Supporting Information for

Activation of Antioxidant and Detoxification Gene Expression in Cucumber Plants exposed to a $Cu(OH)_2$ Nanopesticide

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Comparison with Cu Ion. In order to elucidate whether the observed response was nano-specific, an additional experiment was conducted. 3-week-old cucumber plants (4 replicates) were exposed to different concentrations of $CuSO_4$ (0, 10, 100 and 500 mg/L) suspension for 1 week, corresponding to 0, 0.21, 2.1 and 10 mg $CuSO_4$ per plant. It is challenging to ensure the same internalized Cu concentration within the leaves; this experiment was simply to determine whether Cu^{2+} would elicit a response similar to the $Cu(OH)_2$ nanopesticide for dosing within the same order of magnitude. $CuSO_4$ dissolves completely, so Cu^{2+} is almost immediately bioavailable.

Table S1. Physicochemical Properties of $Cu(OH)_2$ Nanopesticide

Property	
Primary particle diameter (nm)	10-1000
Hydrodynamic diameter (nm)	1532±580
Copper content (wt%)	26.5±0.9
Other elements present	C, O, Na, Al, Si, S, Zn, Ni, Ti, V, Co, Se, Ag, Pb
Copper phase	orthorhombic $Cu(OH)_2$
Helium density (g/cm^3)	2.399
BET surface area (m^2/g)	15.71±0.16
Zeta potential (mV)	-47.6±4.3

Table S2. Primers used for real-time RT-PCR assays

Name	Sequence	Amplicon size (bps)	Database entry#
RBOH F	AAGGTTGCTGTTTATCC	213	Cucsa.181960.1
RBOH R	AATGGTCTTGAGTTGGG		
MAPK1 F	ATTGATGTGTGGTCTGTAGG	188	Cucsa.231940.1
MAPK1 R	GGAGAGTATGGAAGGGATTT		
MAP3K3 F	GGAACAGATACGAAGGGGT	149	Cucsa.100890.1
MAP3K3 R	TTCAGCAGCAGAAGGACGG		
WRKY30 F	CATCTTCACCCTTCTTCAT	231	Cucsa.259110.1
WRKY30 R	CGCATCTCTGCTTCTACTG		
WRKY6 F	GAGGAGTTGATAGTGGTGG	167	Cucsa.250350.1
WRKY6 R	TTCTTGCTCTGATTTGGTT		
HSP70 F	GTTATTGGGATAGATTTG	104	Cucsa.303940.1
HSP70 R	GAAGGTGTGATACGGTTT		
Dnaj F	CAAGGAAGGAATGGGAGGT	321	Cucsa.282790.1
Dnaj R	TGCTGAATCATAGAGGGGC		
GST F	TTTGAGGAGGTGAAGGTAA	151	Cucsa.106850.1
GST R	ACGCACAAGAAATGTAGAT		
POD F1	GAGTGCTTGTCCAGGAGTTG	146	Cucsa.153430.1
POD R1	AGTTTGAGGTTCCAGTTCTATTG		
CAT F1	CTCCCAAATGTCCTCACCAT	181	Cucsa.077510.1
CAT R1	TGATTCTCTTTCGGTATCACACAC		
cAPX F1	GTTCCTTTCCACCCTGGTAGAG	131	Cucsa.213340.1

cAPX R	ACAATGTCCTGGTCCGAAAG		
MDAR F1	TAAACAACTTTGATGTCAGCATG	145	Cucsa.083200.1
MDAR R	GTGAAGCCTACAGCGACT		
GPX4 F1	CGCTATGGCACAACCACTACT	97	Cucsa.184280.1
GPX4 R1	AGTTCAGGTACATTACAAGCTCAC		
GPX2 F1	CAAAGAAGGGAAGGTAGTTGGTAG	108	Cucsa.094950.1
GPX2 R1	TGGTTCTGACCTTGTTTTCAAG		
GPX F1	GTGGAACTTCTCCAAGTTCTTG	114	Cucsa.303050.1
GPX R1	GCAACTCCCAGCAGTTTCTTC		
SOD F1	TTGTCCATGCAGATCCTGATG	110	Cucsa.133590.1
SOD R1	TCACTCTTGCAGCCCAATAAC		
FRO4 F1	AACAACTTTCTTCTTCTTTGGATC	101	Cucsa.108040.1
FRO4 R1	TATCAGCAGCAGAGATTCATGC		
IRT1 F1	CAACAAACGATACTTGCACAATC	118	Cucsa.236470.1
IRT1 R1	TTCTTCATCCACTTGTATTCTGC		
actin F1	GGAAATACAGTGTCTGGATTGGAG	130	Cucsa.250050.1
actin R1	TGAACTTAGAAGCACTTCCTGTG		

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Table S3. Effect of $CuSO_4$ at different doses (0, 0.21, 2.1 and 10 mg as Cu) on cucumber plant essential nutrients (mg/kg dry weight)

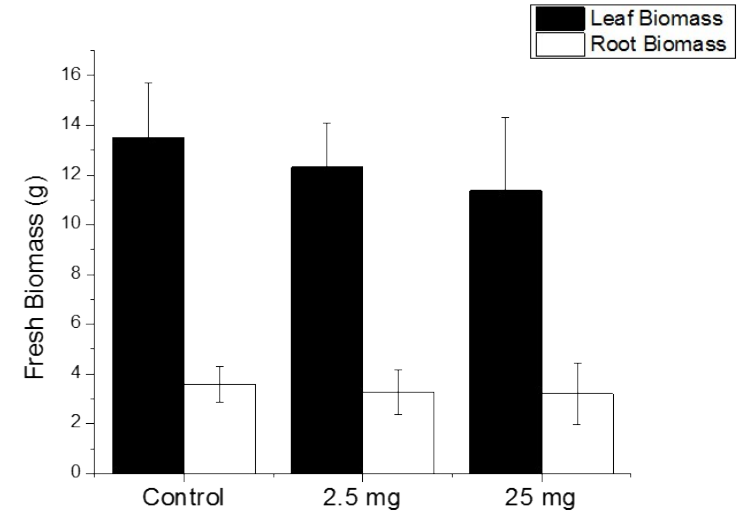
	Na	Mg	P	K	Ca	Mn	Fe	Mo
				Leaf				
Control	910 ± 73	7401 ± 586.7	4007 ± 289	26766 ± 1203	23270 ± 2070	25 ± 3.9	45 ± 3.7	1.2 ± 0.17
0.21 mg	723 ± 161	7710 ± 215	4173 ± 137	28404 ± 1949	23726 ± 1368	27 ± 1.5	53 ± 2.8	1.4 ± 0.56
2.1 mg	689 ± 122**	9721 ± 1094**	4768 ± 390*	29195 ± 2362	28534 ± 2777	29 ± 0.6	61 ± 5.4**	1.3 ± 0.46
10 mg	482 ± 120**	10808 ± 938**	7074 ± 1932**	32499 ± 335**	32166 ± 3340**	33 ± 6.8	62 ± 9.0**	1.6 ± 0.3
				Stem				
Control	6397 ± 486	4619 ± 391	11113 ± 703	85602 ± 6626	11027 ± 1258	9 ± 1.1	18 ± 2.9	0.23 ± 0.02
0.21 mg	7143 ± 1312	4902 ± 269	11288 ± 437	96058 ± 14077	11138 ± 685	8 ± 1.7	22 ± 10	0.24 ± 0.02
2.1 mg	5720 ± 798	4849 ± 955	11457 ± 1285	93825 ± 7820	10414 ± 797	9 ± 1	20 ± 2.3	0.25 ± 0.05
10 mg	4157 ± 566**	4963 ± 818	11305 ± 729	84773 ± 6420	9776 ± 887	8 ± 1.1	24 ± 5.2	0.30 ± 0.06
				Root				
Control	14481 ± 504	14567 ± 594	24152 ± 1552	1E+05 ± 8033	17894 ± 397	27 ± 1.9	242 ± 29	0.38 ± 0.06
0.21 mg	7594 ± 1281	8247 ± 830	13646 ± 938	83663 ± 5820	9136 ± 634	15 ± 1.8	124 ± 22	0.22 ± 0.01
2.1 mg	6614 ± 732	6866 ± 839	12259 ± 630	70790 ± 1884	8381 ± 280	13 ± 1.8	99 ± 24	0.17 ± 0.02
10 mg	4992 ± 522**	5181 ± 326**	10373 ± 489**	55674 ± 3871**	8463 ± 328	13 ± 1.7	187 ± 9.3**	0.20 ± 0.03

All data are mean±SD (n=4). *$P < 0.05$, **$P<0.01$, as compared to the control.

Table S4. Effect of $CuSO_4$ at different doses (0, 0.21, 2.1 and 10 mg as Cu) on cucumber plant nonessential nutrients (mg/kg dry weight)

	Al	Co	Ni	Ti	V	Ag	Pb
			Leaf				
Control	12 ± 4.0	0.028 ± 0.002	0.21 ± 0.04	196 ± 93	0.028 ± 0.010	0.004 ± 0.000	0.072 ± 0.018
0.21 mg	15 ± 2.3	0.031 ± 0.002	0.37 ± 0.17	200 ± 135	0.064 ± 0.068	0.005 ± 0.001	0.086 ± 0.041
2.1 mg	23 ± 8.3*	0.052 ± 0.022*	1.09 ± 1.34	337 ± 124	0.054 ± 0.013	0.008 ± 0.001**	0.065 ± 0.015
10 mg	14 ± 3.4	0.037 ± 0.007*	1.17 ± 0.96	158 ± 20	0.030 ± 0.006	0.021 ± 0.002**	0.062 ± 0.031
			Stem				
Control	6 ± 2.3	0.052 ± 0.007	0.15 ± 0.04	131 ± 34	0.013 ± 0.003	0.003 ± 0.001	0.031 ± 0.013
0.21 mg	10 ± 8.6	0.053 ± 0.010	0.14 ± 0.03	223 ± 171	0.017 ± 0.012	0.003 ± 0.001	0.030 ± 0.005
2.1 mg	6 ± 0.5	0.052 ± 0.006	0.24 ± 0.15	150 ± 33	0.015 ± 0.002	0.003 ± 0.000	0.030 ± 0.004
10 mg	11 ± 9.4	0.044 ± 0.005	0.89 ± 1.39	192 ± 114	0.026 ± 0.024	0.004 ± 0.000	0.038 ± 0.025
			Root				
Control	148 ± 34	0.316 ± 0.037	1.55 ± 0.84	1286 ± 373	0.30 ± 0.085	0.017 ± 0.008	2.413 ± 2.413
0.21 mg	137 ± 23	0.313 ± 0.046	1.07 ± 0.1	1344 ± 307	0.25 ± 0.029	0.016 ± 0.003	1.032 ± 1.032
2.1 mg	110 ± 31	0.289 ± 0.029	0.99 ± 0.09	1088 ± 427	0.19 ± 0.030	0.016 ± 0.003	1.170 ± 1.170
10 mg	229 ± 19**	0.286 ± 0.044	4.44 ± 6.36	2136 ± 205**	0.44 ± 0.052**	0.017 ± 0.006	1.757 ± 1.757

All data are mean±SD (n=4). *P < 0.05, **P<0.01, as compared to the control.



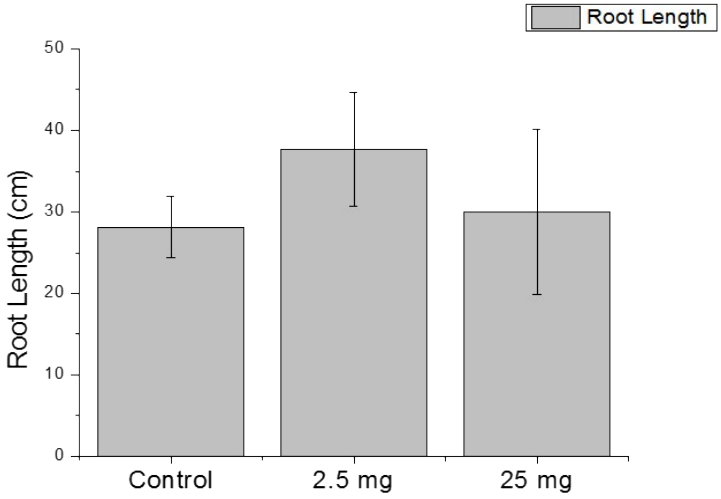


Figure S1. Effects of different doses of $Cu(OH)_2$ nanopesticides (0, 2.5 and 25 mg Cu) on cucumber seedlings biomass (**A**) and root length (**B**). The data are the means of six replicates. Error bars represent standard deviation. There is no significant difference between control and $Cu(OH)_2$ nanopesticide treated plants (p<0.05).

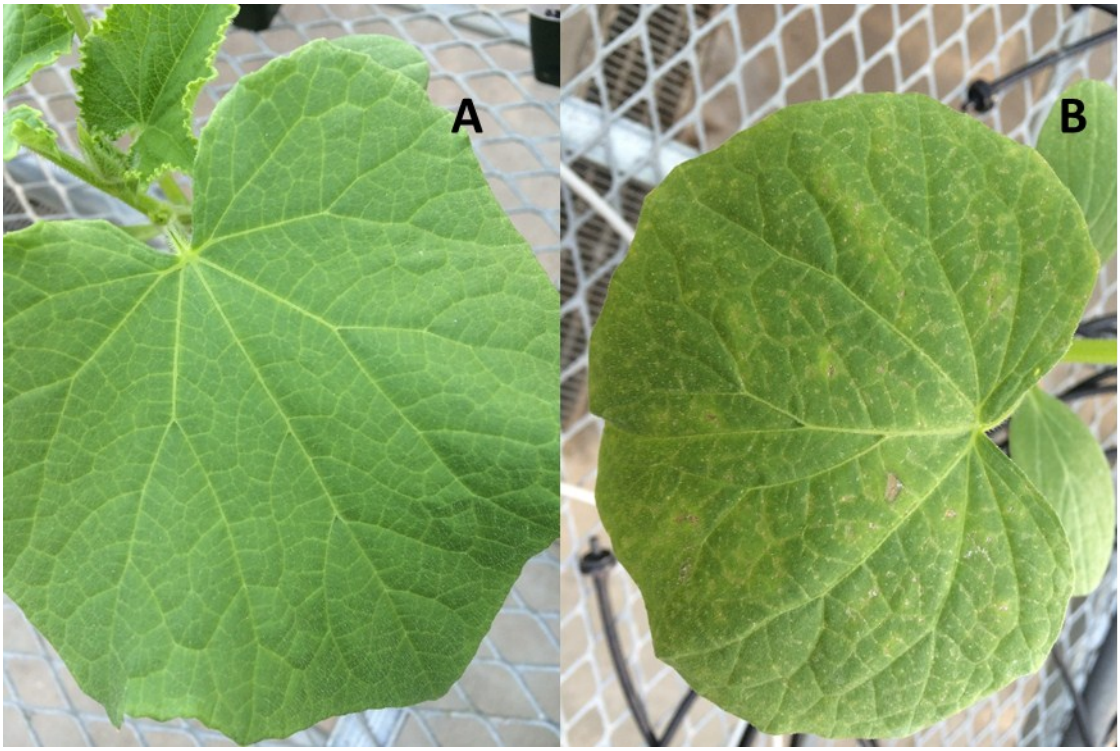
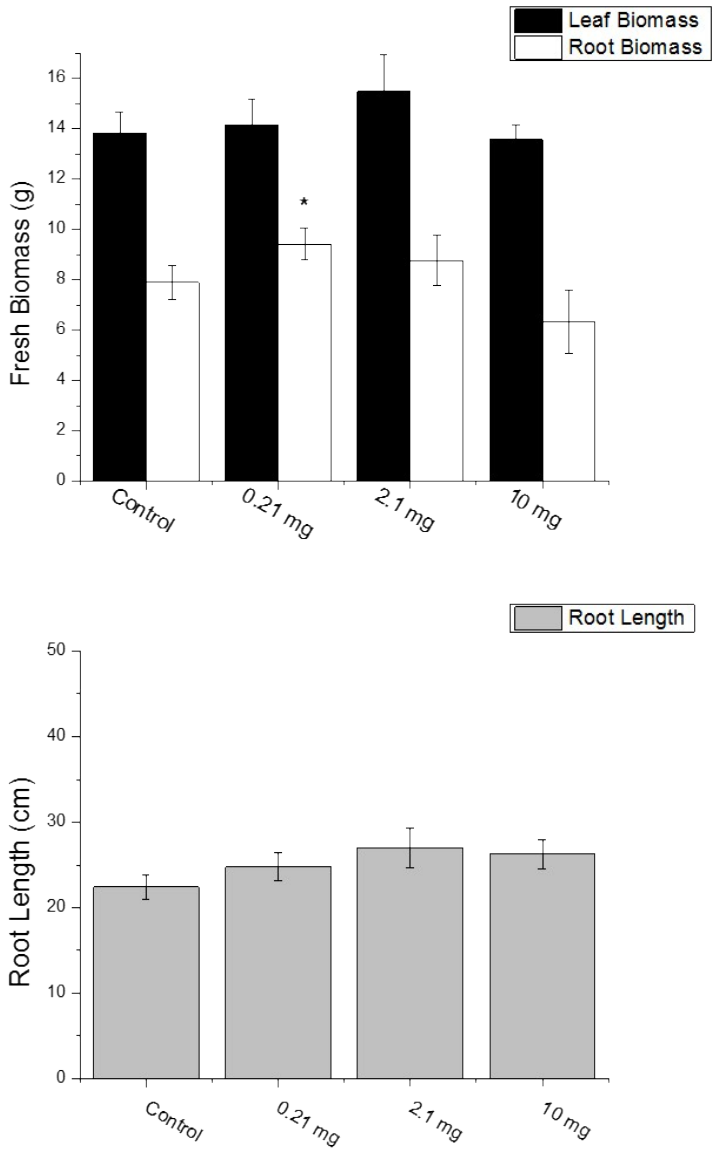


Figure S2. Image of 4-weeks cucumber leaves treated with (**A**) water without $CuSO_4$ and (**B**) 10 mg Cu



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74 **Figure S3.** Effects of different doses of $CuSO_4$ (0, 0.21, 2.1 and 10 mg) on cucumber

75 seedlings biomass (**A**) and root length (**B**). The data are the means of four replicates.

76 Error bars represent standard deviation. *$P < 0.05$ as compared to the control.

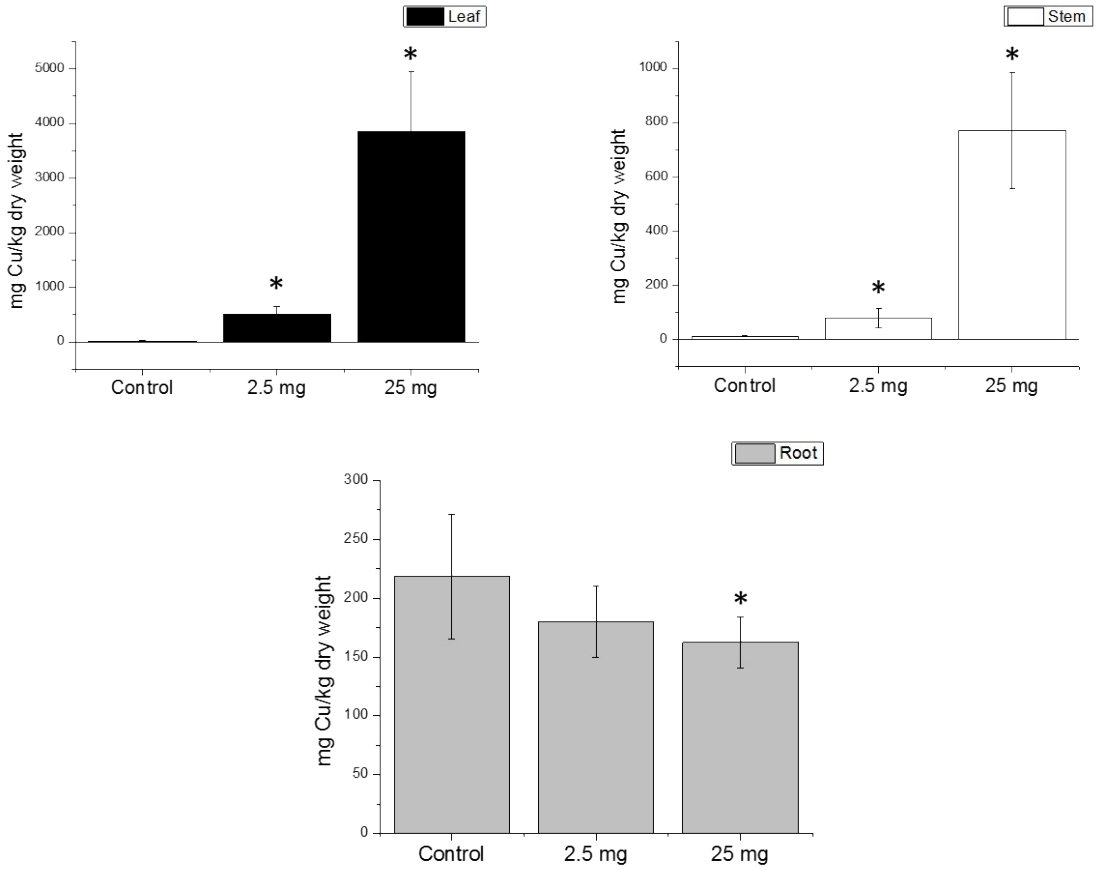


Figure S4. Cu bioaccumulation in leaf, stem and root of cucumber plants after foliar exposure to 0, 2.5 and 25 mg Cu as $Cu(OH)_2$ nanopesticide for one week. The data are the means of six replicates. Error bars represent standard deviation. *$P \leq 0.05$ as compared to the control.

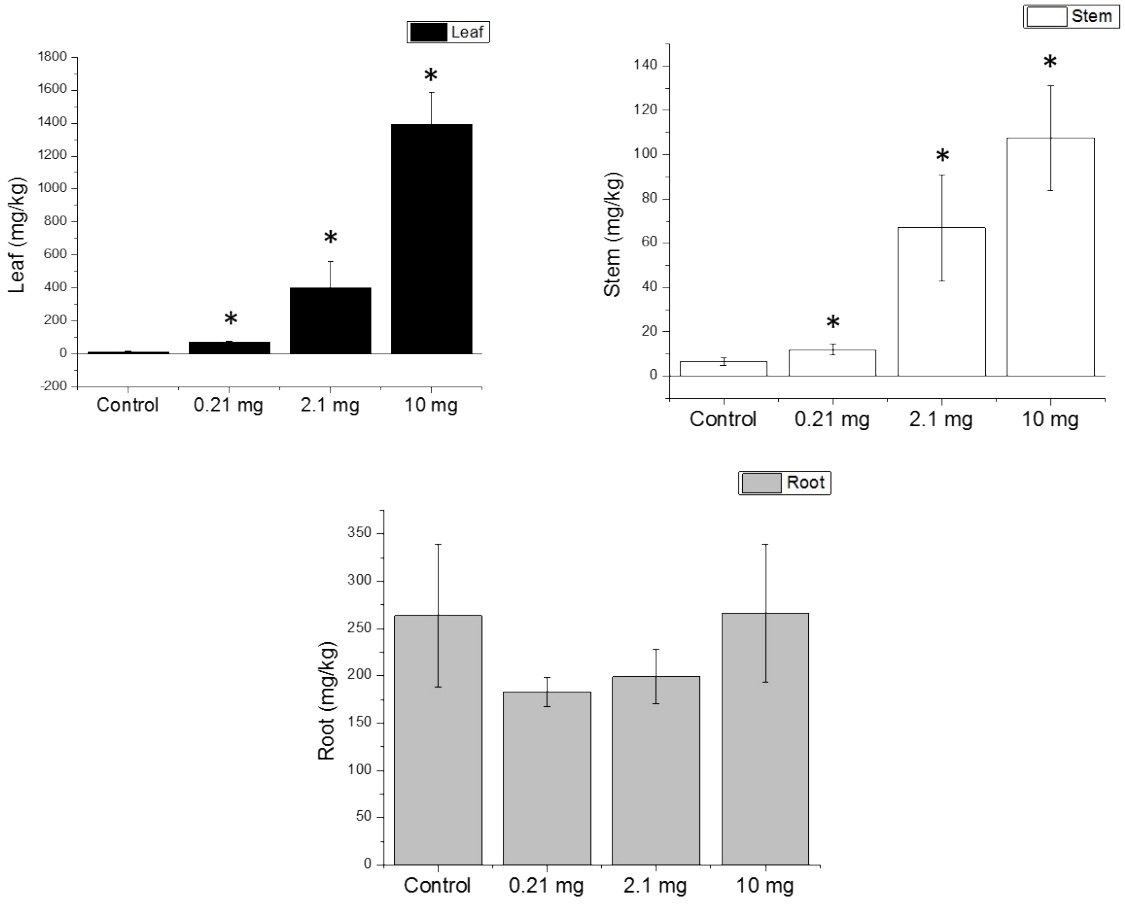


Figure S5. Cu bioaccumulation in leaf, stem and root of cucumber plants after foliar exposure to 0, 0.21, 2.1 and 10 mg Cu as $CuSO_4$ for 1 week. The data are the means of four replicates. Error bars represent standard deviation. *$P < 0.05$ as compared to the control.

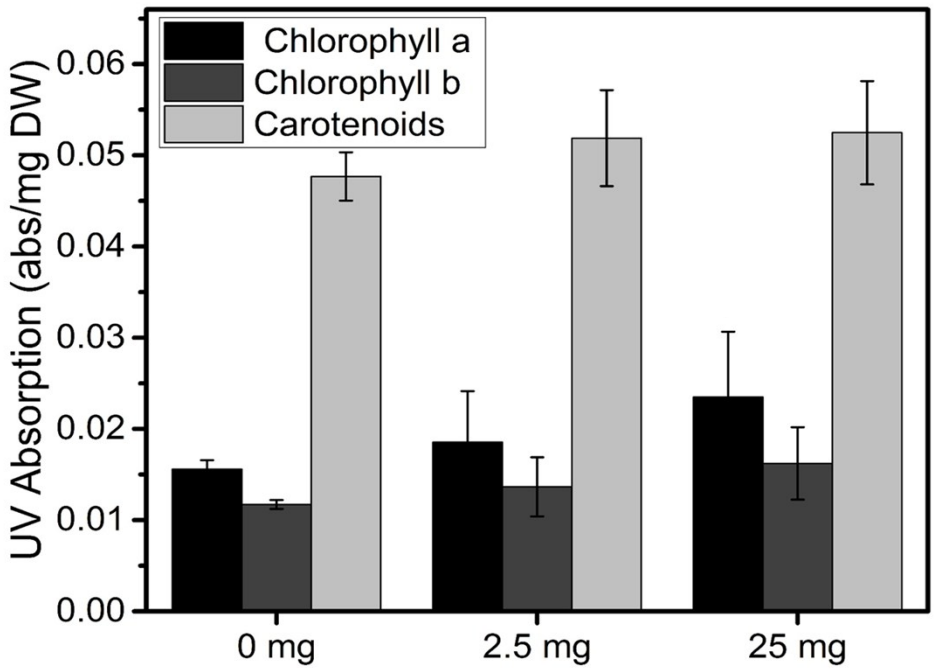


Figure **S6**. Effects of different doses of $Cu(OH)_2$ nanopesticide (0, 2.5 and 25 mg as Cu) on photosynthetic pigment content. The data are the means of six replicates. Error bars represent standard deviation.

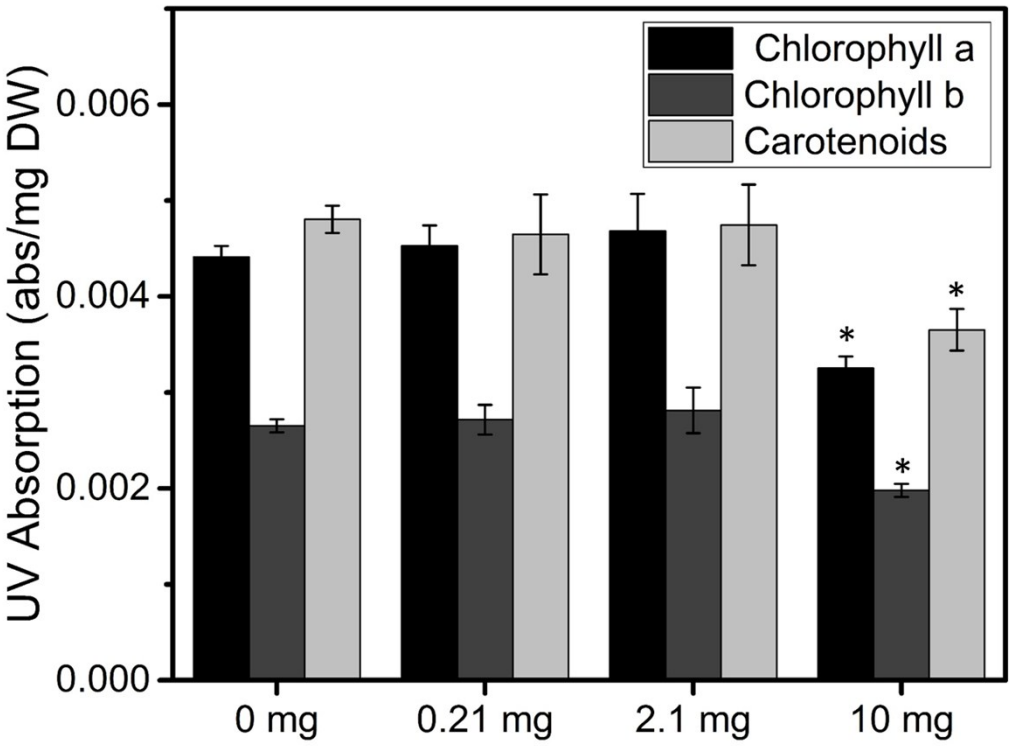


Figure S7. Effects on photosynthetic pigment content of different dose levels of $CuSO_4$ applied (0, 0.21, 2.1 and 10 mg Cu). The data are the means of four replicates. Error bars represent standard deviation. *$P < 0.05$ as compared to the control.

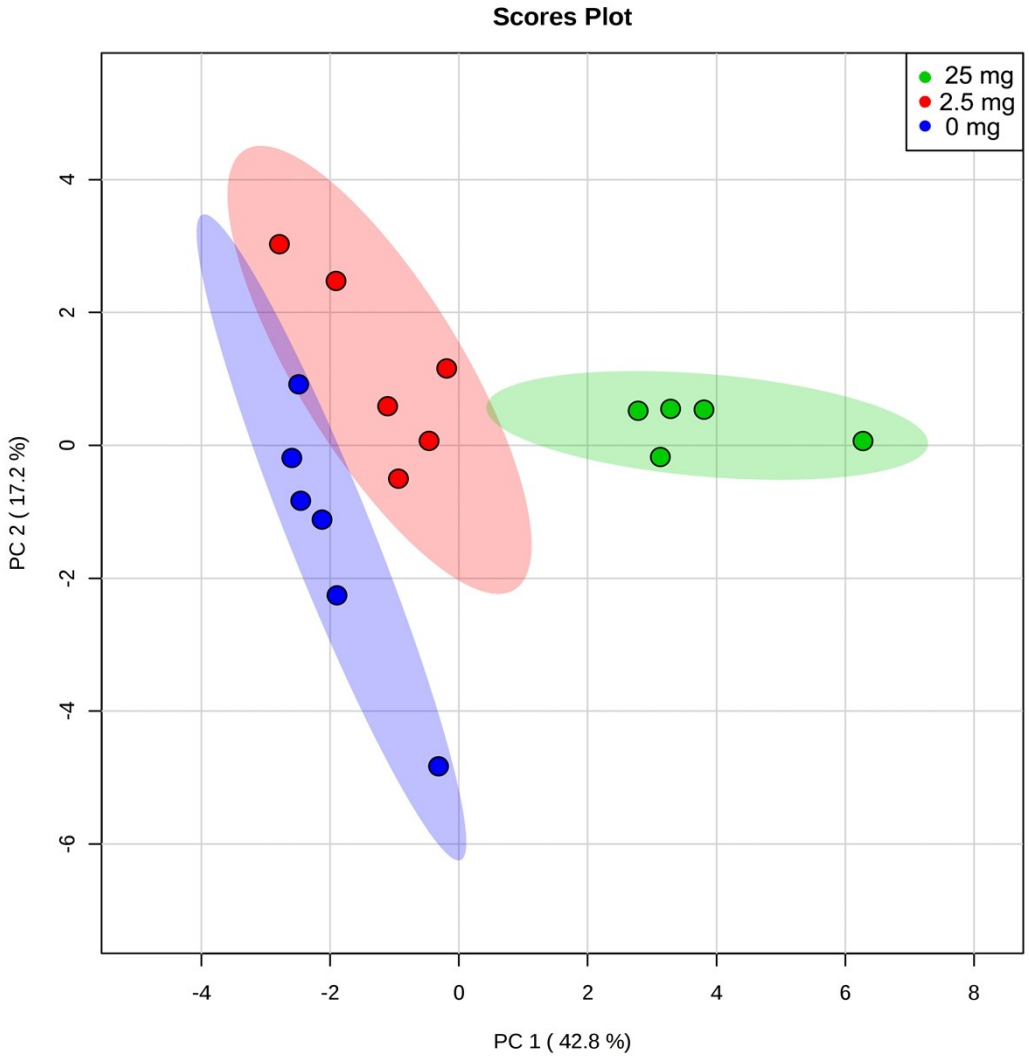
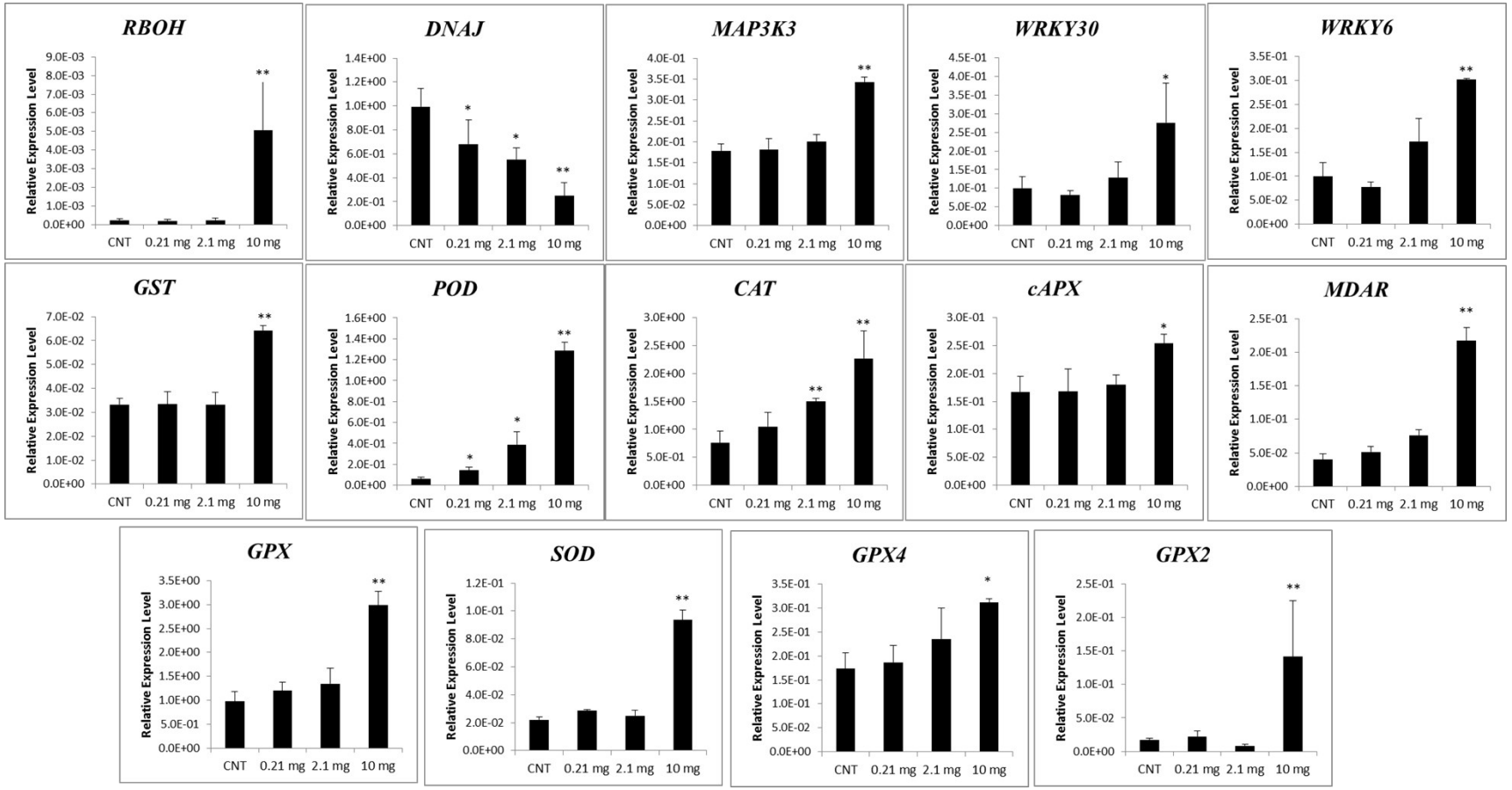


Figure S8. Principal component analysis (PCA) analysis of gene expression in cucumber leaves exposed to different doses of $Cu(OH)_2$ nanopesticides (0, 2.5 and 25 mg Cu). This data set is based on the expression of 18 genes in cucumber leaves analyzed by QPCR.



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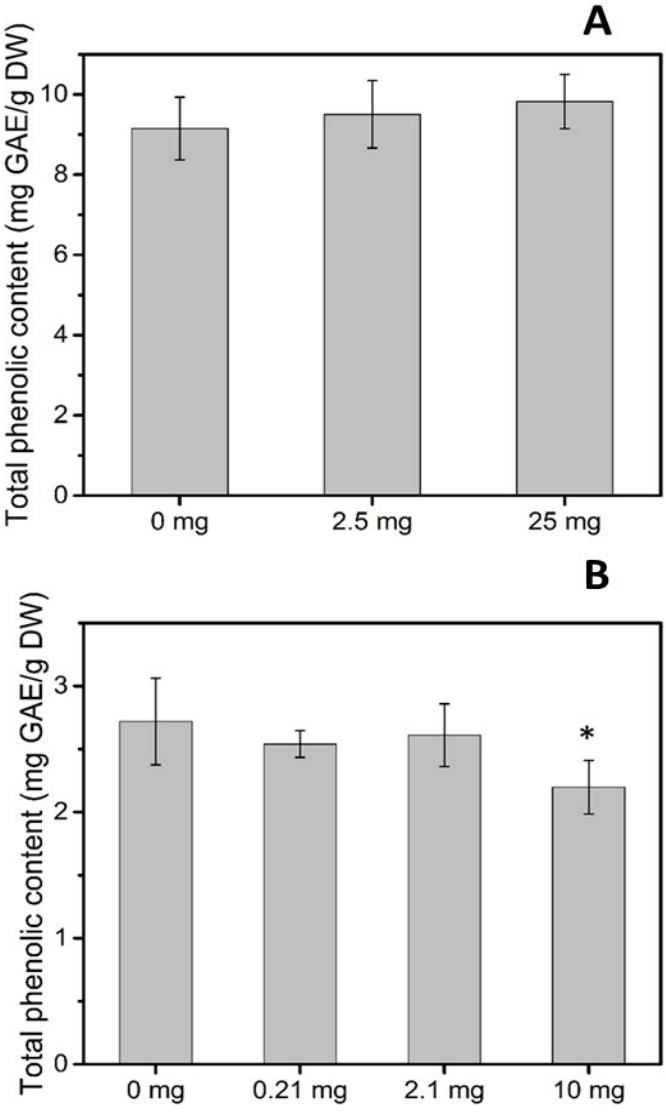
130 **Figure S9.** Expression levels of 14 significantly changed genes in cucumber leaves after foliar application of CuSO4 for 1 week at

131 different doses (0, 0.21, 2.1 and 10 mg as Cu). The data are the means of four replicates. Error bars represent standard deviation. *$P <$

132 0.05, **$P < 0.01$, as compared to the control.



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134 **Figure S10.** Effects of $Cu(OH)_2$ nanopesticide (**A**) and $CuSO_4$ (**B**) on total phenolics

135 content in cucumber leaves. The data are the means of six and four replicates in A and B

136 respectively. Error bars represent standard deviation. *$P < 0.05$ as compared to the

137 control.

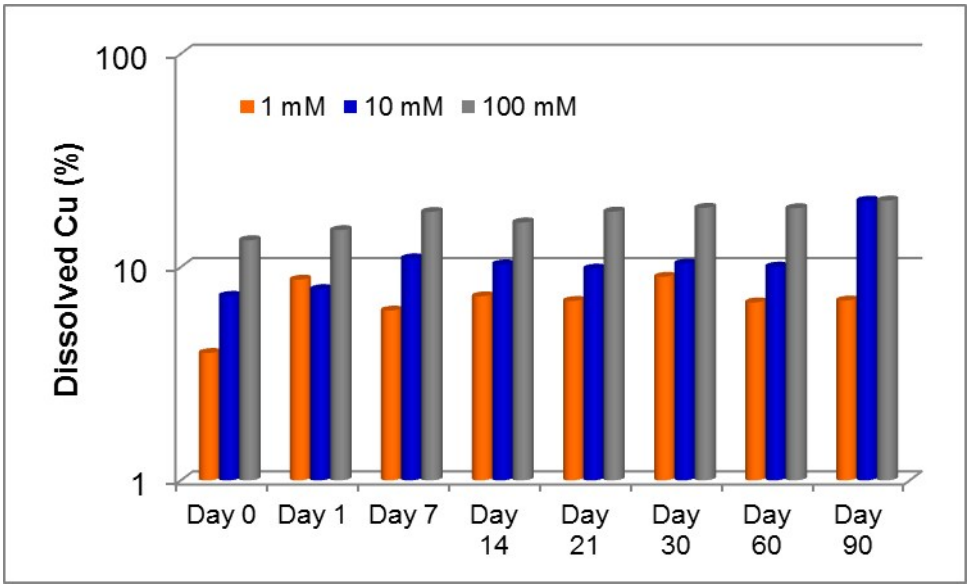

Figure S11. Dissolution kinetics of $Cu(OH)_2$ nanopesticide at pH 7 and 1, 10, and 100 mM ionic strength (Source: Adeleye et al., 2014)

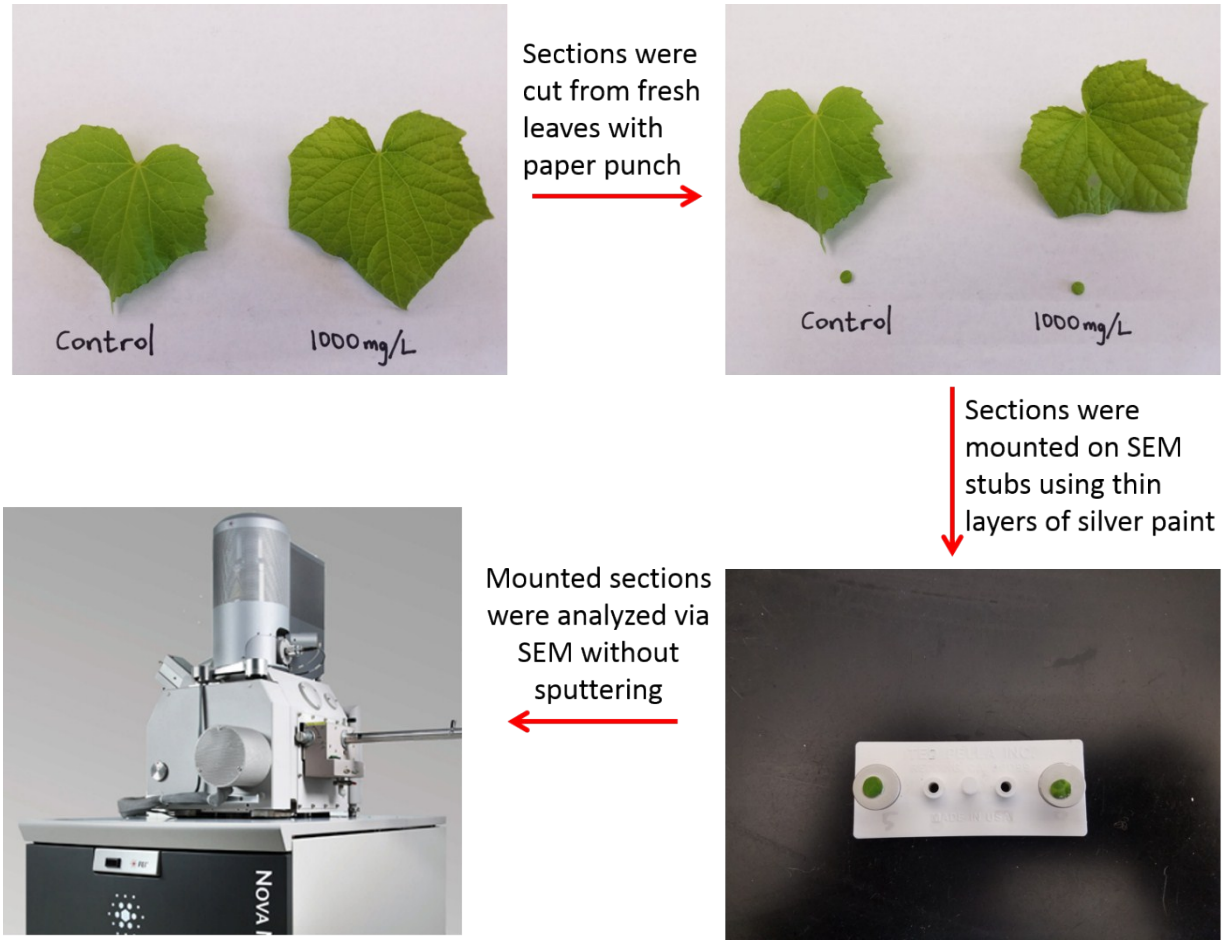

Figure S12. Sample preparation for scanning electron microscopy of cucumber leaves with or without 1000 mg/L $Cu(OH)_2$ nanopesticide