A Water-soluble Essential Oil Product for Controlling Fire Blight



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Fire blight caused by a bacterium

Pathogen is the gram-negative Erwinia amylovora

Affecting tree fruits of apple, pear, and others

Residing in the flower buds

Infecting flowers during spring bloom and causing blights





Current treatments

- IR-4 designate the disease as "Pest Problem Without Solution."
- Huge unmet market need in WA, OR, CA, MI, PA.
- Under test:
 - Firewall (streptomycin)
 - Fireline (oxytetracycline)
 - Blossom Protect (biopesticide)
 - Alum (potassium aluminum sulfate)
 - TDA-NC-1
 - RejuAgro
 - Nanja
 - Others
 - ET91 (Water-soluble Essential Oils)



EO-WSC Product Features

- Active ingredients: Eugenol and Thyme
- The essential oil droplets: uniformly dispersed in water with diameter < 10 nm and encapsulated to remain stable.
- Clarity: clear and transparent (pictures on right) in concentrate and dilutions.
- Soluble stability: freely dilutable to any concentrations (e.g., 5% to 0.5%) without losing clarity.
- Efficacy in cell culture: instantly bactericidal or fungicidal against a number of pathogens (Tables 1 and 2 next).



0.5%





Table 1. Summary of *in vitro* bactericidal activity of a water-soluble essential oils product (5000ppm) after exposure

Organism	Туре	30-seconds to 1-day exposure	
Ralstonia solanacearum	Gram Negative	>99.9%	
Xanthomonas perforans	Gram Negative	>99.99%	
Pseudomonas syringae	Gram Negative	>99.99%	
Clavibacter michiganensis	Gram Positive	>99.99%	
Erwinia amylovora	Gram Negative	>99.9%	
Staphylococcus aureus	Gram Positive	>99.99%	
Enterococcus fecalis	Gram Positive	>99.99%	
Salmonella enterica	Gram Negative	>99.99%	
Escherichia coli	Gram Negative	>99.99%	
Listeria monocytogenes	Gram Positive	>99.99%	
Dickeya dadantii (formerly Erwinia chrysanthemi)	Gram Negative	>99.99%	

Table 2. Summary of in vitro fungicidal activity of a water-soluble essential oils product

Organism	Diseases caused	Spore growth
Ceartocystis fimbriata	Root rot	No growth at 5000ppm
Rhizopus stolonifera	Root rot	No growth at 5000ppm
Rhizoctonia spp	Damping-off	No growth at 1000ppm
Furarium spp	Damping-off	No growth at 1000ppm
Phytophthora spp	Damping-off	No growth at 1000ppm
Pythium spp	Damping-off	No growth at 1000ppm

ET91 – IR4 test protocol

- ET91 was tested in 3 locations over 2 years:
 - UC-Riverside (Dr. Adaskaveg): apple pear
 - Washington State University (Dr. DuPont): apple (red delicious)
 - Pennsylvania State University (Dr. Peter): apple (gala).
- The concentration of the product tested:
 - 0.5% ai (64-oz/A) in 2020
 - 0.5% ai (64-oz/A)/0.25% ai (32-oz/A) in 2021.

IR-4 Efficacy and Safety results on ET91

- The results shown next were extracted from the investigators' reports.
- Infections were normalized over the infections recorded for the untreated control (100%). The lower the less infection.
- Included for comparisons were antibiotic standards firewall and fireline, a chemical product Alum, and a biopesticide product Blossom Protect.
- Efficacy % means % protection from getting infected. The higher the more effective.
- Safety means severity of commercial downgrades.



IR-4 Efficacy and Safety results on ET91

Washington St Univ 2020

UC-Riverside 2020

Penn St Univ 2020 (Not used)

Treatment	Infection (%)	Safety (Fruit russet)	Treatment	Infection (%)	Safety (Fruit russet)	Treatment	Infection (%)	Safety (Fruit russet)
Untreated control	100 a*	No symptoms	Untreated		No			
Streptomycin standard (Firewall)	9 c	No symptoms	control	100 a*	symptoms	Untreated control		
Oxytetracycline standard (Fireline)	26 b	No symptoms	Streptomycin standard (Firewall)	65 ab	Black streaking on leaves	Streptomycin std (Firewall)		ita due to
Alum	71 a	Minimal	,				cold s	<mark>eason</mark>
Blossom Protect	31 b	Minimal	Alum	60 ab	No symptoms	Alum		
ET91 (Essential Oils)	32 b	Some fruit marking	ET91 (Essential Oils)	57 ab	Brown petals	ET91 (Essential Oils)		

Washington St Univ 2021

UC-Riverside 2021

Penn St Univ 2021

Treatment	Infection (%)	Safety (Fruit russet)	Treatment	Infection (%)	Safety (Fruit russet)	Treatment	Infection (%)	Safety (russet severity)
Untreated control	100 a*	0	Untreated control	100 a*	No symptoms	Untreated control	100 a*	1.54
Streptomycin std (Firewall)	42 c	0.06	Streptomycin std (Firewall)	5 c	No symptoms	Streptomycin std (Firewall)	45 b	1.3
Oxytetracycline std (Fireline)	44 c	0	Alum	55 ab	No symptoms	Oxytetracycline std (Fireline)	82 ab	0.86
,					No	Alum	74 ab	1.3
Blossom Protect 46 c	0.69	Blossom Protect	50 b	symptoms	Blossom Protect	73 ab	1.08	
ET91 (Essential Oils)	57 bc	0.06	ET91 (Essential Oils)	20 bc	No symptoms	ET91 (Essential Oils)	74 ab	0.86

^{*}Values followed by different letters are statistically different.



Efficacy and Safety of ET91

Treatment	Control (%)	Average Control (%)	Safety (Fruit russet)
Untreated control	0	0 (n=5)	No symptoms
Streptomycin standard (Firewall)	35 – 95	63 (n=5)	Minimal
Oxytetracycline standard (Fireline)	56 – 74	65 (n=2)	Minimal
Alum	29 – 45	39 (n=4)	Minimal
Blossom Protect	50 – 69	58 (n=3)	Minimal
ET91 (Essential Oils)	43 - 80	55 (n=5)	Minimal



Summary and Outlook

- The water-soluble Essential Oils product ET91 was effective in reducing fire blight infection by an average 55% based on five test results.
- ET91 significantly reduced fire blight infections over untreated control, and was comparable to the antibiotic standards and Blossom Protect, and better than Alum.
- ET91 was safe, producing no concerns of commercial downgrades.
- Unique product features: water-soluble, oil droplets encapsulated, freely dilutable, no shaking required, and reasonably light-stable.
- All ingredients are FDA GRAS. All but one or two are Minimum Risk Ingredients.
- We are seeking registration clearance and are open for collaboration to speed up commercialization.

