Supporting Information

Bio-based Lignin Nanocarriers Loaded with Fungicides as Versatile Platform for Drug Delivery in Plants

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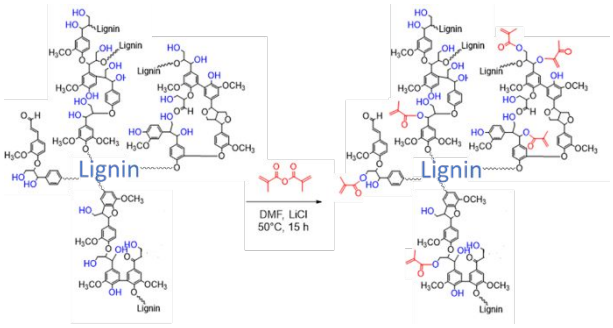
Scheme S1. Scheme of the Kraft lignin modification into lignin-methacrylate.



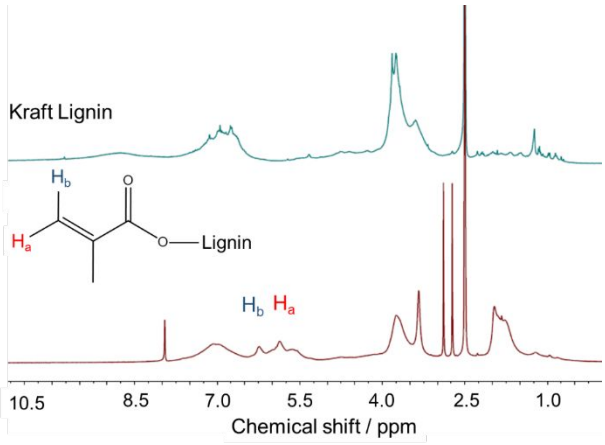


Figure S1. ^{1}H NMR spectrum of Kraft lignin and lignin methacrylate. The hydrogens relative to the double bond are shown in the figure.

Determination of the hydroxyl groups in lignin. The samples for the ^{31}P-NMR spectra were prepared following the method of Balakshin et al.[1]. Herein, lignin's hydroxyl groups are first fully converted with 2-chloro-4,4,5,5-tetramethyl- 1,3,2-dioxaphospholane and quantified afterward using the phosphorylated endo-N-hydroxy- 5-norbornene-2,3-dicarboximide as an internal standard. Lignin's hydroxyl groups are calculated as follows:

$$c(OH-lignin) = \frac{n(std.) \times Integral\ (150-137\ ppm)}{m(lignin)} \left[\frac{mmol}{g}\right] \qquad \text{(S1)}$$

Table S1. Number average and weight average molecular weights, M_n and M_w respectively, and molecular weight dispersity ($Đ$) from Kraft lignin and lignin-MA measured by GPC (vs. PS standards).

Entry	M_n / kDa	M_w / kDa	$Đ$
Kraft Lignin	1.9	3.1	1.6
Lignin-MA	6.8	25.4	3.7

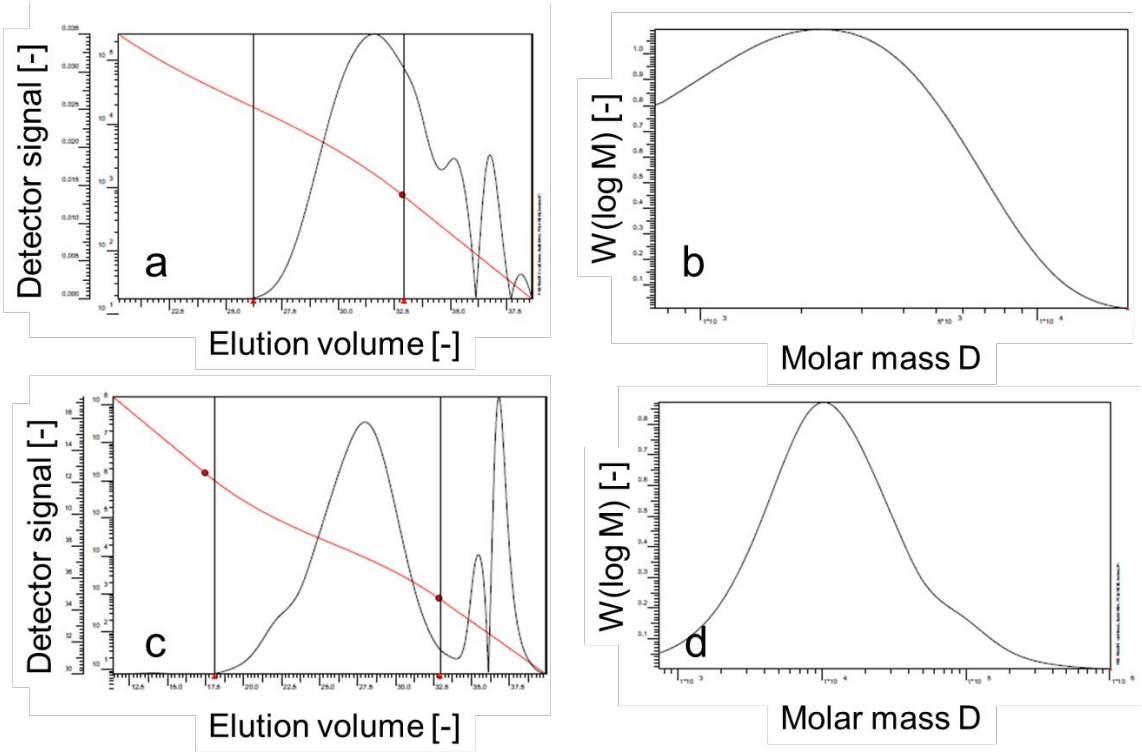


Figure S2. Molecular weight distributions raw data: Detector signal vs. elution for Kraft lignin (a) and lignin-MA (c), W(log M) vs. molar mass for Kraft lignin (b) and lignin-MA (d). The curve in red is the calibration curve from PS standards.

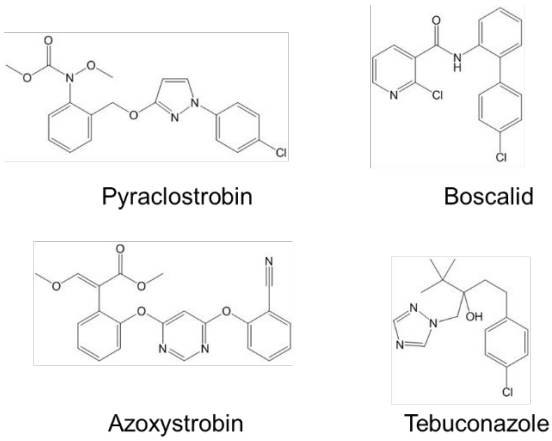


Figure S3. Chemical structures of the drugs: pyraclostrobin, boscalid, azoxystrobin and tebuconazole.

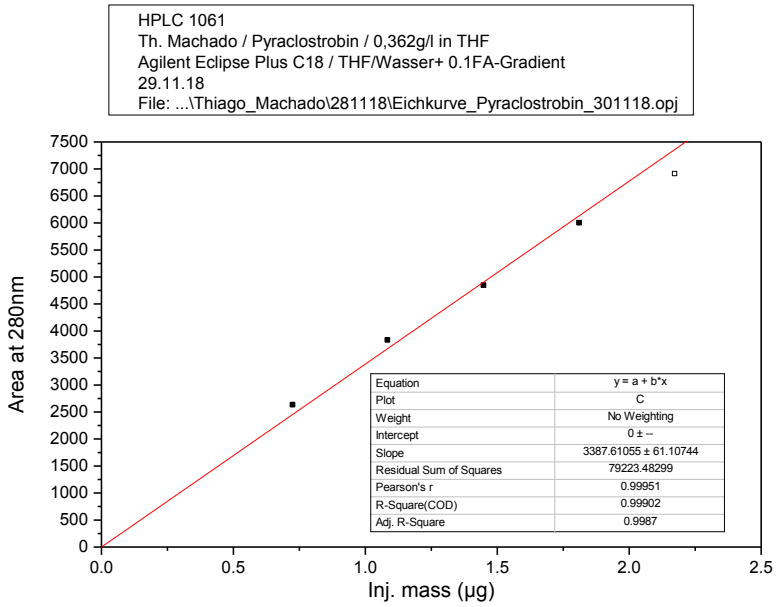


Figure S4. HPLC calibration curve for pyraclostrobin.

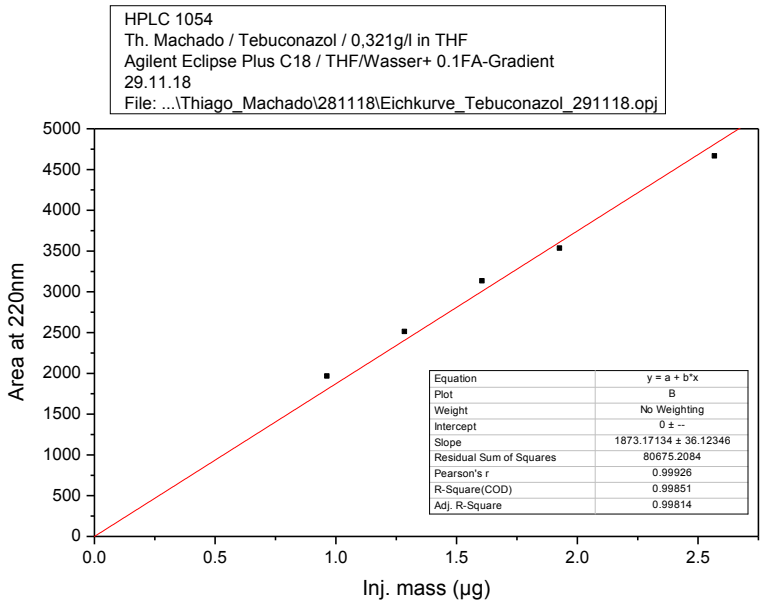


Figure S5. HPLC calibration curve for tebuconazole.

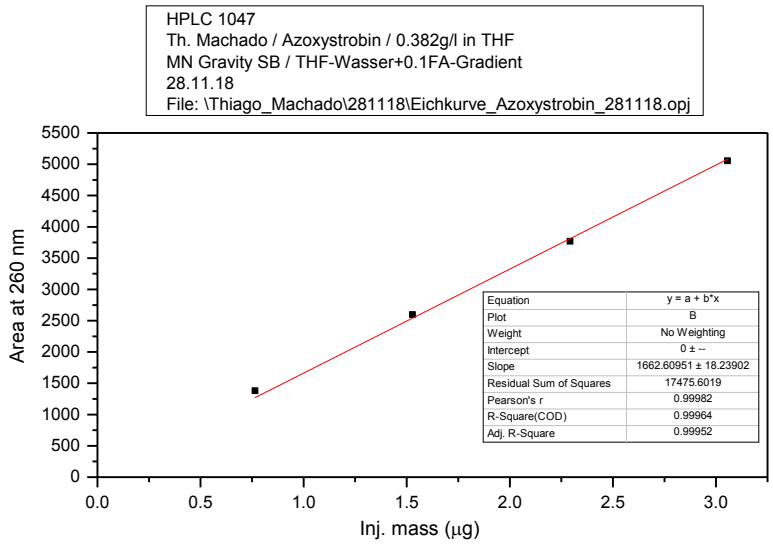


Figure S6. HPLC calibration curve for azoxystrobin.

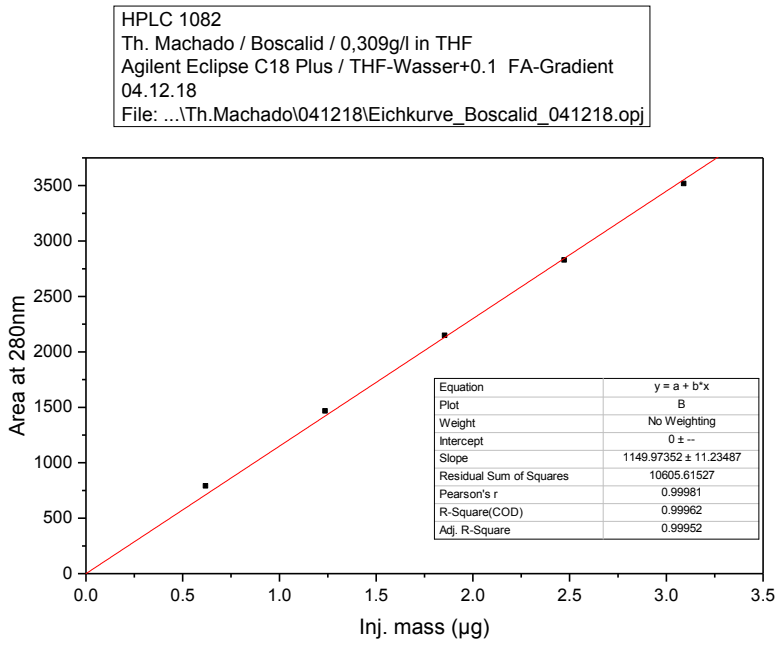


Figure S7. HPLC calibration curve for boscalid.

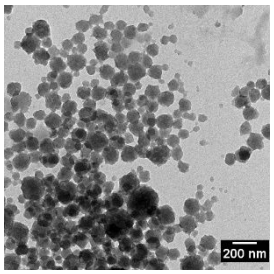


Figure S8. TEM image of lignin-based nanocarriers crosslinked with spermidine and containing 1 mg·mL^{-1} of boscalid in the dispersion (sample BS01).

Table S2. Drug-loaded lignin nanocarriers according to cross-linkers utilized. Average particle size (D_i) and PDI measured by DSL and encapsulation efficiency (EE) measured by HPLC.

Entry	Drug	Drug / mg·mL^{-1}	Cross-linker	D_i/ nm	PDI	EE / %
AE02	Azoxystrobin	3	EDBEA	210	0.20	> 99
AS02		3	Spermidine	(596)	(0.29)	93
TE02	Tebuconazole	3	EDBEA	230	0.23	88
TS02		3	Spermidine	272	0.22	> 99
PE02	Pyraclostrobin	3	EDBEA	186	0.37	90
PS02		3	Spermidine	(> 1000*)	-	97

* Aggregates that could not be measured by DLS.

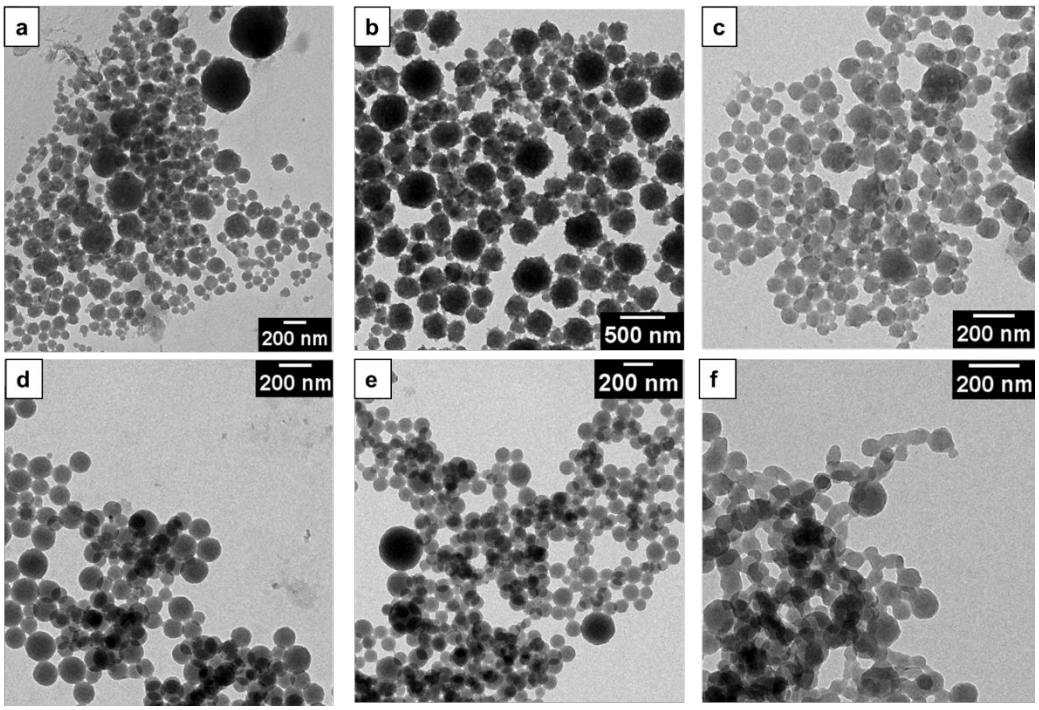


Figure S9. TEM images of the drug-loaded cross-linked lignin nanocarriers a) AE02, azoxystrobin 3 mg·mL^{-1}, EDBEA; b) PE02, pyraclostrobin 3 mg·mL^{-1}, EDBEA; c) TE02, tebuconazole 3 mg·mL^{-1}, EDBEA; d) AS02, azoxystrobin 3 mg·mL^{-1}, spermidine; e) PS02, pyraclostrobin 3 mg·mL^{-1}, spermidine; and f) TS02, tebuconazole 3 mg·mL^{-1}, spermidine.

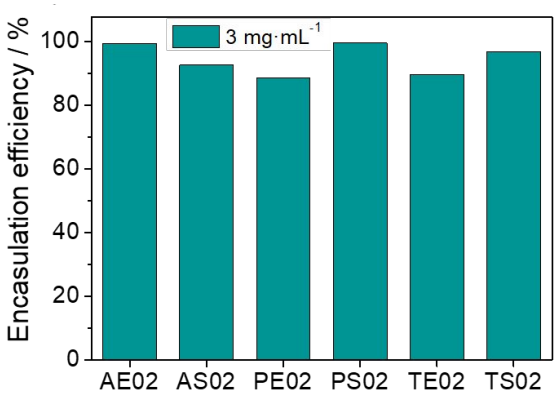


Figure S10. Determined amounts of encapsulated drugs in lignin nanocarriers in % of the total amount encapsulated.

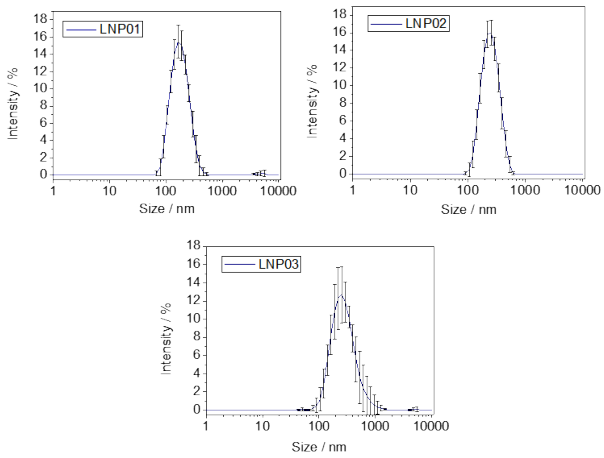


Figure S12. Particle size distributions obtained by DLS of lignin nanocarriers cross-linked with EDBEA (LNP01), spermidine (LNP02) and spermine (LNP03). Error bars are the standard deviation from DLS measurements of triplicates.

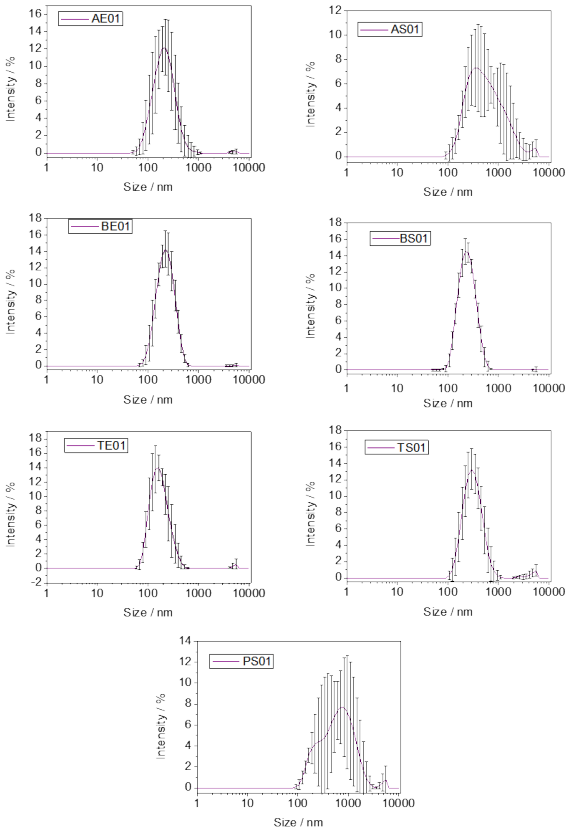


Figure S13. Particle size distributions of latexes containing 20 wt.% of azoxystrobin (AE01, AS01), tebuconazole (TE01, TS01) and pyraclostrobin (PS01), and 10 wt.% of boscalid (BE01, BS01). Error bars are the standard deviation from DLS measurements of triplicates (note the aggregates in AS1 and PS1).

Table S1. Raw data of trunk injections determined by yearly scoring.

		Year:				
Plant n°	Lignin Nanocarrier	**2015**	**2016**	**2017**	**2018**	**2019**
1-13	Lignin empty	1	1.5	3	5	5
1-40	Lignin empty	1	1	1	0	4
1-49	Lignin empty	1	2	3	3	1
2-7	Lignin empty	1	3	3	5	5
6-30	Lignin Boscalid	2	0	0	0	0
7-23	Lignin Boscalid	2	2	2	1	0
8-28	Lignin Boscalid	2	0	0	0	0
9-14	Lignin Boscalid	2	1.5	0	0	0

Additional References

(1) Balakshin, M.; Capanema, E. On the Quantification of Lignin Hydroxyl Groups with ^{31}P and ^{13}C NMR Spectroscopy. *J. Wood Chem. Technol.* **2015**, *35* (3), 220–237. https://doi.org/10.1080/02773813.2014.928328.