Enhanced aryltetralin lignans production in *Linum* adventitious root cultures

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Supplementary Materials:

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1. TLC plate of lignans extracts after reaction with DPPH (Figure S1)

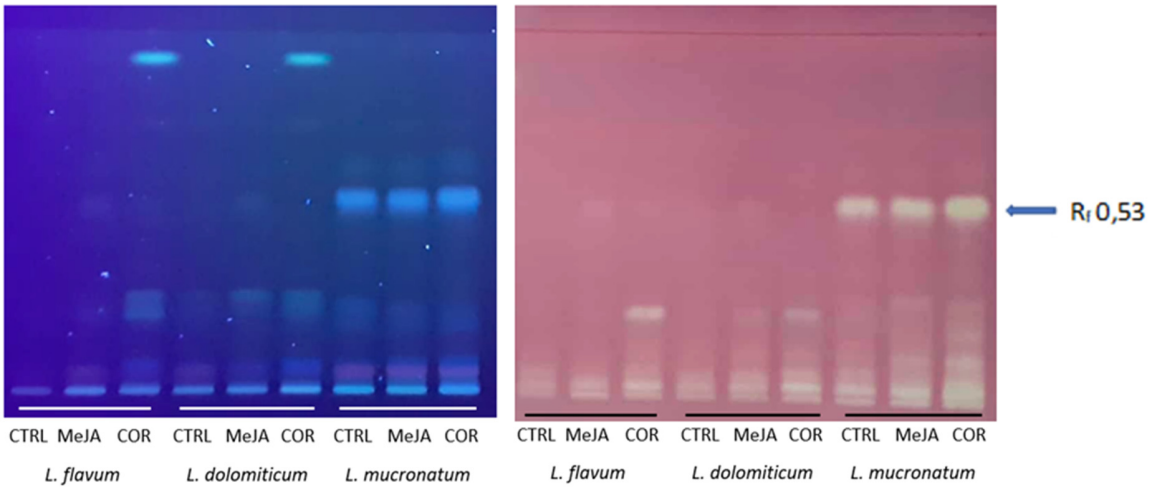


Figure S1. TLC plate of lignans extracts of the *Linum* species at 366 nm (left), and after reaction with DPPH (right).

2. HPLC chromatogram (Figure S2)

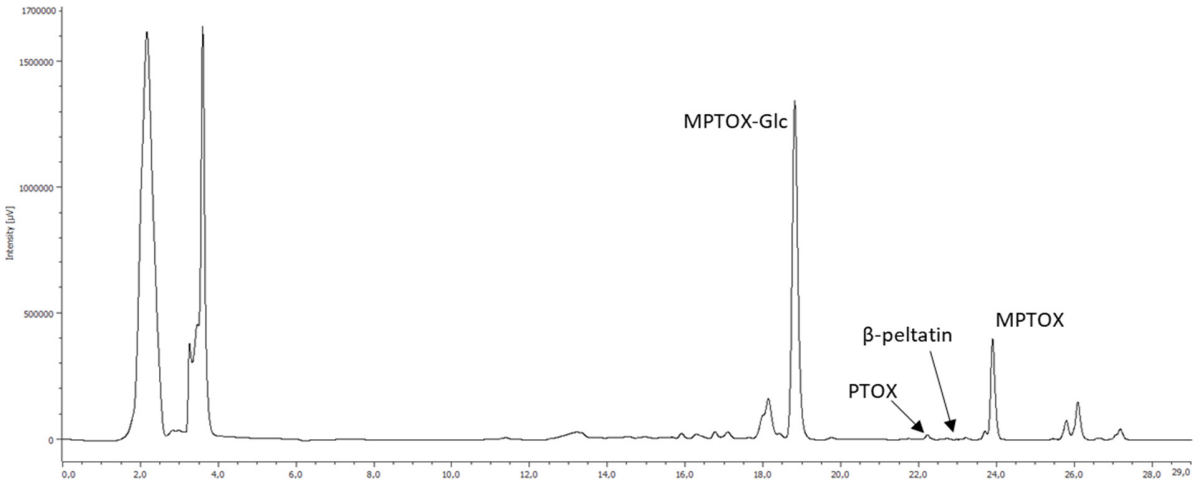


Figure S2 HPLC chromatograms from *L. dolomiticum* COR-treated extract. MPTOX—Glc (Rt 18.8 min), PTOX (Rt 22.2 min) and MPTOX (Rt 23.9 min) were identified. The peak corresponding to β-peltatin was no found.

3. NMR spectra (Figures S3, S4)

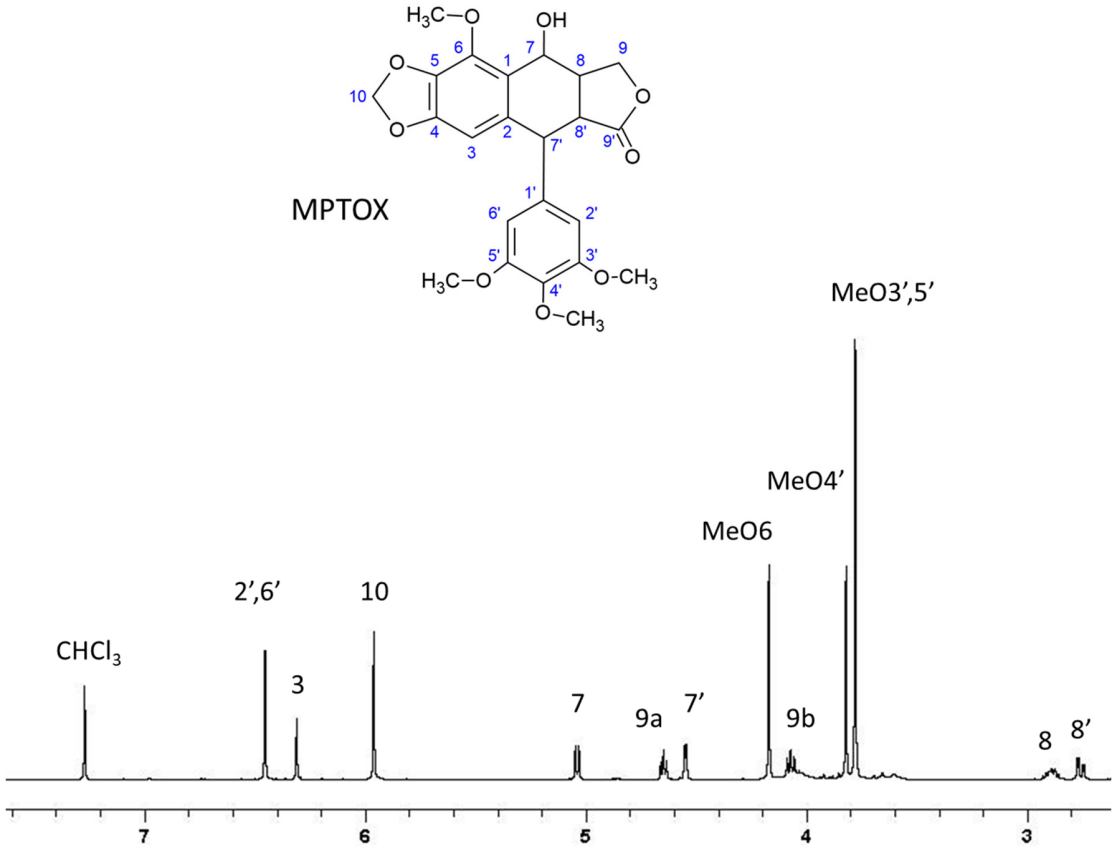


Figure S3 [1]H NMR spectra obtained from *L. flavum* sample with signals corresponding to 6-methoxypodophyllotoxin

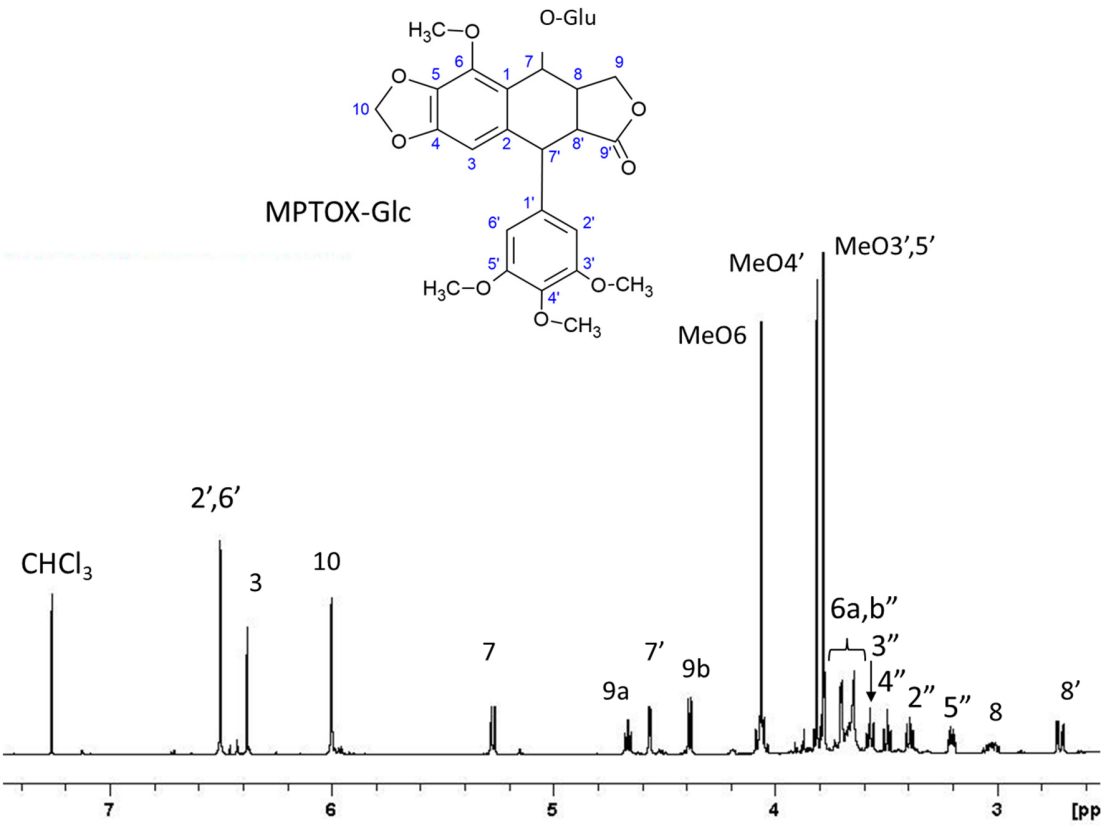


Figure S4 [1]H NMR spectra obtained from *L. flavum* sample with signals corresponding to 6-methoxypodophyllotoxin—7-O-β-glucoside

4. Statistical Report

Statistical analyses were performed using R, library rstatix, tidyverse, agricolae, corrr.

Two-way ANOVA of growth, phenols, flavonoids and antioxidant capacity

Residual analysis was performed to test for the assumptions of the two-way ANOVA. Outliers were assessed by box plot method; normality was assessed using Shapiro-Wilk's normality test and homogeneity of variances was assessed by Levene's test. There were no extreme outliers, residuals were normally distributed ($p > 0.05$) and there was homogeneity of variances ($p > 0.05$).

A two-way ANOVA was conducted to examine the effects of species and elicitor treatments on growth, accumulation of phenols, flavonoids and antioxidant capacity, as showed in **Table S1**.

Table S1 Two-way ANOVA of growth, phenols, flavonoids and antioxidant capacity

Growth					
Effect	DFn	DFd	F	p	η_g^2
Species	2	24	115.212	4.96e-13	0.906
Treatment	3	24	81.498	1.00e-12	0.911
Species x Treatment	6	24	18.200	7.41e-08	0.820
Phenols					
Effect	DFn	DFd	F	p	η_g^2
Species	2	24	112.748	6.28e-13	0.904
Treatment	3	24	402.706	1.19e-20	0.981
Species x Treatment	6	24	25.553	2.63e-09	0.865
Flavonoids					
Effect	DFn	DFd	F	p	η_g^2
Species	2	24	205.901	7.78e-16	0.945
Treatment	3	24	29.541	3.17e-08	0.787
Species x Treatment	6	24	5.128	2.00e-03	0.562
Antioxidant capacity					
Effect	DFn	DFd	F	p	η_g^2
Species	2	24	248.048	9.32e-17	0.954
Treatment	3	24	56.403	5.12e-11	0.876
Species x Treatment	6	24	8.863	3.79e-05	0.689

DFn, degrees of freedom in the numerator; DFd, degrees of freedom in the denominator; F, F-value; p, p-value; η_g^2, generalized eta squared.

4.1 Pairwise comparison of growth, phenols, flavonoids and antioxidant capacity

Tukey HSD post hoc tests were carried out. All pairwise comparisons were analyzed between the different species groups organized by elicitor treatments; the results are showed in **Figure S5** for growth, **Figure S6** for phenols, **Figure S7** for flavonoids and **Figure S8** for antioxidant capacity. Duncan test was performed considering $p < 0.05$.

Figure S5 Tukey HSD post hoc test for growth

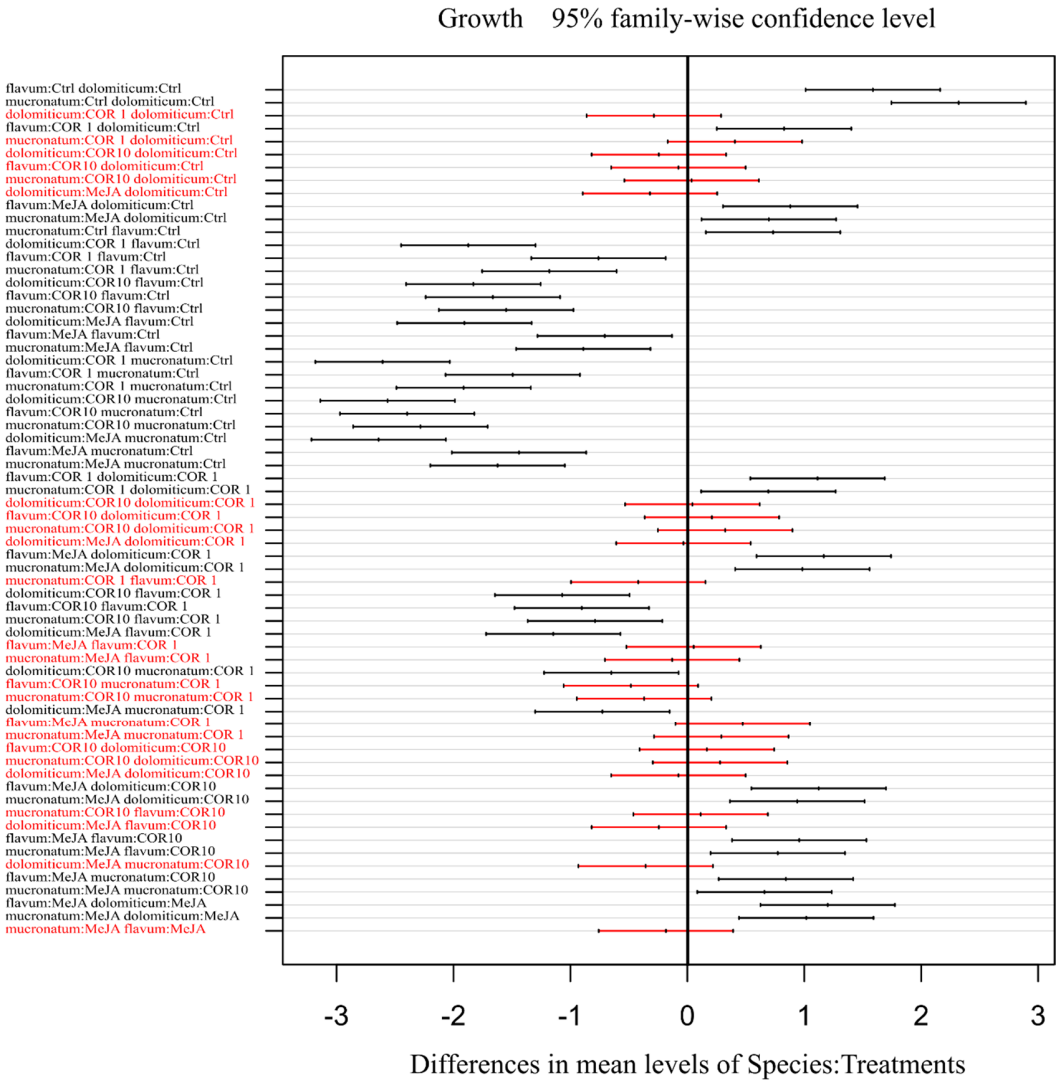


Figure S6 Tukey HSD post hoc test for phenols

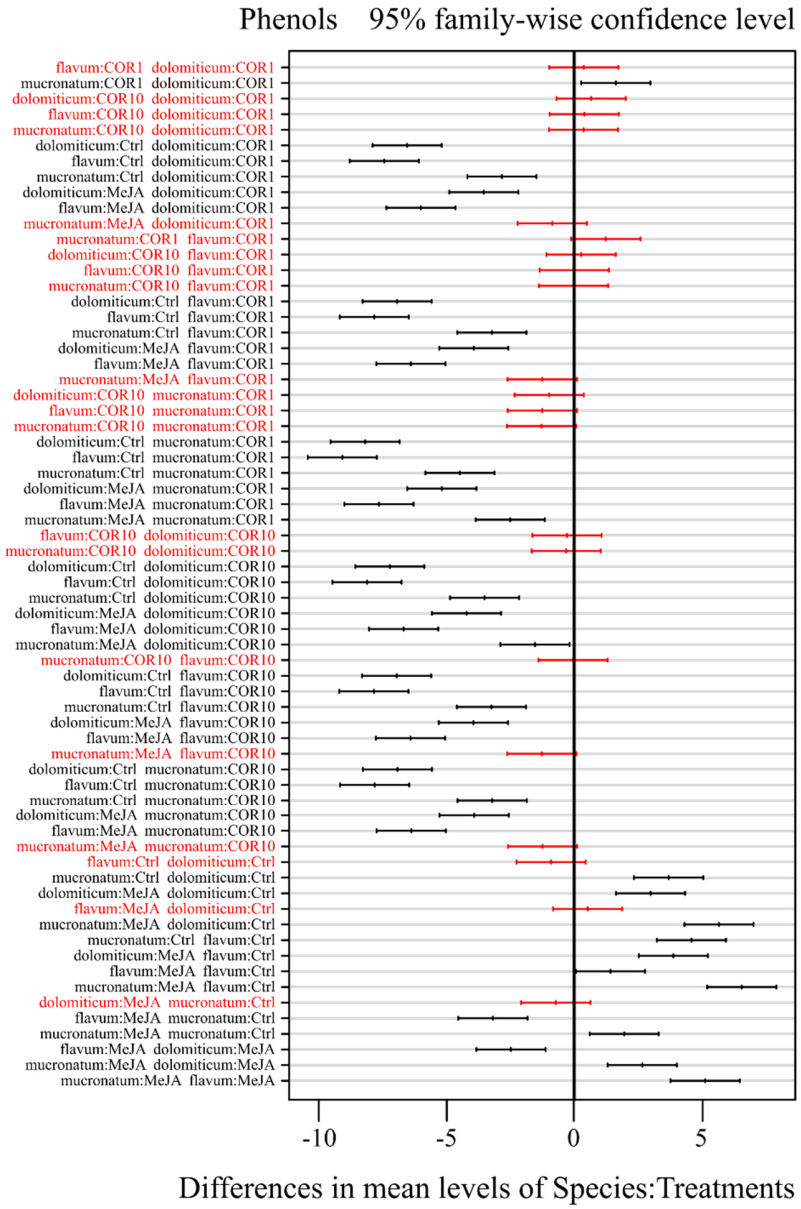


Figure S7 Tukey HSD post hoc test for flavonoids

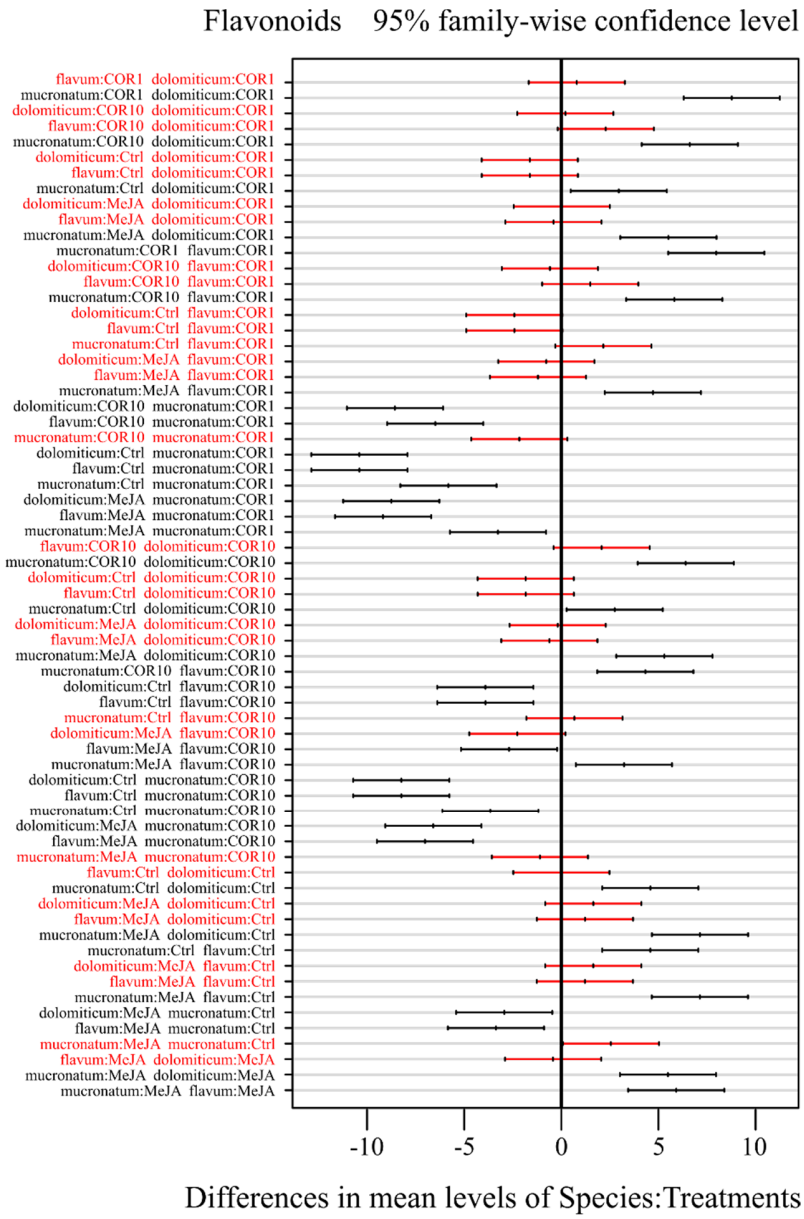
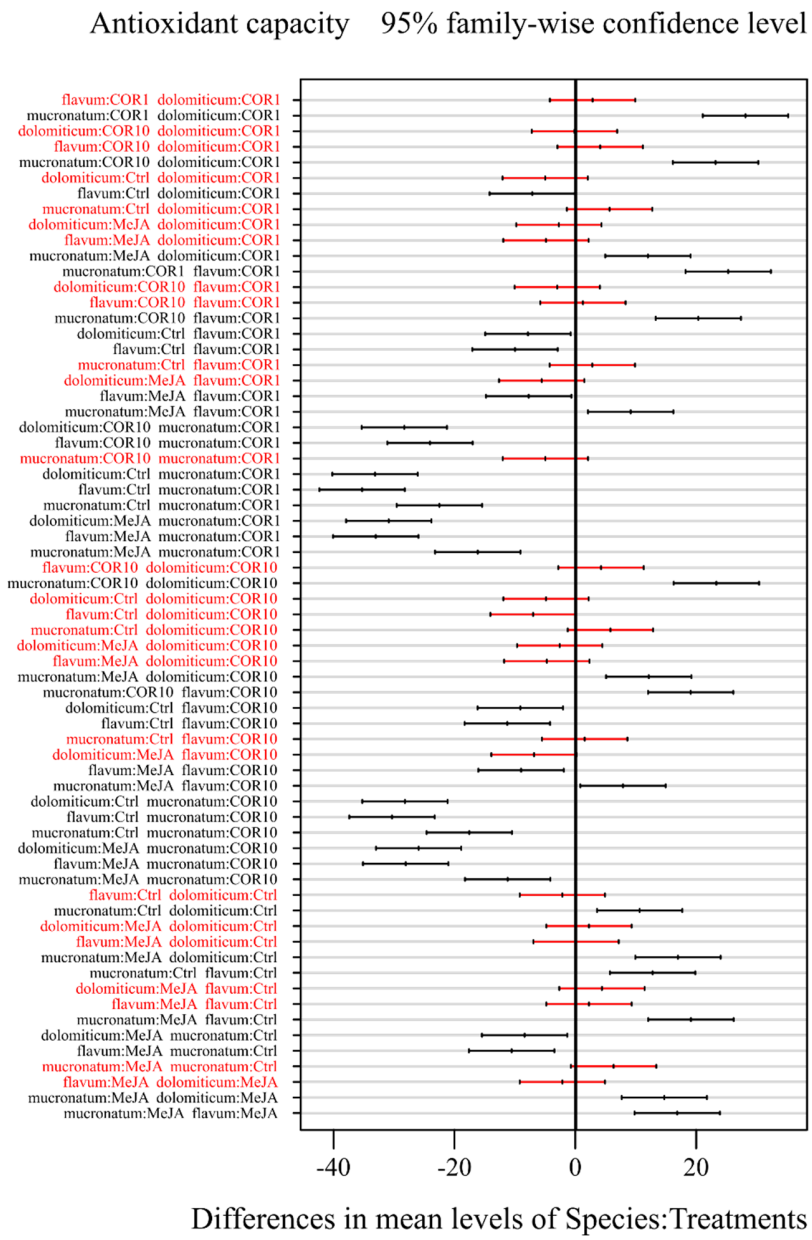


Figure S8 Tukey HSD post hoc test for antioxidant capacity



4.2 Two-way ANOVA on ATLs

Residual analysis was performed to test for the assumptions of the two-way ANOVA. Outliers were assessed by box plot method; normality was assessed using Shapiro-Wilk's normality test and homogeneity of variances was assessed by Levene's test. There were no extreme outliers,

residuals were normally distributed ($p > 0.05$) and there was homogeneity of variances ($p > 0.05$).

A two-way ANOVA was conducted to examine the effects of species and elicitor treatments on accumulation of PTOX, MPTOX and MPTOX—Glc, as showed in **Table S2.**

Table S2 Two-way ANOVA on ATLs

PTOX					
Effect	DFn	DFd	F	p	η_g^2
Species	1	16	55.553	1.37e-06	0.776
Treatment	3	16	138.832	1.15e-11	0.963
Species x Treatment	3	16	185.703	1.22e-12	0.972
MPTOX					
Effect	DFn	DFd	F	p	η_g^2
Species	2	24	61.943	3.34e-10	0.838
Treatment	3	24	25.125	1.40e-07	0.758
Species x Treatment	6	24	13.995	8.37e-07	0.778
MPTOX-Glc					
Effect	DFn	DFd	F	p	η_g^2
Species	2	24	335.469	2.88e-18	0.965
Treatment	3	24	2.682	6.90e-02, ns	0.251
Species x Treatment	6	24	7.655	1.14e-04	0.657

DFn, degrees of freedom in the numerator; DFd, degrees of freedom in the denominator; F, F-value; p, p-value; η_g^2, generalized eta squared

4.3 Pairwise comparison on ATLs

Tukey HSD post hoc tests were carried out. All pairwise comparisons were analyzed between the different species groups organized by elicitor treatments; the results are summarized in **Figure S9** for PTOX, **Figure S10** for MPTOX and **Figure S11** for MPTOX—Glc. Duncan test was performed considering $p < 0.05$.

Figure S9 Tukey HSD post hoc test for PTOX

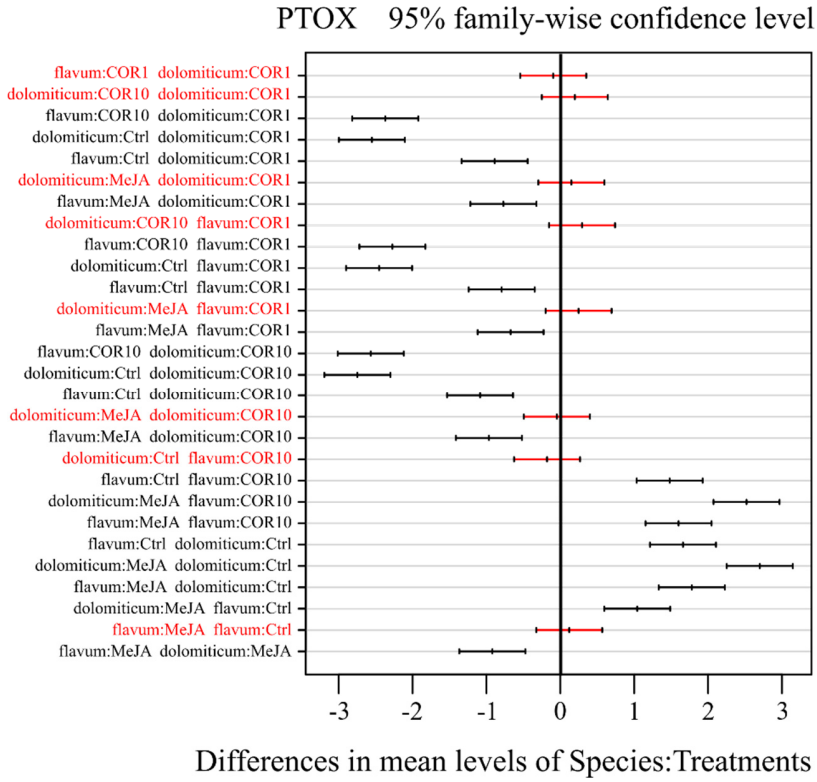


Figure S10 Tukey HSD post hoc test for MPTOX

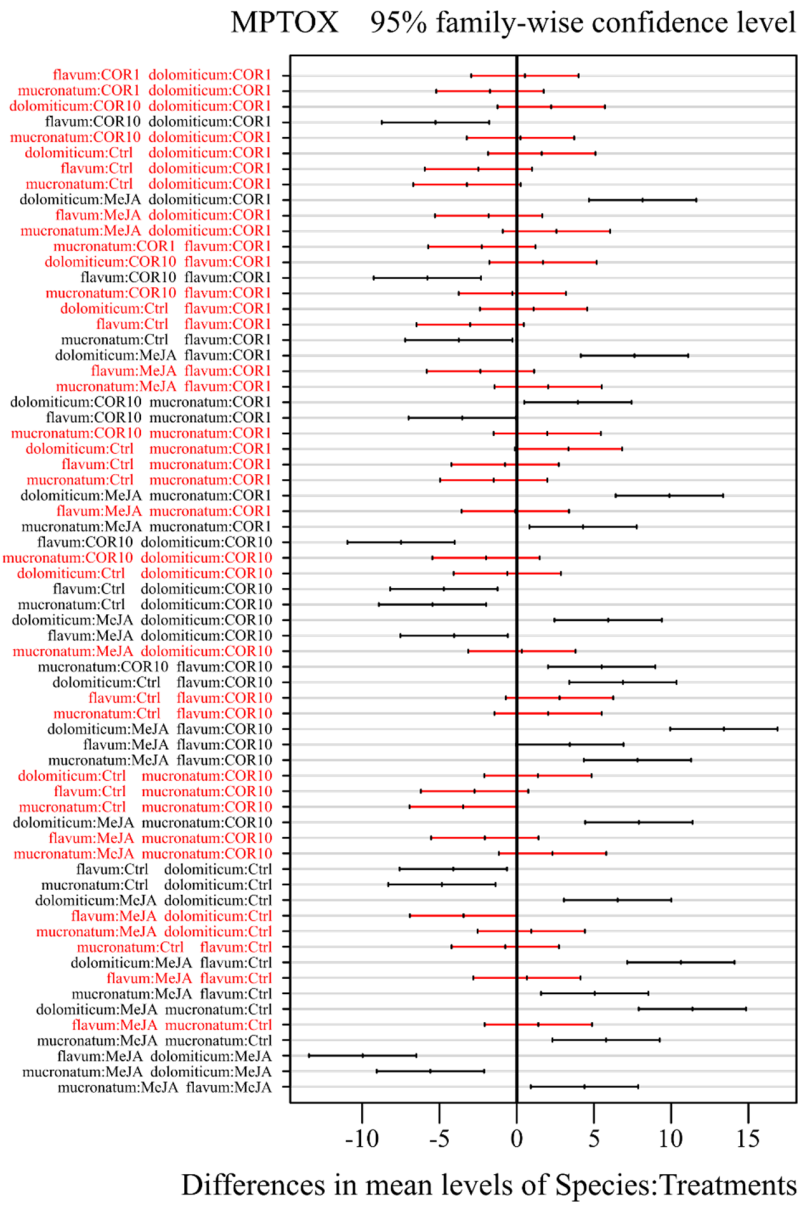
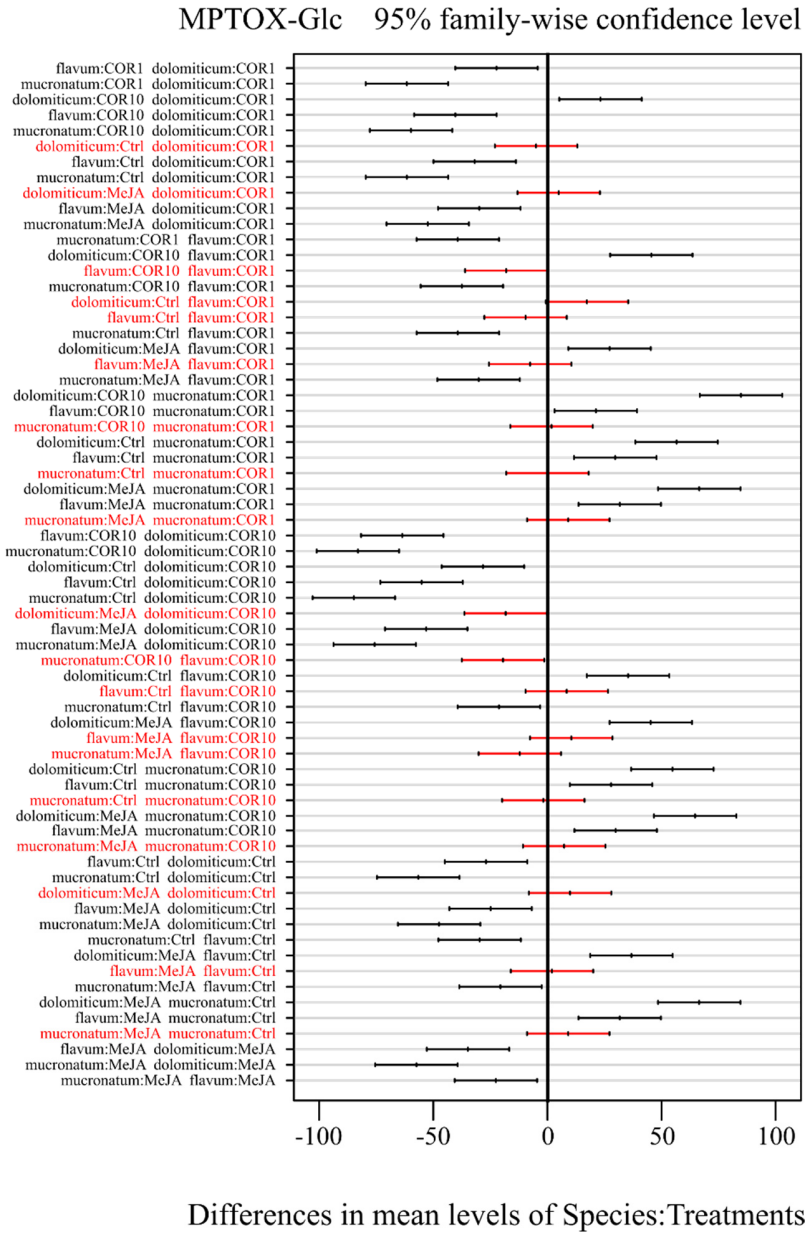


Figure S11 Tukey HSD post hoc test for MPTOX-Glc



4.4 Pearson correlation

Figure S12 Correlation graphs for control (n = 9), MeJA (n = 9), COR 1µM (n = 9) and COR 10µM treated samples (n = 9). *, p ≤ 0.05; **, p ≤ 0.01; ***, p ≤ 0.001