

Supplementary Tables

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Hypothesis-driven dragging of transcriptomic data to analyze proven targeted pathways in *Rhinella arenarum* larvae exposed to organophosphorus pesticides.

Table S1. List of available annotated genes from *R. arenarum* transcriptome

GROUP A, HOUSEKEEPING GENES	
Elongation Factor 1-alpha	EF1A0, 1A1, 1A2
Elongation Factor 1-beta	EF1B
Elongation Factor 1-gamma A	EF1GA
Elongation Factor 1-delta	EF1D
Glyceraldehyde-3-phosphate dehydrogenase	G3P
60S ribosomal protein L8	RL8
Tubulin alpha chain	TBA, TBA1
Tubulin beta chain	TBB, TBB4B
cytoplasmic Actin	ACTB
Actin alpha sarcomeric	ACT3
GROUP B, POLYAMINE METABOLISM GENES	
Ornithine decarboxylase	DCOR1
Ornithine decarboxylase antizyme	OAZ1, 2
Antizyme inhibitor	AZIN1, 2
S-adenosyl methionine decarboxylase precursor	AMD1
Spermidine synthase	SPEE
Amiloride-sensitive Cu-containing amine oxidase / diamine oxidase	AOC1 to 4
N(1)-acetyl-spermine/spermidine oxidase	PAOX
Spermine oxidase	SMOX
Diamine acetyltransferase	SSAT
GROUP C, OXIDATIVE STRESS - ANTIOXIDANT RESPONSE GENES	
Superoxide dismutases	SODC, D, E, 5
Catalases	CATA
Glutathione peroxidases	GPX1 to 8
Glutathione reductase	GSHR
Glutathione synthetase	GSHB
GROUP D, OP- METABOLIZING AND TARGET ENZYME GENES	
Acetyl cholinesterases	ACHE
Butyryl cholinesterases	BCHE
Carboxylesterases	EST3B, EST5A

Serum paraoxonase/arylesterase	PON
Glutathione transferases alpha	GSTA
Glutathione transferases kappa	GSTK
Glutathione transferases mu	GSTM
Glutathione transferases omega	GSTO
Glutathione transferases pi	GSTP
Glutathione transferases theta	GSTT
Glutathione transferases microsomal forms	M-GST1 to 3
Cytochrome P450 1A1	CYP1A1
Cytochrome P450 2C19	CYP2C19
GROUP E, TRANSCRIPTION FACTORS AND PHOSPHORYLATION CASCADES	
Mitogen-Activated protein kinase pathway	
MAPKK/ MEK	MP2K1, K2
MAPK	MK p38, JUNK
Aryl hydrocarbon receptor pathway	
Aryl hydrocarbon receptor	AHR
AhR repressor	AHRR
nuclear translocator	ARNT
heat-shock protein 90	HSP90AB1
Aryl-hydrocarbon-interacting proteins	AIP and AIPL1
Prostaglandin E synthase 3	PTGES3
Transcription factor AP-1 JUN	JUN1, 2, B, D
Proto-oncogene c-Fos	CFOS
Nuclear factor erythroid 2-related factor 2	NRF2

Table S2. Gene transcripts showing no significant or not relevant changes in *R. arenarum* larvae after exposure to OP pesticides.

Transcript	Fold-change					p-value
PA pathway						
	control	AZM6	AZM24	CPF6	CPF24	
OAZ2	1 ± 0.04	1.09 ± 0.00	1.04 ± 0.05	1.04 ± 0.04	1.09 ± 0.03	0.35
AZIN1	1 ± 0.07	1.08 ± 0.10	0.85 ± 0.03*	1.11 ± 0.09	0.95 ± 0.05	0.04*
AZIN2	1 ± 0.11	0.90 ± 0.12	0.77 ± 0.12	0.88 ± 0.29	0.75 ± 0.08	0.41
AOC2	1 ± 0.06	0.52 ± 0.15	0.54 ± 0.02	0.31 ± 0.10	0.46 ± 0.05	0.17
AOC3	1 ± 0.02	0.68 ± 0.33	1.08 ± 0.14	1.13 ± 0.40	1.70 ± 0.01	0.29
AOC4	1 ± 0.37	1.19 ± 0.03	0.98 ± 0.16	1.48 ± 0.07	1.04 ± 0.37	0.40
PAOX-2	1 ± 0.08	0.89 ± 0.07	1.00 ± 0.05	1.04 ± 0.10	0.91 ± 0.03	0.51
Antioxidant enzymes						
GPX2	1 ± 0.26	1.02 ± 0.10	0.46 ± 0.02	0.70 ± 0.09	0.84 ± 0.40	0.41
GPX7	1 ± 0.39	1.00 ± 0.27	0.66 ± 0.10	0.70 ± 0.12	0.86 ± 0.08	0.41
GSHR	1 ± 0.12	0.75 ± 0.04	0.74 ± 0.02	0.73 ± 0.02	0.69 ± 0.08	0.41
Detoxifying enzymes						
EST3B	1 ± 0.08	0.65 ± 0.09	0.84 ± 0.05	0.64 ± 0.17	0.87 ± 0.26	0.35
GSTC	1 ± 0.06	0.92 ± 0.03	0.94 ± 0.00	0.98 ± 0.05	1.05 ± 0.05	0.32
GSTM1	1 ± 0.07	1.00 ± 0.10	0.81 ± 0.10	0.73 ± 0.10	0.90 ± 0.12	0.24
GSTP1-2	1 ± 0.00	1.07 ± 0.06	1.15 ± 0.03	1.08 ± 0.05	1.15 ± 0.05	0.20
Signaling pathways and transcription factors						
MP2K1	1 ± 0.02	1.03 ± 0.01	0.95 ± 0.02	0.92 ± 0.02*	0.97 ± 0.01	0.09*
MP2K2-2	1 ± 0.04	0.91 ± 0.05	0.81 ± 0.05	0.91 ± 0.03	0.87 ± 0.03	0.26
p38	1 ± 0.01	0.92 ± 0.04	0.87 ± 0.03*	0.89 ± 0.03*	0.94 ± 0.00	0.09*
JUN-K	1 ± 0.04	1.00 ± 0.01	0.92 ± 0.06	1.02 ± 0.06	0.89 ± 0.01	0.32
JUN-1	1 ± 0.14	1.13 ± 0.06	1.16 ± 0.01	1.23 ± 0.15	0.83 ± 0.13	0.27
JUNB	1 ± 0.17	1.17 ± 0.33	1.22 ± 0.24	1.37 ± 0.06	0.80 ± 0.13	0.40
JUND-1	1 ± 0.05	0.98 ± 0.04	0.81 ± 0.05	0.94 ± 0.08	0.86 ± 0.01	0.94
AHR	1 ± 0.06	0.85 ± 0.04	0.90 ± 0.26	0.78 ± 0.15	0.74 ± 0.09	0.14
AIP	1 ± 0.03	0.98 ± 0.04	1.00 ± 0.00	1.08 ± 0.03	1.00 ± 0.01	0.35
PTGES3	1 ± 0.09	0.83 ± 0.05	0.68 ± 0.02	0.87 ± 0.06	0.79 ± 0.03	0.14

AZM: azinphosmethyl; CPF: chlorpyrifos. *Significant, not relevant changes

TABLE S3. Comparison between non-parametric tests and edgeR performances.

Transcript (I) ¹	KW/ Median	paired edgeR (Treated vs Control) analysis							
	p value (n) ²	p values ³				DEG Ranking ⁴			
		AZM6	AZM24	CPF6	CPF24	AZM6	AZM24	CPF6	CPF24
PA pathway									
DCOR1	0.09 (6)	0.33	0.44	1	0.42				
SPEE	0.09	0.51	0.28	0.11	0.05	#-1281			
OAZ1	0.05 (4)	0.79	0.001	0.002	0.03	#-819		#-1038	#-1429
AMD1-a	0.09	Data not found in edgeR							
AMD1-b	0.09	0.24	0.06	0.06	0.07	#-1206		#-1330	#-1340
AOC1 (4)	0.0001 (8)	0.14	0.11	0.01	0.13	#-246			
		0.13	0.02	0.008	0.12	#-417		#-363	
		0.06	0.01	0.002	0.01	#-62	#-411	#-253	#-422
		0.30	0.07	0.007	0.05	#-577		#-222	#-529
PAOX	0.09	0.43	0.95	0.26	0.94				
SMOX	0.09	0.49	0.56	0.40	0.76				
SAT1	0.09	0.18	0.0009	0.002	0.001	#-472		#-843	#-587
SAT2-a	0.09	0.12	0.02	0.36	0.0008				
SAT2-b	0.08	0.47	0.72	0.73	0.55				
OXIDATIVE STRESS									
GPX1	0.08	0.77	5x10 ⁻⁷	1x10 ⁻⁶	2x10 ⁻⁵	#+24		#+58	#+96
GPX3	0.01 (4)	0.56	0.63	0.07	0.04			#+375	#+210
GPX4	0.07 (4)	1	0.40	0.17	0.14				
GPX8B	0.09	0.47	0.13	0.04	0.12	#-796			
GSHB	0.09	0.65	0.80	0.64	0.71				
SODC	0.09	0.54	0.05	0.005	0.09	#-1310		#-1071	#-1468
SOD E (2)	0.0007(12)	0.31	0.54	0.15	0.36				
CATA (2)	0.09 (4)	0.64	0.28	0.23	0.17				
DETOXIFYING ENZYMES									
EST 5A	0.09	Data not found in edgeR							
PON2	0.05	0.74	0.74	0.20	0.75				

CYP1A1	0.007 (4)	0.90	0.90	0.80	0.60				
CYP2C19	0.09	0.06	0.003	0.06	0.003	#-41	#-65	#-656	#-59
GSTA3	0.09	0.01	0.09	0.08	0.59	#-72	#-1331	#-1438	
GSTK1	0.04 (4)	0.66	0.58	0.13	0.03				#+285
GSTM3	0.09	0.89	0.0001	0.0003	0.0001		#+38	#+89	#+13
GSTO1	0.05 (6)	0.41	0.74	0.38	0.25				
GSTP1 (2)	0.0001 (8)	0.23	0.003	0.009	0.01		#-346	#-674	#-728
		0.98	0.04	0.05	0.01		#-997	#-1208	#-719
GSTP2	0.08	0.31	0.01	0.004	0.24		#-1236	#-1177	
GSTT3	0.09	0.95	0.01	0.004	0.04		#-1133	#-1012	#-1404
M-GST1	0.09	0.88	0.01	0.02	0.002		#+294	#+430	#+223
M-GST2-1	0.09	0.18	0.99	0.37	0.19				
M-GST2-2	0.09	0.95	0.28	0.44	0.34				
M-GST3-1	0.09	0.88	0.02	0.03	0.003		#+297	#+432	#+215
M-GST3-2	0.09	0.93	0.95	0.80	0.15				
TRANSCRIPTION FACTORS, SIGNALING									
MP2K2	0.09	0.89	0.38	0.52	0.74				
NRF2	0.09	0.21	0.01	0.01	0.008		#-96	#-1051	#-948
ARNT	0.09	0.001	0.35	0.26	0.05				
ARNT2	0.09	0.30	0.90	0.0006	0.60				
AHRR	0.09	0.80	1	0.90	0.50				
HSP90AB1	0.09 (4)	0.47	0.91	0.25	0.01				#-1409
AIPL1	0.09	0.31	0.80	0.40	0.25				
FOS	0.08	0.01	0.25	0.27	0.70				

The p-values for annotated transcripts in hypothesis-driven selected pathways, showing statistically significant differential expressions in non-parametric tests (filtered database), were included for comparison with edgeR analysis performed on not filtered database. AZM: azinphos methyl; CPF: chlorpyrifos; 6 and 24 h of treatment.

¹ (I) isoforms for one annotated gene are indicated by separate in edgeR analysis, if more than one

² KW, Kruskal-Wallis ANOVA and Median non-parametric tests, best p value for (n) number of data per treatment, when n is higher than 2.

³ edgeR p-value ranges when more than one fragment and/or isoform were determined for a single annotated gene, for the different pairs of treatments. Red numbers indicate no coincidence with non-parametric statistics for the same pair of treatments.

4 Ranking only for significant DEG; #- for downregulation; #+ for upregulation. Total DEG in each treatment: AZM6 #- 185, #+ 121; AZM24 #-1379, #+ 374; CPF6 #- 1513, #+ 492; CPF24 #- 1504, #+ 347.

Table S4. Summary of transcripts and steps performed to develop further expression analysis by RT-PCR and qPCR

Transcript	RT-PCR			qPCR		
	Primer	Amplification	Sequence Validated	Primer	Amplification	Expression Testing
Polyamine pathway						
DCOR1	Y	Y	Y	Y	(Y)	-
PAOX	Y	Y	Y	Y	(Y)	-
AOC1	Y	Y	Y	Y	(Y)	-
SMOX	Y	N	-	Y	(Y)	-
AMD1	Y	Y	N	Y	Y	Y
SPEE	Y	Y	Y	Y	(Y)	-
Antioxidant response						
CATA	Y	Y	N	-	-	-
GSHR	Y	N	-	-	-	-
GSTP	Y	Y	N	-	-	-
SODC	Y	Y	N	Y	Y	Y
Signaling and transcription factors						
FOS	Y	Y	Y	Y	(Y)	-
NRF2	Y	Y	N	-	-	-
JUN	Y	Y	Y	-	-	-
JUNK	Y	N	-	-	-	-
Housekeeping genes						
ACTB	Y	Y	Y	Y	Y	Y
RL8	Y	Y	Y	Y	Y	Y

Y indicates succesful step; (Y) indicates amplification at high cycle numbers that diffculted validation and further testing of differential expression levels. Expression testing refers to transcript expression analysis by qPCR in *R. arenarum* larvae exposed to azinphos methyl and chlorpyrifos.