



## PROUD 3<sup>®</sup> Antibacterial Efficacy Study

### Research Report

Legend Technical Services  
Phoenix, AZ  
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#### Summary

Huma Gro<sup>®</sup> PROUD 3<sup>®</sup> organic pest control manufactured by Bio Huma Netics, Inc. was tested at varying concentrations on Romaine lettuce (*Lactuca sativa* L. var *longifolia*) leaves with the bacteria: *Escherichia coli* O157:H7 and *Salmonella enterica*. The purpose of the testing was to evaluate the antimicrobial efficacy of the PROUD 3<sup>®</sup> product against *E. coli* O157:H7 and *S. enterica*. The efficacy was assessed using standard aerobic plate count (APC) procedures following treatment of the infected lettuce with PROUD 3<sup>®</sup> at concentrations of 5mL/L and 7mL/L.



#### Purpose

*E. coli* O157:H7 is a cause of foodborne illness & disease, and infection can lead to hemorrhagic diarrhea and kidney failure. *S. enterica* is also a cause of foodborne illness & diseases such as systemic infections and gastroenteritis. Because these strains of bacteria are particularly dangerous to consume, using PROUD 3<sup>®</sup> at the recommended concentrations for treating lettuce and other produce immediately before harvest or post harvest could have a positive impact by preventing serious illness for people consuming produce with *S. enterica* and *E. coli* O157:H7 bacteria.

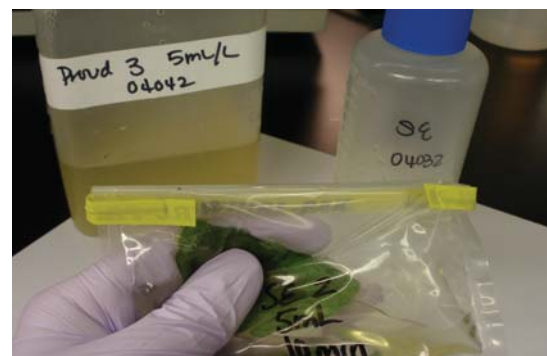
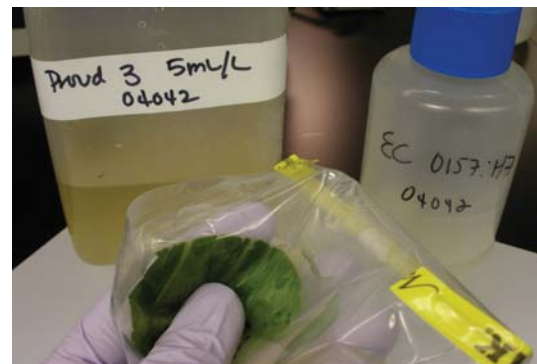
#### Description

Lettuce leaves were cut into circles using a clean cookie cutter and disinfected to ensure they were relatively free from background bacteria. Then the lettuce leaves were transferred into individual sterile plastic bags for testing at time intervals of 5, 10, and 20 minutes. Four replicates of *E. coli* O157:H7 and *S. enterica* for each PROUD 3<sup>®</sup> concentration (5mL/L and 7mL/L) per time interval were prepared and analyzed.

For quality control purposes, negative controls (blanks) were prepared to assess the disinfection of the leaves (prior to bacterial inoculation) and sterility of reagents and media used in the study.

Five (5) mL of the bacterial suspension were applied to each lettuce leaf in the test groups and hand-massaged. The infected lettuce leaves were then immersed and hand-massaged in the PROUD 3<sup>®</sup> test solutions.

After the designated contact times, a portion of the solution was transferred to tubes of Lethen broth and serially diluted in Butterfield's phosphate buffer (BFB) for aerobic plate counts. Lethen broth is a common neutralizer used in antimicrobial studies to ensure that the action of the product is limited to the specified contact time.

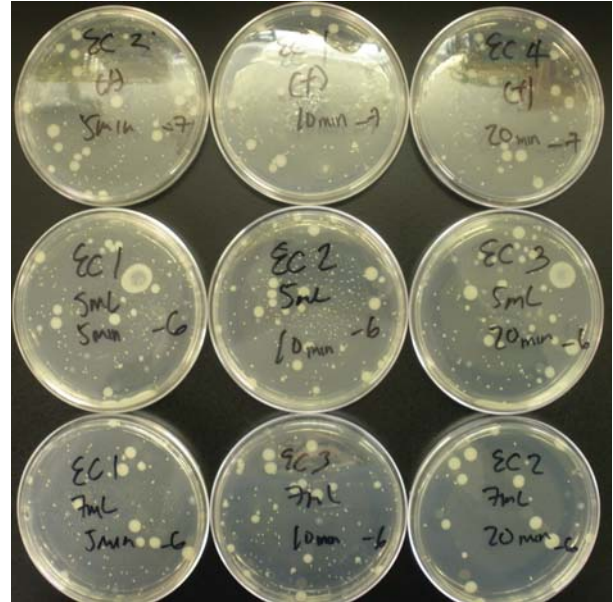


## HUMA GRO® PROUD3® Antimicrobial Efficacy Study

Aerobic plate counts were performed using Plate Count Agar (PCA) and incubated at 35°C for 48 ± 3 hours. The resulting colonies were counted and reported. Using the mean values of the four replicates in each test group/contact times, the percent (%) kill was calculated.



*S. enterica* - Representative Agar Plate Counts (APCs)



*E. coli* - Representative Agar Plate Counts (APCs)

## Results

The study results demonstrate the antimicrobial efficacy of PROUD 3® when applied to lettuce leaves infected with *E. coli* O157:H7 or *Salmonella enterica*. When applied at a concentration of 5mL/L to lettuce leaves inoculated with *E. coli*, PROUD 3® reduced bacterial counts by 88.57 to 93.67% over the test period of 5 to 20 minutes. At a 7mL/L concentration, the product reduced *E. coli* counts by 95.00 to 98.20% over the test period of 5 to 20 minutes. When applied at a concentration of 5mL/L to lettuce leaves inoculated with *S. enterica*, PROUD 3® reduced bacterial counts by 99.77 to 99.96% over the test period of 5 to 20 minutes. At a 7mL/L concentration, the product reduced *S. enterica* counts by 99.96 to 99.99% over the test period of 5 to 20 minutes.

## Conclusion

**This research study demonstrate a significant antimicrobial activity against both *S. enterica* and *E. coli* O157:H7 using PROUD 3® diluted with water at concentrations of 5mL/L and 7mL/L.**

PROUD 3® organic pest control is a safe, effective, organic foliar applied insecticide, miticide and fungicide when applied using recommended methods and rates. PROUD 3 is OMRI® listed for organic farming.



**If you would like to learn more about this top quality product, contact us directly at 480-423-6805 or visit our website at [www.humagro.com](http://www.humagro.com).**