Effectiveness of Cinnamon Oil Nano-emulsion for antimicrobial properties on Alfalfa Seeds

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Introduction

- According to CDC, about 50 million people are infected and around 3000 people die annually due to foodborne illness in the US.

- Risk of foodborne illness due to consumption of raw sprouts especially Alfalfa sprouts is mainly associated with *Salmonella Newport*. Alfalfa sprouts are recognized as “Father of all food” due it is high mineral and vitamin content and low calorific value.

- In recent years Alfalfa sprouts is linked with foodborne illness mainly because they are grown and germinate in moist and warm temperatures which acts as a favorable ground of pathogenic infestation.

- Unlike processed or cooked food, prevention of microbial infestation on raw food needs new methods and strategies for safety of consumption.
• Evaluation of Nano-emulsion of cinnamon oil for its effectiveness as an antimicrobial agent (Salmonella Newport) is done under laboratory settings by simulating natural ways.

• Broth dilution tests were performed for Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC).
Preparation of Alfalfa seeds and inoculum:

- Alfalfa seeds which are used for the experiment are procured from local food store which are washed, dried, and packed in airtight zip-lock bags.
- Three strains of Salmonella used in the study are *Salmonella enterica* S11975, *Salmonella enterica* E2002001708, *Salmonella enterica* E20002725.
- Bacteria is cultured separately in Tryptic Soy broth for 24 hours and centrifuged under 4000 rpm for 20 minutes and washed with Phosphate Buffer Saline (PBS) and run in the centrifuge twice after discarding the supernatant. 2 ml of each strain is mixed to make bacterial cocktail. $10^9$ concentration CFU/ml was obtained by adding 2ml of the cocktail to 18ml of PBS.
Formulation, Characterization and Application of 5% Cinnamon Oil Nano-emulsion:

- Ultrasonication method was used to prepare 5% Nano-emulsion of Cinnamon oil using Tween 80 as emulsifier.
- Predetermined concentration of Cinnamon oil, Tween 80 and distilled water was used and sonication is done for 20 minutes at the Amplitude of 60.
- 0.75\% and 1.00\% concentration are used on the seeds after seeds are artificially treated with 10^9 concentration of bacterial cocktail and dried.
- Seeds are dipped in the emulsion for 1 minute and hand agitated to ensure complete coating which is followed by 30 minutes drying. The dried seeds are stored in zip-lock bags at 4 degree Celsius.
0.75% and 1% Nano emulsion used to treat seeds showed significant amount of log reduction when compared to control (DI water).

Greatest reduction was seen after 24 hours of treatment with Cinnamon oil Nano-emulsion with 1.16 log reduction using 0.75% and 1.32 log reduction using 1.00%.

Cinnamon oil Nano-emulsion proved to be effective in inhibiting foodborne pathogens like Salmonella. Where the average droplet size of Nano-emulsion is 19.6 nm.

Inhibitory concentration of Cinnamon oil Nano-emulsion for Salmonella was found to be 0.039% and Bactericidal concentration was found to be 0.078%.
REFERENCES

ACKNOWLEDGMENTS
This research is funded by USDA NIFA Foundation Grant 2019-06872 awarded to University of Central Oklahoma

QUESTIONS?