Denele Analytical Inc.
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Evaluation of Efficacy of Aquacode
for Reduction of *Aspergillus flavus* the causative
agent of Aflatoxin

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OBJECTIVE:

The objective of this study was to assess the efficacy of a liquid disinfectant solution on the reduction of *Aspergillus flavus* on pistachios.

MATERIALS AND METHODS:

Test Product

Pistachios were disinfected and stored in a climate chamber until testing. The solution Aquacode was provided by the client.

Test Organism

Pistachios were inoculated with a culture of Aspergillus flavus prepared and maintained in Denele Microbiology Culture Library.

Preparation of test sample:

The pistachios were rinsed with a 10% bleach solution, rinsed with sterile distilled water to remove surface fungi and other microbes, blotted dry and aseptically inoculated with the culture by dipping the pistachios. The pistachios were agitated for 60 seconds in the inoculum, then removed and allowed to dry for 24 hours at room temperature.

Preparation and Treatment for Disinfectant Solution:

16 to 20 pistachios were treated with the disinfectant at 30 seconds and 60 seconds of exposure, one portion was treated with de-ionized water for similar amounts of time, and similar amounts were left untreated (Negative Process Control). After processing a spore suspension and hemocytometer were prepared. Approximately 9 microliters of thoroughly mixed suspension was pipetted of the cell suspension into one of the two counting chambers. The spores were counted in each of the four 0.1 mm³ corner squares.

Sample Analysis

Each portion was analyzed by plate count methods, providing a minimum of three replicates per treatment. The method of analysis is outlined in the following table.

Test	Medium	Incubation time/ Temperature and Atmosphere
Aspergillus flavus	AFPA	48 hours/35°C/ Aerobic

RESULTS AND DISCUSSION

Data for the recovery of A. flavus in inoculated pistachios after wash are shown in Table 1 and 2.

Control Samples	Dilution	Results (Log)
Average 60 seconds with DI Water	10,000	7.52
Uninoculated Pistachios	10,000	--

Spore Counts (Table #1) in Log of spores/mL 30 seconds

	Aquacode
Rep #1	0.00
Rep #2	0.00
Rep #3	0.00
Average	0.00

Spore Counts (Table #1) in Log of spores/mL 60 seconds

	Aquacode
Rep #1	0.00
Rep #2	0.00
Rep #3	0.00
Average	0.00

Reduction of Spores (Table #2) in Log of spores/mL

	Aquacode	Water
Average	7.52	0.00

The wash showed a complete reduction in the amount of Aspergillus flavus. Water showed a minimal reduction. The reduction of spore is a method of comparison of Aquacode to sterile distilled water. A separate set of infested pistachios were used and rinsed with water to make sure the process was working and to have a baseline of what a conventional method might look like. The water removed almost none of the A. flavus while the Aquacode completely eliminated the fungi.