SMART ORGANICS

TESTING RESULTS FOR HARVEST CLEAN

Comparison of sanitizing efficacy of Origin 3-6-9 and sodium hypochlorite

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Introduction

Sanitizers are typically added to the wash water in produce wash facilities to reduce the microbial load on produce surfaces, and to prevent cross contamination. Sodium hypochlorite (150-200 ppm free chlorine: HOCl) is one of the most frequently used antimicrobial agent in produce washes. This study compared the pathogen removal efficacy of the proposed antimicrobial agent Origin 3-6-9 to that of sodium hypochlorite, from *Salmonella* inoculated green tomatoes.

Materials and Methods

The inoculum cocktail was prepared by combining overnight cultures of five rifampicin (rif) resistant *Salmonella enterica* serovars (*S*. Braenderup, *S*. Montevideo, *S*. Newport, *S*. Anatum and *S*. Javiana). Three treatments (HOCl, Origin 3-6-9 and water), two contact times (30 or 60 s) and three holding times (0, 24 or 48 h post-wash) were tested.

Unwashed green tomatoes from a local packinghouse in FL were inoculated with the *Salmonella* cocktail (10^7 log CFU/tomato), around the blossom scar, and dried for 2 h under a laminar flow hood. Three separate circulating water baths containing HOCl (100 ppm), Origin 3-6-9 (1000 ppm active ingredient-as claimed by manufacturer (unconfirmed)) or distilled water (control) were set up to mimic a standard flume. Inoculated tomatoes (3/treatment) were placed in respective waterbaths for 30 or 60 s (mimicking standard fluming conditions), and removed for microbial enumeration performed 0, 24 or 48 h post-wash. Inoculated unwashed (washing time = 0 s), and uninoculated washed tomatoes were used as positive and negative controls respectively. The tomatoes were stored at 23°C for 0, 24 or 48 h post-treatment, and enumerations were conducted as described below. For each treatment, three tomatoes were tested per trial and each study was replicated three times (n=9).

Microbial analysis

For enumeration, tomatoes were placed in sterile sample bags containing 100 ml 0.1% peptone water (1 tomato/bag). The bagged tomatoes were then subjected to shake-rub-shake for 90 s to remove bacteria from the surface. One ml of the rinsate was removed from each sample bag, ten-fold serial dilutions were performed, and pour plated on tryptic soy agar (TSA) plates supplemented with 80 ppm rif. The plates were incubated at 37°C for 24 h, colonies counted and log CFU/tomato was calculated.

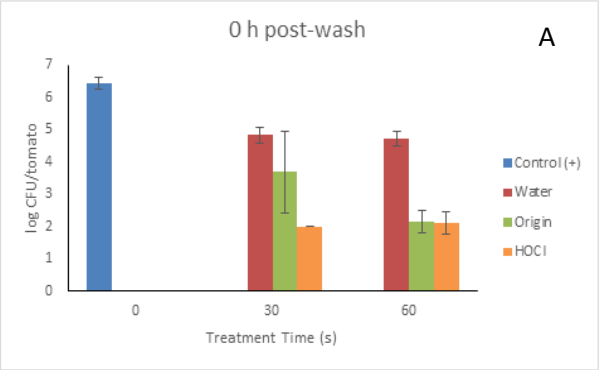
Statistical Analysis

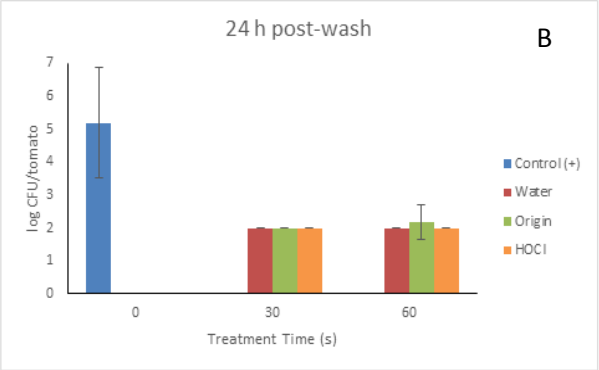
Analysis of Variance (ANOVA) was carried out for six different treatments: unwashed (positive control), washed with water for 30s, washed with water for 60s, washed with origin 3-6-9 for 30s, washed with origin 3-6-9 for 60s, washed with HOCl for 30s and washed with HOCl for 60s. Tukey's test was utilized to determine the difference levels between each treatment ($p < 0.05$). All data were analyzed using Statcrunch™ on: statcrunch.stat.ncsu.edu.

Results

All wash treatments (water, HOCl or Origin 3-6-9) significantly ($p < 0.05$) reduced *Salmonella* levels on inoculated tomatoes compared to the unwashed positive control, regardless of the wash time (30 or 60 s). Compared to washing with water alone, washes with water containing a sanitizer (either HOCl or Origin 3-6-9) significantly reduced initial (0 h post-wash) *Salmonella* levels on inoculated tomatoes, regardless of wash time. At 30 s wash time, reduction in initial *Salmonella* levels (day 0) was significantly higher with HOCl (4.4 log CFU reduction) compared to Origin (2.7 log CFU reduction). Additionally, the standard error or variation seen with Origin was greater than either water or HOCl. However, when the wash time was increased to 60 s, both HOCl and Origin had a similar effect on reducing *Salmonella* levels (4.3 log CFU reduction).

After washing, tomatoes were then stored for either 24 or 48 h at 23°C (73°F). All washed tomatoes, regardless of treatment, (water, HOCl or Origin) or wash time (30 or 60 s), reduced *Salmonella* levels to close to the enumerable levels (~2 log CFU/tomato). *Salmonella* levels on the unwashed positive control remained significantly higher.





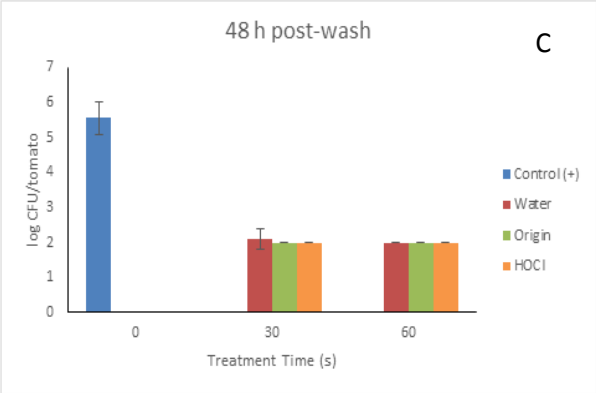


Fig. 1. *Salmonella* surface levels on inoculated tomatoes after a 0, 30 or 60 s wash with water, Origin 3-6-9 or HOCl. Post-wash, the tomatoes were incubated at 23^{o}C for 0 h (A), 24 h (B) or 48 h (C) post washed.

Conclusions

Washing tomatoes with water alone reduced the initial (0 h post-wash) microbial load on the tomato surface, and further reduced it upon holding the tomatoes for an additional 24-48 h. Regardless of the wash time (30 or 60 s), addition of HOCl to the wash water reduced *Salmonella* levels to close to the enumerable limit at all three time points (0, 24 and 48 h). When Origin 3-6-9 was added to the wash water, a wash time of 60 s was more effective in reducing initial (day 0) *Salmonella* levels compared to 30 s. *Salmonella* counts were below the enumerable limit when the tomatoes were held for an additional 24-48 h post-wash, regardless of the wash time.

The results show that all treatments were effective in removing the inoculated *Salmonella* from the intact surface of the tomatoes. The use of water only is not recommended for commercial applications since it does not prevent cross contamination nor does it aid in the prevention/reduction of biofilm formation. Of the two sanitizers tested, HOCl was more effective at shorter contact times, though no difference was seen at longer exposure times. Additionally, it was noted with the Origin, that a strong odor accompanied the treatment due to the nature of the compound (unknown) and the high concentration necessary. Additional ventilation would be required for everyday use. Another observation was the tomatoes were 'slicker' to the touch, making handling post washing more problematic. Additional work on how this would affect waxing, packing and shelf life would need to be evaluated.

Silliker, Inc. Food Science Center Report

RPN 16508

May 13, 2013

Validation of Efficacy of Harvest Clean (Amino Acid Complex) Against Bacteria and Fungi on Fresh Produce
Part II

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Background and Objectives
Smart Organics wished to conduct a validation study to assess the efficacy of Harvest Clean, an amino acid complex product at 1,000 ppm as a post harvest wash solution against bacteria and fungi on fresh produce.

There are a number of foodborne microbial pathogens associated with the consumption of fresh produce**.** Consumption of fresh fruits and vegetables contaminated with bacterial foodborne pathogens including *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* spp. can cause illness or death. Fresh produce favors the growth of yeasts and molds. If they are not properly cleaned and sanitized, fresh fruits and vegetables were spoiled during storage by these microorganisms.

Materials and Methods
Test Product-Harvest Clean (Amino Acid Complex)
NACMCF guidelines for conducting validation studies recommend evaluating three production lots, representing three separate manufacturing dates to account for variability among lots *(1)*. However, one lot of product was used in this study at the client's request due to investigational nature of the study.

One gallon of product labeled Harvest Clean #304080 was received from Smart Organics in good condition on April 11, 2013. Product temperature upon receipt was 17.1°C. Product was stored at ambient temperature until use.

Fresh Produce
Roma tomato was chosen as the test product to validate the efficacy of Harvest Clean. Roma tomato samples were purchased form a local grocery store.

Test Microorganisms
The following strains obtained from the Silliker Inc., Food Science Center (FSC) culture collection (FSC-CC) were used in this study.

Composite 1- *Escherichia coli* O157:H7	**FSC-CC Number**
E. coli O157:H7	2842
E. coli O157:H7	1428
E. coli O157:H7	2841
E. coli O157:H7	1431
E. coli O157:H7	2118

Composite 2- *Listeria monocytogenes*	**FSC-CC Number**
L. monocytogenes	525
L. monocytogenes	1797
L. monocytogenes	2450
L. monocytogenes	2453
L. monocytogenes	2473

Composite 3- *Salmonella*	**FSC-CC Number**
Salmonella Panama	266
Salmonella Enteritidis	2415
Salmonella Montevideo	2972
Salmonella Newport	551
Salmonella Typhimirium	2489

Mold	**FSC-CC Number**
Aspergillus niger	1204

Yeast	FSC-CC Number
Candida albicans	1221

Confirmation of Culture

1. Bacterial Cultures

The purity of each strain of *E. coli* O157:H7, *Salmonella* and *L. monocytogenes* was verified by streak plating on eosin methylene blue (EMB), xylose lysine desoxycholate (XLD) and modified oxford (MOX), respectively. The plates were incubated for 24 h at 35°C. The appearance of typical colonies was considered confirmatory.

2. Fungi

The purity of *A. niger* and *C. albicans* was verified by plating on potato dextrose agar (PDA). The plates were incubated for 5 days at 25°C. The appearance of typical colonies was considered confirmatory.

Culture Preparation

1. Bacterial Cultures

Strains of *E. coli* O157:H7 and *Salmonella* were cultivated in tryptic soy broth (TSB) and incubated at 35°C for 24 h. Strains of *L. monocytogenes* were cultivated in tryptic soy broth with 0.6% yeast extract (TSBYE) and incubated at 35°C for 24 h. Each strain was enumerated by the pour plate technique using trypticase soy agar (TSA) for *E. coli* and *Salmonella* and TSA with 0.6% yeast extract (TSA-YE) incubated at 35°C for 24 h. Cell suspensions were mixed to prepare a cocktail (*1*) culture, which contained approximately equal numbers of cells of each strain.

3. Fungi

A. niger and *C. albicans* were cultivated on PDA and incubated for 5 days at 25°C. Following incubation, approximately 10 ml of 0.1% peptone water were added to each plate. *A. niger* and *C. albicans* were loosened with a sterile spreader and a sterile pipette was used to collect the culture.

Preparation of Harvest Clean-Amino Acid Complex Solution

Harvest Clean 1000 ppm was prepared according to the client's instructions. 300 ml of the concentrate were combined with 900 ml water to achieve 1000 ppm solution (25%).

Inoculation Procedure

Ten tomatoes were placed in sterile plastic polyethylene bags (12 in x 20 in) and surface inoculated with 1 ml of the cocktail cultures of *E. coli* O157:H7, *L. monocytogenes, Salmonella, A. niger* or *C. albicans.* The bags were closed and mixed by hand through inversion for 1 minute to uniformly distribute the inoculum on the surface. Inoculated samples were stored at ambient temperature 4°C for 1.5 hour for bacterial attachment.

Treatment

The flow diagram of the test procedure is summarized below:

Purchase tomato from a local store
↓
Wash samples with deionozed water and dry at ambient temperature
↓
Inoculate washed samples with test microorganisms
↓
Store inoculated samples for 1 to 1.5 h at 4°C
↓
Dip 25 inoculated samples into 1.2 L of 1,000 ppm Harvest Clean solution for 2 min
↓
Remove samples from the solution, remove excess moisture with air and store at ambient temperature
↓
Analyze samples for surviving microorganism at 0, 1, 2 and 24 h

Microbiological Analyses
Samples of the inoculated control and treated samples were individually placed into sterile plastic polyethylene bags (12 in x 20 in) containing 100 neutralizing buffer. Each bag was shaken vigorously for 2 min and after standing for 5 minutes were shaken an additional five times before serial dilution and plating. Ten fold serial dilutions were performed using Butterfield's phosphate buffer. Untreated inoculated samples were used to establish the initial inoculation level. All samples were analyzed by the pour plate technique as outlined in Table 1. The appearance of typical colonies was considered confirmatory.

Table 1. Methods of analysis

Test	Medium	Incubation Time/ Temperature/ Atmosphere
Aerobic plate counts	Tryptone Glucose Yeast Agar	48 hours/35°C/aerobic
E. coli O157:H7	Trypticase soy agar with violet red bile agar (VRBA) overlay	48 hours/35°C/aerobic
L. monocytogenes	Trypticase soy agar with yeast extract with modified oxford (MOX) agar overlay	48 hours/35°C/aerobic
Salmonella	Trypticase Soy Agar with xylose lysine desoxycholate (XLD) overlay	48 hours/35°C/aerobic
Yeast/Mold Count	Potato Dextrose Agar with antibiotics	5 days/25°C/aerobic

Data Analysis:
The log base 10 counts were averaged and the difference between the average inoculated control count and the treated count represents the effectiveness of the treatment. Test results are reported per 100 ml rinse solution

Report
The aerobic plate counts of tomato samples were 16000 CFU/tomato. Microbiological test results for *E. coli* O157:H7, *L. monocytogenes*, *Salmonella*, *A. niger* and *C. albicans* trials are presented in Tables 2 thru 6, respectively. Harvest Clean at 1000 ppm was effective reducing the counts of the challenge microorganism significantly during 24 h storage. It was more effective against bacterial cultures compared to yeast and mold. Sporadic reduction values were observed for each microorganism. This may be attributed to sample to sample variations.

References
1. National Advisory Committee on Microbiological Criteria for Foods (NACMCF). 2010. Parameters for Determining Inoculated Pack/Challenge Study Protocols. J. Food Protect. 73: 140-202.

Table 2. Counts of *E. coli* O157:H7 on tomato treated with 1,000 ppm Harvest Clean solution for 2 min and held at ambient temperature for up to 24 h

Organism	Pull Time	Replicate	Log CFU/100 ml rinse (per tomato)	Log reduction from inoculated average
E. coli O157:H7	Inoculated control	1	5.00	
		2	5.23	
		3	4.36	
		4	5.15	
		5	5.11	
		Average	**4.97**	
	1 hour	1	2.49	2.48
		2	<1.00	>3.97
		3	1.60	3.37
		4	<1.00	>3.97
		5	2.11	2.86
	2 hour	1	<1.00	>3.97
		2	3.63	1.34
		3	<1.00	>3.97
		4	<1.00	>3.97
		5	2.08	2.89
	24 hour	1	3.34	1.63
		2	2.23	2.74
		3	2.15	2.82
		4	1.48	3.49
		5	<1.00	>3.97

Table 3. Counts of *L. monocytogenes* on tomato treated with 1000 ppm Harvest Clean solution for 2 min and held at ambient temperature for up to 24 h

Organism	Pull Time	Replicate	Log CFU/100 ml rinse (per tomato)	Log reduction from inoculated average
L. monocytogenes	Inoculated control	1	5.49	
		2	5.40	
		3	6.04	
		4	5.20	
		5	5.61	
		Average	**5.55**	
	1 hour	1	2.00	3.55
		2	2.08	3.47
		3	2.48	3.07
		4	2.30	3.25
		5	1.30	4.25
	2 hour	1	2.82	2.73
		2	3.04	2.51
		3	1.78	3.77
		4	1.60	3.95
		5	2.41	3.14
	24 hour	1	2.95	2.60
		2	<1.00	>4.55
		3	<1.00	>4.55
		4	1.95	3.60
		5	<1.00	>4.55

Table 4. Counts of *Salmonella* on tomato treated with 1000 ppm Harvest Clean solution for 2 min and held at ambient temperature for up to 24 h

Organism	Pull Time	Replicate	Log CFU/100 ml rinse (per tomato)	Log reduction from inoculated average
Salmonella	Inoculated control	1	6.11	
		2	6.15	
		3	6.58	
		4	5.72	
		5	6.00	
		Average	**6.11**	
	1 hour	1	1.30	4.81
		2	1.00	5.11
		3	1.00	5.11
		4	1.70	4.41
		5	1.30	4.81
	2 hour	1	1.30	4.81
		2	1.00	5.11
		3	1.30	4.81
		4	<1.00	>5.11
		5	1.00	5.11
	24 hour	1	1.48	4.63
		2	1.00	5.11
		3	<1.00	>5.11
		4	2.69	3.42
		5	1.00	5.11

Table 5. Counts of *A. niger* on tomato treated with 1000 ppm Harvest Clean solution for 2 min and held at ambient temperature for up to 24 h

Organism	Pull Time	Replicate	Log CFU/100 ml rinse (per tomato)	Log reduction from inoculated average
A. niger	Inoculated control	1	5.15	
		2	5.08	
		3	5.04	
		4	5.40	
		5	5.26	
		Average	**5.18**	
	1 hour	1	3.61	1.57
		2	3.84	1.34
		3	3.97	1.21
		4	4.04	1.14
		5	3.85	1.33
	2 hour	1	3.54	1.64
		2	3.83	1.35
		3	3.68	1.50
		4	3.87	1.31
		5	3.53	1.65
	24 hour	1	3.34	1.84
		2	3.62	1.56
		3	3.88	1.30
		4	3.36	1.82
		5	2.81	2.37

Table 6. Counts of *C. albicans* on tomato treated with 1000 ppm Harvest Clean solution for 2 min and held at ambient temperature for up to 24 h

Organism	Pull Time	Replicate	Log CFU/100 ml rinse (per tomato)	Log reduction from inoculated average
C. albicans	Inoculated control	1	6.78	
		2	6.90	
		3	7.41	
		4	6.58	
		5	6.99	
		Average	**6.93**	
	1 hour	1	5.86	1.07
		2	5.36	1.57
		3	5.49	1.44
		4	5.49	1.44
		5	5.71	1.22
	2 hour	1	5.70	1.23
		2	5.70	1.23
		3	4.93	2.00
		4	4.71	2.22
		5	5.38	1.55
	24 hour	1	4.81	2.12
		2	4.83	2.10
		3	4.15	2.78
		4	4.18	2.75
		5	4.40	2.53

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CERTIFICATE OF ANALYSIS

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COA No:	CHG-36630578-0
Supersedes:	None
COA Date	11/27/13
Page 1 of 1	

TO:
Mr. Ray W. Allbert
General Manager
DiMare Fresh Dallas
1049 Avenue H East
Arlington, TX 76011

Received From:	Arlington, TX
Received Date:	11/22/13
P.O.# / ID:	NA
Location of Test: (except where noted)	Crete, IL

Analytical Results

Desc. 1: 5 OZ HCTW 1000
Desc. 2: Smart Organics - Harvest Clean @ 1000
Desc. 3: PPM

Laboratory ID: 342809392
Condition Rec'd: NORMAL
Temp Rec'd (°C): 14.1

Analyte	Result	Units	Method Reference	Test Date	Loc.
Almond Allergen by EIA	<2.5	ppm (w/w)	Neogen Veratox Test	11/23/13	
Egg Allergen by EIA	<2.5	ppm (w/w)	Neogen Veratox Test	11/25/13	
Gliadin (Component of gluten)	<5	ppm (w/w)	Neogen Kit Insert	11/26/13	
Hazelnut Allergen by EIA	<2.5	ppm (w/w)	Neogen Kit Insert	11/27/13	
Peanut Allergen by EIA	<2.5	ppm (w/w)	Neogen Veratox Test	11/23/13	
Soy Allergen	<2.5	ppm (w/w)	Neogen Veratox Test	11/25/13	
Total Milk Allergen	<2.5	ppm (w/w)	Neogen Veratox Test	11/23/13	

Randy Fleener — Laboratory Director

Preliminary Findings for Testing Ambient Air Micro-organisms on Stainless Steel Surfaces Using Harvest Clean

JOHN H. STUMPF

Biological Research Service
Waco, Texas

ABSTRACT

The antimicrobial efficacy of Harvest Clean against ambient micro-organisms on the surface of stainless steel was examined. Stainless Steel surfaces were treated with 10% solution of Harvest Clean and allowed to dry for 24 hours. The treated surfaces were swabbed and tested for ambient micro-organism survivability. Results showed that no ambient micro-organisms were recovered.

MATERIAL AND METHODS

Surface Preparation. Stainless steel surfaces wash cleaned and thoroughly rinsed and then dried. An application of 10% Harvest Clean was applied and allowed to dry for 24 hours.

Ambient air sampling. A petri dish containing Sabourand Dextrose Agar (SBA, Hardy Diagnostics Santa Maria, CA) was exposed for 24 hours after the application of 10% Harvest Clean.

Surface swabbing and sample preparation. An area 12"sq. was swabbed using a sterile sampling sponge w/10 ml neutralizing buffer manufactured by Solar Biologicals Inc. Ogdensburg, NY. The swabs were blended in 225 ml Buffered Phosphate Solution. From the buffered solution 1.0 ml aliquot was surface plated into TSA incubated at 37ºC for 48 hours and into SBA incubated at 25ºC for 72 hours.

Results

Control data. Temperature was 55ºF with a relative humidity of 52%. Control pour plates for TSA and SBA were negative.

Ambient air plate findings: The SBA exposed plate had yeast growth 120 CFU/ 12 inches^2.

Efficacy findings. All swabbing trials showed no bacterial growth in the agar plates after 24 hours of incubation.

Discussion

The dried surface treat with a 10% Harvest Clean exhibited residual lethality for bacterial and yeast-type organisms. The temperatures and humidity levels were similar to that found in food manufacturing facilities. The implication is that Harvest Clean gives a continual guard against ambient air-borne micro-organisms. A continuing time study is in progress to determine the extent to which Harvest Clean provides lethality of air-borne micro-organisms. Harvest Clean is a 50% activity ratio.

Preliminary Findings for Reducing Levels of Pathogenic Bacteria Inoculated on Stainless Steel Surfaces Using Harvest Clean

JOHN H. STUMPF

Biological Research Service
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ABSTRACT

The antimicrobial efficacy of a 10% aqueous solution of Harvest Clean, an antimicrobial derived from Amino Based Acids complexes, was tested for lethality of pathogenic bacteria on the surface of stainless steel was examined. Stainless Steel surfaces were inoculated with a cocktail of *L. monocytogenes 4b, E. coli 0157:H7, and S. typhynurium.* Surface counts were found to be 5.5 X 10^7 cfu/cm². The treated surfaces were swabbed and tested for pathogen survivability at time zero, time 30 minutes, time 60minutes and time 120 minutes. Results showed that pathogenic bacteria was reduced through time and eliminated by time 120 minutes in all trials.

MATERIAL AND METHODS

Cultures. Cultures of Listeria, E. coli 0157:H7 and S. typhimurium were obtained from Microbiologics Inc. of St. Cloud, MN. *Listeria monocytogenes* ATCC #19115, *E. coli* ATCC #35150, and *S. typhimurium* ATCC# 13311 were grown on Typtic Soy Agar, Hardy Diagnostics Santa Maria, CA. One loop of each bacterium was harvested from TSA and each placed into 100 ml of TSB broth and cultured 24 hours at 37ºC.

A cocktail of the three bacteria was prepared by combining equal portions of cultures into an applicator. Cocktail was tested by performing three 10X dilutions to establish cell count per milliliter. The dilutions counts were determined by aliquoting 1 ml into a pour plate of TSA media (Difco) and incubating for 48 hours at 37ºC.

Surface Preparation. Stainless steel surfaces wash cleaned and thoroughly rinsed and then dried. An application of Harvest Clean was applied and allowed to dry for 2 hours. A visible sheen could be existed at the time of inoculation of bacteria. Cocktail of bacteria was applied before a second application of Harvest Clean. Temperature of the surface was 56ºF with ambient relative humidity of 52%.

Surface swabbing and sample preparation. An area 12"sq. was swabbed using a sterile sampling sponge w/10 ml neutralizing buffer manufactured by Solar Biologicals Inc. Ogdensburg, NY. The swab was blended in 225 ml Buffered Phosphate Solution. From the buffered solution 1.0 ml aliquot was pour plated into EMB (Eosine Methylene Blue) and Mox (Modified Oxford Media) liquid media and incubated at 37ºC for 48 hours. Swabs samples were taken immediately after second application (cover coat) of 10% aqueous Soap With A Purpose, and at time, 30 minutes, 60 minutes, and 120 minutes.

Results

Efficacy findings. Results for the three trials showed a reducing level of pathogens through time with total elimination of pathogens by time 120 minutes. EMB media was used to show results for levels of E. coli and Salmonella. Mox media show the efficacy on the *Listeria monocytogenes 4b*. Counts <10 were from observations of **no growth.**

Table 1. Trial 1 with control EMB with and without Harvest Clean and Mox with and without Harvest Clean. Bacterial counts are in units cfu.

Time	EMB w/o SCP	EMB w/ SCP	Mox w/o SCP	Mox w/ SCP
0	TNTC	TNTC	TNTC	3000
30	TNTC	11,840	TNTC	600
60	TNTC	<10	TNTC	<10
120	1500	<10	<10	<10

Table 2. Trial 2 with control EMB with and without Harvest Clean and Mox with and without Harvest Clean. Bacterial counts are in unit's cfu. In this trial bacterial application was doubled effecting the survivor rate in the control.

Time	EMB w/o SCP	EMB w/ SCP	Mox w/o SCP	Mox w/ SCP
0	TNTC	TNTC	TNTC	TNTC
30	TNTC	TNTC	TNTC	TNTC
60	TNTC	TNTC	TNTC	3440
120	TNTC	<10	TNTC	<10

Table 3. Trial 3 with control EMB with and without Harvest Clean and Mox with and without Harvest Clean. Bacterial counts are in units' cfu. Bacterial population was applied as in trial 1

Time	EMB w/o SCP	EMB w/ SCP	Mox w/o SCP	Mox w/ SCP
0	TNTC	TNTC	TNTC	16,800
30	TNTC	37,600	TNTC	2,080
60	TNTC	260*	TNTC	90
120	TNTC	<10	TNTC	<10

* Only *Salmonella typhimurium* survived.

Discussion

In trials #1 and #3 very similar results were achieved with application of 10% Harvest Clean. *Listeria monocytogenes 4b* shows least resistance to Harvest Clean than both *E. coli* 0157:H7 and *Salmonella typhimurium*. In trial #3 time 60 minutes shows only Salmonella typhimurim was recovered.

In trial #2 bacterial populations were increased to see if the 10% Harvest Clean could by overwhelmed by populations of 1.1×10^8 cfu/cm². Survival times in controls were effectively increased and the efficacy of Harvest Clean showed an expanded time for lethality. These results were consistent with expectations.

From the data, 10% aqueous Harvest Clean destroys pathogens within two hours of application. Interestingly at conditions of testing, it was between hour one and two that the stainless surface had dried. Implications are that as the surface with Harvest Clean dries, the killing power seems to completely destroy the three pathogens used in this study. Harvest Clean has a 50% activity ratio.

This study was performed in the laboratory of Biological Research Service in Waco, Texas. Smart Organics supplied the Food Antimicrobial (Harvest Clean) and funding for this project.

Preliminary Findings for Reducing Levels of Pathogenic Bacteria Coagulase Positive *Staphylococcus aureus* Inoculated on Stainless Steel Surfaces Using Harvest Clean for Lethality

JOHN H. STUMPF

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ABSTRACT

The antimicrobial efficacy of Harvest Clean against pathogenic bacteria on the surface of stainless steel was examined. Stainless Steel surfaces were inoculated with *Staphylococcus aureus* ATCC # 25923, (2.0 X 10^8 CFU/ml), allowed to dry for 15 minutes and treated by spraying a 20% solution of Harvest Clean across inoculated surface. The treated surfaces were swabbed and tested for pathogen survivability. Results showed a reduction of bacteria.

MATERIAL AND METHODS

Cultures. A culture of *Staphylococcus aureus,* ACTT #25923 was obtained from Microbiologics Inc. of St. Cloud, MN. *S. aureus* was grown on Typtic Soy Agar, Hardy Diagnostics Santa Maria, CA. One loop of bacteria was harvested from TSA and placed into 100 ml of TSB broth and cultured 24 hours at 37ºC. Bacteria was washed three times and suspended in Buffered Phosphate Solutions for application.

Bacteria population was tested by performing three 10X dilutions to establish cell count per milliliter. The dilutions counts were determined by aliquoting 1 ml into a pour plate of TSA media (Difco) and incubating for 48 hours at 37ºC.

Surface Preparation. Stainless steel surfaces wash cleaned and thoroughly rinsed and then dried. An application of Harvest Clean was applied and allowed to dry for 24 hours. A visible sheen could be existed at the time of inoculation of bacteria. Bacteria were allowed to stand 15 minutes before a second application of Harvest Clean was applied. The second coat of Harvest Clean was allowed to dry 15 minutes before first swabbing occurred. The second swabbing was performed 24 hours later.
Environmental conditions showed temperature of 65°F and relative humidity at 45%.

Surface swabbing and sample preparation. An area 12"sq. was swabbed using a sterile sampling sponge w/10 ml neutralizing buffer manufactured by Solar Biologicals Inc. Ogdensburg, NY. The swab was blended in 225 ml Buffered Phosphate Solution. From the buffered solution 1.0 ml aliquot was surface plated into TSA and incubated at 37ºC for 48 hours.

Results

Control data. A control for surface contamination was performed using bacteria applied to stainless steel, let stand for 24 hours, and swabbed. Sample preparation was performed as fore-mentioned. Control findings showed *Staphylococcus aureus* could survive a stainless steel surface for 24 hours.

Efficacy findings. Initially there was a near half reduction in detected target bacteria. The 24 hour sample shows a significant reduction of log 4.

Table 1. CFU determination after swabbing surfaces treated with Harvest Clean

Sample No.	Untreated surface	Treated surface
# 1 Time 15 min.	30,000 cfu / 12 in²	12,500 cfu / 12 in²
# 2 Time 24 hr.	8,640 cfu / 12 in²	<10.0 cfu / 12 in²

Discussion

Harvest Clean at 20% worked effectively over a 24 hour period to reduce pathogenic *Staphylococcus aureus* population to undetectable levels of the experimental method. The initial decrease in the swabbing for time 15 minutes may have a result due to a dilution factor since the Harvest Clean had not dried in 15 minutes. Since *Staphylococcus aureus* survives well on dried surfaces of animal and human skin, the experiment focus on time was extended to 24 hours.

Previous experimentation with Harvest Clean at reduced concentrations did not exhibit a substantial lethality in a 24 hour exposure. Based on this study, a 20% in a aqueous solution is recommended when *Staphylococcus aureus* is the target micro-organism on dry surfaces. Harvest Clean has a 50% Activity by ratio.

This study was performed in the laboratory of Biological Research Service in Waco, Texas. Smart Organics supplied the Food Antimicrobial (Harvest Clean) and funding for this project.

Report of Findings and Observations on Tomato Crop Performance Using Harvest Clean to Control Blossom Drop and Other Tomato Pests

JOHN H. STUMPF

Smart Organics
Hewitt, Texas

ABSTRACT

Harvest Clean is an antimicrobial soap derived from food components. The benefits of such a compound were explored on tomato crops to control blossom drop, insects, and spider mites. This report found that Harvest Clean controlled insects and mites. Blossom drop was reduced. Additional observations showed other benefits for treatment with Harvest Clean.

INTRODUCTION

Tomato plants drop their blossoms in response to stresses to the plant. Stress factors can be singular or compounded. Soil temperature, dehydration, poor fertilization and soil type all play a role in blossom drop. Additionally biological stresses cause blossom drop. Insects, mites, and micro-organisms comprise this group. The bottom line is: no blossom, no tomato.

Grower for years have used many pesticides and other compounds to control the biologics. Included in this arsenal was insecticidal soap. These forms of chemotherapy have their limitations and generally do not enhance plant growth.

Harvest Clean 's unique design and formulation allows for both a soap action and an anti-microbial control. Harvest Clean is easily applied to follage or can be administer in a soil application for root uptake. Because Harvest Clean is a food and not a chemical, plant stress is minimal at recommended rates.

MATERIAL AND METHODS

Applications of Harvest Clean. Since tomatoes are sensitive, application of Harvest Clean must be carefully measure to be a 1.0 % solution. Stronger concentration injure plant tissues causing reduced tomato output.

Fertilization Tomato plants were fertilized with 13-13-13 with trace elements. Application rates were established at 200 pounds per acre. Side applications of 20-20-20 solubelized fertilizer were performed twice a month at low rates.

Soil Type The soil type is a swelling black clay with a depth of four feet deep on top of limestone type rock. Soil pH is in the range of 7.5-7.8. Rain fall ceased by mid June. Water was supplied by use of city well-water. Study was performed for 5 years.

Results

Control data. Comparable data is subjective in that over the years consistently tomato crop is destroyed by mid July by fungal, mites and insect attack. Insect and mite pests were identified tomato horn worm and spider mites. Blossom drop occurred throughout the growing season.

Efficacy findings. All plants survived until August with irrigation and applications of Harvest Clean. Plant life was terminated by heat. Plants produced fruit continuously throughout the growing season. No horn worms were observed. Spider mite treatments were topical to the underside of the leaf and were performed as needed (every two days). Blossom drop was measured by fruit production. The treated plots were 40% greater in total pounds produced as compared to the control patch.

Discussion

While the findings for the soil application of Harvest Clean were subjective in nature, the results were compelling. First the tomato plant survived through the entire Central Texas season : especially in light of the severity of the drought (city well-water was being tolerated). Secondly, fruit production continued through the season.
Fruit production in tomato will cease when soil temperature is too hot. Blossoms will be produced by the plant but fruit set will not occur.

As long as the soil temperature is not prohibitive to fruit set, the treated tomatoes produced fruit. Stress factors of fungal, insect and mites all contribute to blossom drop and that means less fruit. From this five year study, Harvest Clean has shown a positive impact for increased tomato production.

Preliminary Findings for Applications of Harvest Clean into Soil to Control Mold and Mildews Effecting Yellow Straight Neck Squash Fruit Production

JOHN H. STUMPF

Biological Research Service
Hewitt, Texas

ABSTRACT

The antimicrobial efficacy of Harvest Clean against soil mold and mildews effecting squash production was examined over a growing season in Central Texas. Growing season was expanded when Harvest Clean was used in the clay soils in Central Texas. The expanded season was contributed to the squash surviving fungal and mildew attack experienced every growing season.

INTRODUCTION

Over the last ten years of squash production, after one month of harvesting the squash plants would die from fungal attack. Additionally the squash bug population would surge to the limits of the squash plants ability to cope. Many interventions such as fungicides and insecticides were employed. However, the use of pesticides yielded results that were not profitable, nor food safe. In the last three years squash were planted and maintained until the onset of fungal attack and insect manifestation at which point the squash season was over.

Recently a new product has been introduced that seems to have application for squash growers in Central Texas. Test results of Harvest Clean have shown efficacy against yeast and molds on surfaces continually exposed to these micro-organisms. The possibilities for fungal control would be in the soil and in the plant if Harvest Clean would have systemic uptake by the target crop. Since Harvest Clean is a FDA approved food, it clearly would have an organic advantage over other alternatives.

MATERIAL AND METHODS

Applications of Harvest Clean. Six yellow straight neck squash plants were purchased from the garden center and planted in the garden in early May, 2009. Before transplanting squash 10 ml of a 2% solution of Harvest Clean was poured onto the potting soil containing the squash plant. Once squash plants were established (approximately one week after planting) additional applications of 50 ml of Harvest Clean were poured onto soil near the squash stem every two weeks.

Fertilization Squash plants were fertilized with 13-13-13 with trace elements. Application rates were established at 200 pounds per acre. No side applications were performed.

Soil Type The soil type is a swelling black clay with a depth of four feet deep on top of limestone type rock. Soil pH is in the range of 7.5-7.8. Rain fall ceased by mid June. Water was supplied by use of city well-water.

Results

Control data. Comparable data is subjective in that over the years consistently squash is destroyed by the end of June by fungal and insect attack. Insect pest is identified as squash bug (*Anasa tristis*). Large amounts of reddish brown eggs (squash bug eggs) were also observed.

Efficacy findings. All squash plants survived through August with irrigation and applications of Harvest Clean. Plant life was terminated by mowing to make ready for fall tilling. Plants produced fruit continuously throughout the growing season. Adult squash bugs were observed but not consistently. No insect eggs on the under side of leaves were found during the entire growing season.

Discussion

While the findings for the soil application of Harvest Clean were subjective in nature, the results were compelling. First the squash plant survival through the entire Central Texas summer was astounding: especially in light of the severity of the drought (city well-water was being tolerated). Secondly, squash bug attack was not a factor. Since no insect eggs were found on the under side of the squash leaves, some systemic control mechanism must have been in place.

A strong possibility exists that the systemic uptake of Harvest Clean was not only combating fungal attack, but in fact may have contributed to insect feeding being greatly reduced. In any case squash was harvested and consumed all summer; a feat which has never occurred during the present ownership of this property.

Testing done in Tap Water

Bacterial Counts (CFU/gram)

Sample #	Test Samples	Day 0	Day 7	Day 14	Day 28
1	Unpreserved Control	5.4×10^6	2.26×10^8	1.67×10^8	3.6×10^7
2	Glydant Plus 100 ppm	2.2×10^6	2.68×10^8	2.73×10^8	3.3×10^7
3	Glydant Plus 1000 ppm	1.9×10^6	< 10	< 10	< 10
4	Glydant 2000 100 ppm	2.5×10^6	7.9×10^5	1.6×10^7	1.08×10^8
5	Glydant 2000 1000 ppm	1.8×10^6	< 10	< 10	< 10
12	Harvest Clean 100 ppm	3.2×10^6	9.3×10^7	1.14×10^8	169×10^7
13	Harvest Clean 1000 ppm	1.5×10^6	< 10	< 10	< 10

Bacterial Mixture:	2.2×10^9

.1% of the concentrate to acheve these results

Testing done in Tap Water

Fungal Counts (CFU/gram)

Sample #	Test Samples	Day 0	Day 7	Day 14	Day 28
24	Unpreserved	5.0×10^5	1.7×10^4	3.5×10^5	2.4×10^7
25	Glydant Plus 100 ppm	1.4×10^6	1.6×10^6	2.2×10^6	1.8×10^6
26	Glydant Plus 1000 ppm	7.3×10^4	< 10	< 10	< 10
27	Glydant 2000 100 ppm	5.0×10^5	7.0×10^3	1.7×10^4	2.4×10^5
28	Glydant 2000 1000 ppm	1.8×10^5	< 10	< 10	< 10
35	Harvest Clean 100 ppm	3.1×10^6	5.1×10^4	4.0×10^4	1.1×10^7
36	Harvest Clean 1000 ppm	2.4×10^5	< 10	< 10	< 10

Fungal Mixture:	7.2×10^7

.1% of the concentrate to acheve these results

Bacterial and Fungicidal Efficacy Ranking- MIC ppm

	gram (-)	gram (+)	gram (-)	gram (-)	Fungus	Yeast
	P. aerug.	**S. aureus**	**E.coli O157:H7**	**S. choler.**	**A. niger**	**C. albicans**
	9027	6538	35339	10708	16404	10231
Quat	10	10	10	10	10	10
Isocil RW	*39*	*39*	*19*	*19*	*312*	*156*
Triclosan	*>10,000*	*20*	*20*	*20*	*>10,000*	*>10,000*
Harvest Clean	2500	2,500	2500	2500	5,000	5,000
DMDMH	5000	2,500	5000	2500	>10000	>10000
IPBC	*>10,000*	*156*	*5,000*	*1,250*	*10*	*10*

.1% of activity for Harvest Clean Concentrate to acheve these results

NOVA
Enhancing Human Life

PROJECT: F0425E						PAGE 1 OF 2
COLLECTED		RECEIVED		COMPLETED		REQUISITION NO.
DATE	TIME	DATE	TIME	DATE	TIME	M17491
06-21-05	NA	06-27-05	1000	08-03-05	0900	

CUSTOMER: Phoenix Plastics Co., Inc.	
STREET: 5400 Jefferson Chemical Rd.	
CITY, STATE, ZIP CONROE, TX 77301	
PHONE NO: 936-760-2311	**ATTENTION:** ROD GARCIA

.Laboratory Report
ASTM G-21

Project:

Standard practice for determining resistance of Synthetic Polymeric Materials to Fungi in that is does not serve as a carbon source for growth of fungi,

Procedure:

All samples were cut into 2 by 2 inch pieces and set up in triplicate along with positive and negative growth controls. Sufficient nutrient-salts agar is poured into suitable sterile Petri dishes to provide a solidified agar layer of 3 to 6 mm in depth. After the agar is solidified, the samples are placed on the surface of the agar, and inoculated with a standard spore suspension of *Aspergillus Niger.* The samples are then incubated under conditions favorable for growth and examined for visible growth at the indicated time intervals.

A rating of trace or no growth is confirmed by microscopic observation.

Fungi Tested: *Aspergillus Niger*

Component Tested: F0425E

Reagents and Materials: Nutrient – Salts Agar
Nutrient – Salts Solution

Incubation Conditions: 28-30ºC At a relative humidity not less than 85%

Incubation Time: 28 days with 7 day reporting intervals

NOVA

Enhancing Human Life

PROJECT: F0425E						PAGE 2 OF 2
COLLECTED		RECEIVED		COMPLETED		REQUISITION NO.
DATE	TIME	DATE	TIME	DATE	TIME	M17491
06-21-05	NA	06-27-05	1000	08-03-05	0900	

Results:

Observed growth on specimen F0425E				
	Day 7	Day 14	Day 21	Day 28
Replicate 1	0	0	0	0
Replicate 2	0	0	0	0
Replicate 3	0	0	0	0

Observation of visible effects is based on the following:

Observed growth on specimen	Rating
None (no growth)	0
Traces of growth (less than 10%)	1
Light growth (10-30%)	2
Medium growth (30-60%)	3
Heavy growth (60% to complete coverage)	4

Summary of Results:
No growth of *Aspergillus Niger* was observed on any of the replicates at all time intervals.

Comments:
Test results indicate the component tested (F0425E) is resistant to fungal/mold growth and does not serve as a carbon source for growth of fungi.

Quality assurance:

For the test to be valid the following parameters have been met:

- Copious growth on all three of the viability controls.
- No observed growth should be seen on the Negative inoculated media control.
- A rating of trace of No Growth must be confirmed by 20x microscopic observation.
- *Aspergillus niger* spore concentration was verified at $2.0x10^5$ to $1.0x10^6$ spores per ml.

Reference:

ASTM G-21. Standard Practice for Determining Resistance of Synthetic Polymeric materials to Fungi.

Tech: _____ Approved: _____

Paul J. Pearce, Ph.D.

Myco CURB® Brand Liquid Mold Inhibitor

Features:

- Contains 65% propionic acid blended with sorbic and benzoic acids.
- Propionic Acid 10 ppm. **Phosphoric Acid1 mg/m3.**
- Inhibits the typical mold growth found in grains or processed grains during storage, handling and
  export shipments.
- Buffered for improved equipment and employee safety.
- Works in a variety of feed situations.
- Tested for use with widely available application equipment.

- Tests were used on sugar cubes freshly made by Bryant Feed and Grain, 38 % cotton seed pellets made fresh by Bryant Feed and Grain. 68% cotton seed pellets made fresh by Bryant Feed and Grain. A 50% cotton seed pellet made fresh by Bryant Feed and Grain. Testing was at 102* F and 95% humidity in a 60 day natural environment, stored at the warehouse. Stored out on the racks in paper bags lined with polymer, and sealed for moisture sake.
- Tests show the Micro Curb liquid lasted two weeks before oxidation and molding.
- Smart Organics Origin3-6-9 Liquid lasted two weeks before oxidation and mold occurred. Smart Organics due to neutral its pH needed no buffering for application and equipment.

Benefits: Noted for Micro Curb liquid

- Complete control of spoilage organisms so that feed remains stable and palatable to the animal.
- Fewer employee safety issues and lower equipment costs.
- Allows purchasing only product needed thus saving money tied up in unused product.
- Higher feeding value.
- Purchaser can negotiate best equipment alternative.
- Flexible and convenient for a wide variety of users.

Myco CURB® Brand Dry Mold Inhibitor

Features:

- Contains 65% propionic acid blended with sorbic and benzoic acids.
- Propionic Acid 10 ppm**. Calcium Hydroxide 5 mg/m3. Amorphous Silicon Dioxide 10 mg/m3. Phosphoric Acid1 mg/m3.**
- Inhibits the typical mold growth found in processed feeds during storage, handling and
  export shipments.
- Buffered for improved equipment and employee safety.
- Package size is 50lb bag.

- Tests were used on sugar cubes freshly made by Bryant Feed and Grain, 38 % cotton seed pellets made fresh by Bryant Feed and Grain. 68% cotton seed pellets made fresh by Bryant Feed and Grain. A 50% cotton seed pellet made fresh by Bryant Feed and Grain. Testing was at 102* F and 95% humidity in a 60 day natural environment, stored at the warehouse. Stored out on the racks in paper bags lined with polymer, and sealed for moisture sake.
- Tests show the Micro Curb dry lasted two weeks before oxidation and molding.
- Smart Organics Origin3-6-9 dry powder lasted six weeks before oxidation and mold occurred. Smart Organics due to neutral its pH needed no buffering for application and equipment.

Benefits: noted for Micro Curb dry

- Maintains nutritive and quality value of feed through short or long-term storage and transportation and for export.
- Reduces mold contamination into complete feeds
- An effective and safer alternative to corrosive, unbuffered propionic acid alone.

Ultra CURB® Brand Liquid Mold Inhibitor

Features:

- Highly concentrated at 82% total acid content.
- **Propionic Acid 10 ppm. Acetic Acid 10 ppm.**
- High inclusion level of acetic, benzoic and sorbic acid effectively target yeast growth in addition to controlling molds.
- Buffered for improved equipment and employee safety.

- Tests were used on sugar cubes freshly made by Bryant Feed and Grain, 38 % cotton seed pellets made fresh by Bryant Feed and Grain. 68% cotton seed pellets made fresh by Bryant Feed and Grain. A 50% cotton seed pellet made fresh by Bryant Feed and Grain. Testing was at 102* F and 95% humidity in a 60 day natural environment, stored at the warehouse. Stored out on the racks in paper bags lined with polymer, and sealed for moisture sake.
- Tests show the Ultra Micro Curb lasted two weeks before oxidation and molding.
- Smart Organics Origin3-6-9 liquid lasted six weeks before oxidation and mold occurred. Smart Organics due to neutral its pH needed no buffering for application and equipment.

Benefits: Noted for Ultra Micro Curb

- By preventing the proliferation of yeast growth, environmental conditions ideal for mold growth are delayed.
- Delays heating of total mixed rations caused by yeast and mold growth.
- Extends shelf life of complete feeds that contain a relatively high percentage of moisture.

Ammo CURB® Brand Dry Mold Inhibitor

Features:

- Inhibits the typical mold growth found in processed corn in storage, handling and complete feeds.
- **Propionic Acid 10 ppm.**
- Buffered for improved equipment and employee safety.

- Tests were used on sugar cubes freshly made by Bryant Feed and Grain, 38 % cotton seed pellets made fresh by Bryant Feed and Grain. 68% cotton seed pellets made fresh by Bryant Feed and Grain. A 50% cotton seed pellet made fresh by Bryant Feed and Grain. Testing was at 105* F and 102% humidity in a 60 day natural environment, stored at the warehouse. Stored out on the racks in paper bags lined with polymer, and sealed for moisture sake.
- Tests show the Ammo Curb lasted two weeks before oxidation and molding.
- Smart Organics Origin3-6-9 dry powder lasted six weeks before oxidation and mold occurred. Smart Organics due to neutral its pH needed no buffering for application and equipment.

Benefits: noted for Ammo Curb Dry

- Helps maintain stability of complete feeds during relatively short-term storage.
- Can be added directly to TMR.
- Convenient and cost effective to use.
- Smart Organics Origin3-6-9 Liquid and dry is 100% food (Amino Acid Complex)

Ingredients:

Smart Organics Unique Process, made ingredients, Triglyceride form: Amino Acids pH 7.5 Neutral.

Alanine	3.0
Arginine	3.3
Aspartic Acid	3.1
Cystine	0.2
Glutamic Acid	38.40
Glycine	3.8
Histidine	2.3
Isoleucine	3.5
Leucine	7.4
Lysine	3.8
Methionine	1.6
Phenylalanine	3.6
Proline	8.5
Serine	5.9
Threonine	6.0
Tyrosine	1.3
Valine	4.3

Glycerin	Soluble
Propylene Glycol	Soluble
Water	Soluble