PROGRESS REPORT

PROJECT TITLE: Value-added use of corn byproducts as nanocarriers of biopesticides
PROJECT NUMBER: 6063-21DD
REPORTING PERIOD: 31 Mar 2023
PRINCIPAL INVESTIGATOR: Lingling Liu
ORGANIZATION: Iowa State University
PHONE NUMBER: 515.294.3939
EMAIL: lingling@iastate.edu

1.) PROJECT ACTIVITIES COMPLETED DURING THE REPORTING PERIOD. (*Describe project progress specific to goals, objectives, and deliverables identified in the project workplan.*)

a. (2,2,6,6-Tetramethylpiperidin-1-yl)oxyl oxidized cellulose nanofibrils (TEMPO-CNF) was prepared from corn stover. Physicochemical properties of nanocellulose (TEMPO-CNF) were characterized. Corn stover derived TEMPO-CNF has a width of ~4.0 nm, length of ~353 nm, zeta potential of -65 mV, and a surface charge of 1.48 mmol -COONa/g.

b. Nanocellulose (TEMPO-CNF) was used to encapsulate lemongrass essential oil (EO). Essential oil (EO) loaded emulsion with different formulations were prepared, containing certain concentration of EO (0 ~ 40%), Tween 80 (EO/Tween 80 ratio 3:1, 1:1, and 1:3), and nanocellulose (0, 0.1 wt%, 0.3 wt%, 0.5 wt%, and 0.7 wt%). Physicochemical properties of the emulsions were characterized, including particle size, zeta potential, transmittance, pH, and color. Emulsions with nanometer size range were achieved for formulations containing EO and nanocellulose. The stability of the nanoemulsion was tested against centrifugation, freeze-thaw, and room temperature storage (tested up to 30 days). One of the optimal formulations was selected for further antifungal test: 2.5% EO, 7.5% Tween 80, and 0.3% TEMPO-CNF.

c. Four concentrations of essential oil (EO) were tested to determine the minimum inhibitory concentration against *A. flavus* growth: 0.03%, 0.05%, 0.08% and 0.1%. Complete colony growth (~8 cm) on plate was observed for the control (without EO) by day 8. In comparison, treatment at 0.03% EO concentration, 43% inhibition was observed, and 100% inhibition was observed at 0.05%~0.1% EO loadings. On day 14, at 0.05% and 0.08% EO, inhibition rates of 20% and 33%, respectively were observed. Lemongrass essential oil at 0.1% exhibited 100% inhibition against *A. flavus* growth for 14 days.

d. Lemongrass essential oil in nanoemulsion format had better fungistatic properties than the pure essential oil treatments. Specifically, the nanoencapsulation formulation contained 2.5% EO, 7.5% Tween 80, and 0.3% TEMPO-CNF. Diluted nanoemulsion was tested for its antifungal activity. Results showed that at 0.08% EO loading, 100% inhibition against *A. flavus* growth for 14 days was observed.

e. The effect of lemongrass essential oil in pure format or nanoemulsion format on spore germination of *A. flavus* was tested using the cavity slide technique. With 10 hr incubation, 100% inhibition was observed at 0.01% EO loadings or higher. Comparing results of EO only and nanoemulsion samples, a significantly greater inhibition rate (97%) was observed in nanoemulsion samples than EO treatments (33%) at 0.005% EO loadings. In conclusion, from the conidial germination assay, better spore germination inhibition was exhibited by nanoemulsion treatments (with 0.005% EO loadings) as compared to the EO only after 10 hr incubation.

2.) IDENTIFY ANY SIGNIFICANT FINDINGS AND RESULTS OF THE PROJECT TO DATE.

We successfully prepared nanocellulose from corn stover and used the corn stover derived nanocellulose to stabilize essential oil loaded nanoemulsion. The prepared nanoemulsion was stable against centrifugation, freeze-thaw and room temperature storage. The essential oil loaded nanoemulsion had better inhibition activity against *Aspergillus flavus* than pure essential oil.

3.) CHALLENGES ENCOUNTERED. (*Describe any challenges that you encountered related to project progress specific to goals, objectives, and deliverables identified in the project workplan.*)

N/A

4.) FINANCIAL INFORMATION (*Describe any budget challenges and provide specific reasons for deviations from the projected project spending.*)

N/A

5.) EDUCATION AND OUTREACH ACTIVITES. *(Describe any conferences, workshops, field days, etc attended, number of contacts at each event, and/or publications developed to disseminate project results.)*

Type	**Status**	**Year Published**	**Citation**

Journal article	Published	2023	Liu, L., Gerard, G., Peng, Z., & Yu, Z. (2023). The Use of Corn Stover-Derived Nanocellulose as a Stabilizer of Oil-in-Water Emulsion. Polymers, 15(3), 757.
Conference	Published abstract in Proceedings	2021	Lingling Liu. The use of corn stover-derived nanocellulose as a stabilizer of nanoemulsion. Poster presentation at the American Society of Agricultural and Biological Engineers (ASABE) conference, 2021, online.