \times 10^{-4} \pm 1.09 \times 10^{-4}$	$9.42 \times 10^{-4} \pm 2.03 \times 10^{-4}$	2.32	positive regulation of apoptotic process, negative regulation of phosphoprotein phosphatase activity
	26S proteasome non-ATPase regulatory subunit 13 (+1)	XP_022164476.1	$3.24 \times 10^{-4} \pm 7.09 \times 10^{-5}$	$8.47 \times 10^{-4} \pm 1.80 \times 10^{-5}$	2.61	protein de-ubiquitination, ubiquitin-dependent protein catabolic process
Signaling pathway	V-type proton ATPase subunit d	XP_022181057.1	$6.05 \times 10^{-4} \pm 1.24 \times 10^{-5}$	$1.44 \times 10^{-3} \pm 7.32 \times 10^{-5}$	2.38	cilium assembly, insulin receptor signaling pathway, regulation pf macroautophagy
	unc-112-related protein-like	XP_022166379.1	0	$3.69 \times 10^{-5} \pm 1.88 \times 10^{-7}$	–	integrin-mediated signaling pathway
	lamin Dm0-like	XP_022162306.1	$2.83 \times 10^{-4} \pm 3.89 \times 10^{-5}$	$1.47 \times 10^{-4} \pm 2.42 \times 10^{-5}$	0.52	signaling receptor binding, adult locomotory behavior, negative regulation of cell proliferation and immune response
Others	synaptic vesicle membrane protein VAT-1 homolog-like	XP_022164309.1	$8.82 \times 10^{-4} \pm 4.41 \times 10^{-5}$	$2.02 \times 10^{-3} \pm 1.34 \times 10^{-4}$	2.29	negative regulation of mitochondrial fusion

TPA_inf: cathepsin B	DAA06113.1 (+1)	$2.10 \times 10^{-4} \pm 6.80 \times 10^{-5}$	$4.35 \times 10^{-4} \pm 4.76 \times 10^{-5}$	2.07	collagen binding, regulation of apoptotic process
slit homolog 1 protein-like	XP_022178244.1	0	$2.67 \times 10^{-4} \pm 9.46 \times 10^{-5}$	–	calcium ion binding, negative control to cell growth, positive regulation of apoptotic process, response to cortisol
ras-related protein Rab-5C	XP_022181507.1 (+2)	$4.84 \times 10^{-4} \pm 6.24 \times 10^{-5}$	$2.45 \times 10^{-4} \pm 2.71 \times 10^{-6}$	0.51	GTP binding, positive regulation of defense response to bacterium
von Willebrand factor A domain-containing protein 8	XP_022159907.1	$4.50 \times 10^{-5} \pm 9.5 \times 10^{-6}$	$1.24 \times 10^{-5} \pm 1.07 \times 10^{-5}$	0.28	identical binding, behavioral response to pain
caspase-1-like	XP_022164969.1	$2.02 \times 10^{-4} \pm 4.61 \times 10^{-5}$	$2.77 \times 10^{-5} \pm 4.80 \times 10^{-5}$	0.14	kinase binding, positive regulation of interleukin-1 beta secretion, regulation of inflammatory response
protein FAM98A	XP_022162395.1	$9.17 \times 10^{-5} \pm 1.77 \times 10^{-6}$	0	–	protein methyltransferase activity, positive regulation of cell proliferation

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