



**NON-GLP STUDY REPORT**

Date: July 2022

Sponsor: Bee Friendly Pest Control, LLC.

Study: Honey Bee Residual 22

Test Method: 310

**REPORT TITLE**

Evaluation of Bee friendly Yard and Garden Concentrate When Applied as a Residual Spray Application Against Honey Bees *Apis mellifera*

**STUDY**

Honey Bee Residual 22

**TRIAL**

APISME

**SPONSOR CODE**

N/A

**EXPERIMENTAL START DATE**

June 14, 2022

**EXPERIMENTAL COMPLETION DATE**

June 16, 2022

**REPORT DATE**

July 21, 2022

**TEST FACILITY/AUTHORS**

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**SPONSOR**

Bee Friendly Pest Control, LLC.

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**STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS**

No claim of confidentiality, on any basis whatsoever, is made for any information contained in this document. I acknowledge that information not designated as within the scope of FIFRA §10(d)(1)(A), (B), or (C) and which pertains to a registered or previously registered pesticide is not entitled to confidential treatment and may be released to the public, subject to the provisions regarding disclosure to multinational entities under FIFRA 10(g).

Submitter: \_\_\_\_\_ Date: \_\_\_\_\_

Typed Name of Signer: \_\_\_\_\_

Typed Name of Company: Bee Friendly Pest Control, LLC.



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**COMPLIANCE STATEMENT**

This study was NOT conducted in compliance with Good Laboratory Practice Standards as described by EPA (40 CFR Parts 160 and 792) and was never intended for that purpose.

Test Facility Management:  
07/21/22

Date: \_

Eric J. Snell

Snell Scientifics, LLC.

Study Director:

Date: 07/21/22

Todd Smith

Snell Scientifics, LLC.

Sponsor:

\_\_\_\_\_  
\_\_\_\_\_

Date: \_

Submitter:

\_\_\_\_\_  
\_\_\_\_\_

Date: \_



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**STUDY OBJECTIVE(S):**

To determine the mortality associated with the Bee Friendly Yard and Garden concentrate when applied as a residual spray application against honey bees *Apis mellifera*.

**TEST SUBSTANCE INFORMATION:**

#	Test Substance	Dilution Rate	Active Ingredients	EPA Reg. No. and/or Est. No.	Lot and/or Batch #	Snell Receipt Code
1	Controls - Untreated	N/A	N/A	N/A	N/A	N/A
2	Bee Friendly Yard & Garden	1:64	N/A	N/A	N/A	052022-1-A-BEE

**TEST SYSTEM INFORMATION:**

Trial	Test System	Strain	Stage	Source	Test Environmental Conditions
APISME	Honey Bee ( <i>Apis mellifera</i> )	Field	Workers	Field Collected	Incubator at 77°F/80%RH with a 12:12 L:D photoperiod

**MATERIALS AND METHODS:**

**310.1 Materials:**

*Test Arena Information:*

- 310.1.1 Surface Types: Camelia plant leaves (4” x 4” section of leaves). The plant leaves were harvested from the plant the day of testing. The test substance was applied to the surface.
- 310.1.2 Cover Type: Tulle mesh cartridge. The test systems were contained on the surfaces using the cover type listed.
- 310.1.3 Post-Treatment Arenas: 20oz SOLO cup. The Post-Treatment arenas were used to contain the test systems in a clean environment after exposure to the test substance(s).
- 310.1.4 Food/Moisture: 50:50 sucrose: water-soaked cotton ball.

*Test Equipment:*

- 310.1.5 Volumetric Measuring Equipment: Graduated cylinders and/or beakers were used as needed in preparing and/or measuring the flow rates of the test substance(s).
- 310.1.6 Digital Balance(s): Balances were used as needed in preparing and/or weighing the test substance canisters before and after applications.

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- 310.1.7 CO<sub>2</sub> and Regulator: A standard 20-pound CO<sub>2</sub> cylinder with regulator was used to anesthetize the test systems and sort them into the test arenas (prior to exposure to the test substances). The test systems were allowed to adequately recover from anesthetizing before being exposed to the test substance(s), and they were not anesthetized at any point following exposure to the test substance(s). Any additional transfers required after exposure to the test substances was conducted using methods that did not involve anesthetizing.
- 310.1.8 Intermediate Sorting/Transfer Containers: Additional sorting and transfer containers were used to aid in moving the test systems from the primary rearing/collection containers and into the treatment and/or post-treatment arenas.
- 310.1.9 Metronome/Timing Equipment: A metronome and/or other timing equipment were used as needed to assist in the timing when conducting the applications and/or when collecting the observations.

*Application Equipment:*

- 310.1.10 Application Equipment: Stihl SR 200 Backpack Mister.

**310.2 Methods:**

*Test Substance Preparation & Applications:*

- 310.2.1 The test substance was diluted with water and adequately shaken prior to applications.
- 310.2.2 The applications were conducted using an approximate 2 gal/10,000 sq. ft. application rate with a Stihl SR 200 backpack sprayer:
  - Sprayer settings
    - Nozzle setting = 2
    - Spray speed setting = low
    - Flow rate = 1 gal/6.5 min (3,785mL/390 sec = 9.7mL/sec)
  - Spray distance/measurements
    - Spray distance = 6 ft
    - Spray swath = ~24 inches
  - Application details
    - 2 gal (7,571mL)/10,000 sq. ft. (1,440,000 sq. in.) = 0.005mL/sq. in.
    - 40 linear inches (960 sq. in.) sprayed in 0.5 second to apply approximately 4.8mL
- 310.2.3 The test systems were exposed to the treated surfaces at approximately 1 hour after the applications were conducted.

*Test Design:*

- 310.2.4 The evaluations of this study followed the photographs in the Appendix A: Photograph section of the report.
- 310.2.5 Prior to applications, each Surface Type and/or Post-Treatment Arena was labeled with a test substance code and a replicate number. The arenas were positioned on a clean tray and grouped together per test substance type. The tray(s) with the Surface Types and/or

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Post-Treatment Arenas were also labeled using the study name, trial name, and the study initiation date (as a duplicate means of ensuring accurate data collection).

- 310.2.6 The test systems were sorted onto the surfaces using the appropriate methods based on the species type.
- 310.2.7 All the test systems were confirmed to be of “good vigor” (alive) prior to testing.
  - 310.2.7.1 Only live test systems were selected for use in the study.
  - 310.2.7.2 After all test systems were transferred onto the surfaces, they were confirmed to be alive and exhibiting normal behavior before continuing with the study.
- 310.2.8 Four surface replicates were conducted for each population (controls and treatments) with ten test systems (40 per population) exposed to each dried surface replicate.

*Observation Methods:*

- 311.2.1 The number of “Alive”, “Knockdown”, and “Dead” test systems per arena was recorded prior to surface exposure (Pre-trt), and at 30 mins, 1 hr, 2 hrs, 4 hrs, 24 hrs, and 48 hrs after the applications.
- 310.2.9 The observations were collected by raising the test arenas and gently blowing air on the test systems to provoke movement, lightly prodding the test systems, or the test arenas were shaken/agitated to provoke test system movement.
- 310.2.10 The test systems were transferred from the surfaces into the clean Post-Treatment Arenas 1 hour after exposure.
- 310.2.11 Definitions of “Alive”, “Knockdown”, and “Dead”:
  - 310.2.11.1 Alive – Test System exhibited normal forward motion and/or the ability to fly.
  - 310.2.11.2 Knockdown (KD) – Test System exhibited some movement but could not crawl and/or fly.
  - 310.2.11.3 Dead - Test System exhibited no movement when stimulated.

*Environmental Conditions:*

- 310.2.12 Average environmental conditions recorded during the study:
  - 310.2.12.1 Applications: Temperature: 85°F Humidity: 70%
  - 310.2.12.2 Laboratory: Temperature: 71°F Humidity: 54%
  - 310.2.12.3 Incubator: Temperature: 77°F Humidity: 80%



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**RESULTS:**

The results of this study are shown in Table 1, which illustrates the percent mortality and Abbott’s corrected percent mortality for the honey bees after exposure to the treated surfaces.

$$\text{Percent Mortality} = \text{Number Dead} / \text{Total Population} \times 100$$

$$\text{Abbott's Percent Mortality} = (1 - (1 - \text{Treatment Mortality}) / (1 - \text{Control Mortality}))$$

The honey bees that were exposed to the leaves treated with Bee Friendly Yard and Garden formulation (1:64 dilution) recorded 5% mortality at 24 and 48 hours after exposure. The untreated population recorded 0% mortality during the study.

**STATISTICAL ANALYSIS:**

Due to the minimal mortality associated with the Bee Friendly Yard and Garden treated population, no statistical analysis was conducted on the results.

**TABLES:**

**Table 1.**

Test Substance:	Percent Mortality						
	Pre-trt	30 min	1 hr	2 hr	4 hr	24 hr	48 hr
Control - Untreated	0%	0%	0%	0%	0%	0%	0%
Bee Friendly Yard and Garden (1:64 dilution rate)	0%	0%	0%	0%	3%	5%	5%
Abbott's Percent Mortality (1925.)							
Bee Friendly Yard and Garden (1:64 dilution rate)	0%	0%	0%	0%	3%	5%	5%

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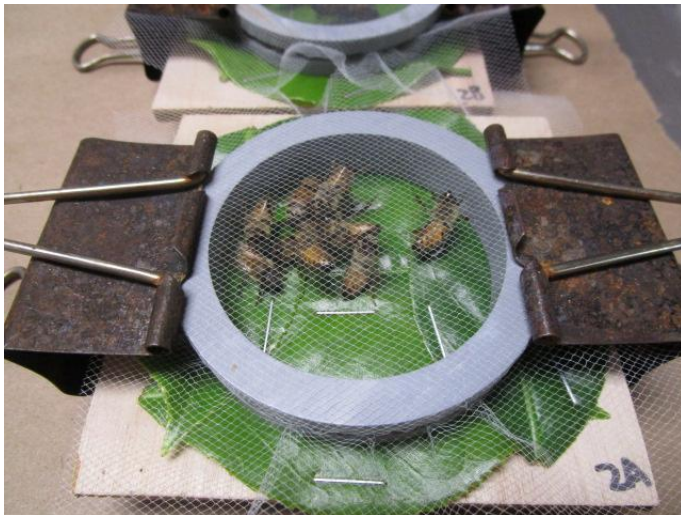


**APPENDIX A: PHOTOGRAPHS**

**Photograph 1.** Test Substance



**Photograph 2.** Treated Leaves with Honey Bees



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**Photograph 3.** Application on Leaves with Stihl SR 200 Sprayer



**Photograph 4.** Honey Bees in Post-Treatment Arena



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**APPENDIX B: RAW DATA**

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Study: Honey Bee Residual 22 Trial: APISME

**Test Arena Info:**  
 Treatment Arena: 4" x 4" panel covered with Camellia leaves, mesh lid as cover.  
 Post-Trt Arena: 20oz SOLO cup with mesh lid.  
 Food/Moisture: 50/50 sucrose water soaked cotton ball.  
 Incubator set to 77°F/80%RH

**Test System:** Honey Bee  
 Strain/Stage/Age: Field/Workers  
 Exp. Time/Date: 10:35AM / 06/14/22  
 Exp. Duration: 1 hr

**Test Substance Info:**  
 Mix Time/Date: 9:19AM / 06/14/22  
 Treatment Time/Date: 9:28AM / 06/14/22  
 Dry Time/Evaluation: 0 Day (Fresh)

**Asterisk Info:**  
 \* = \_\_\_\_\_  
 \*\* = \_\_\_\_\_  
 \*\*\* = \_\_\_\_\_

**Start Date/Environmental Conditions:** Rep #s: A-D Date(s): 06/14/22 Temp (F): 85 RH%: 70  
 Rep #s: \_\_\_\_\_ Date(s): \_\_\_\_\_ Temp (F): \_\_\_\_\_ RH%: \_\_\_\_\_

**Application:** Temp (F): 70 RH%: \_\_\_\_\_  
 Temp (F): \_\_\_\_\_ RH%: \_\_\_\_\_

**Laboratory:** Temp (F): 71 RH%: 54  
 Temp (F): \_\_\_\_\_ RH%: \_\_\_\_\_

Test Substance/Mix Rate:		Control - Untreated							
Application Details:		N/A							
Rep	Cond.	Pre-trt	30 min	1 hr	2 hr	4 hr	24 hr	48 hr	
A	Alive	10	10	10	10	10	10	10	
	KD	0	0	0	0	0	0	0	
	Dead	0	0	0	0	0	0	0	
B	Alive	10	10	10	10	10	10	10	
	KD	0	0	0	0	0	0	0	
	Dead	0	0	0	0	0	0	0	
C	Alive	10	10	10	10	10	10	10	
	KD	0	0	0	0	0	0	0	
	Dead	0	0	0	0	0	0	0	
D	Alive	10	10	10	10	10	10	10	
	KD	0	0	0	0	0	0	0	
	Dead	0	0	0	0	0	0	0	
Initials:		AK	AK	AK	AK	AK	AK	AK	
Date:		06/14/22	06/14/22	06/14/22	06/14/22	06/14/22	06/15/22	06/14/22	

Test Substance/Mix Rate:		Bee Friendly Yard and Garden (1:64 dilution rate, 29.6ml/1892.71ml water)							
Application Details:		40 linear inches in 0.5 second spray from a 6' distance using 5Bhl 2.5 gallon gas powered sprayer set on low speed with misting head set on 2							
Rep	Cond.	Pre-trt	30 min	1 hr	2 hr	4 hr	24 hr	48 hr	
A	Alive	10	10	10	10	10	9	9	
	KD	0	0	0	0	0	0	0	
	Dead	0	0	0	0	0	1	1	
B	Alive	10	10	10	10	10	10	10	
	KD	0	0	0	0	0	0	0	
	Dead	0	0	0	0	0	0	0	
C	Alive	10	10	10	10	10	10	10	
	KD	0	0	0	0	0	0	0	
	Dead	0	0	0	0	0	0	0	
D	Alive	10	10	10	10	9	9	9	
	KD	0	0	0	0	0	0	0	
	Dead	0	0	0	0	1	1	1	
Initials:		AK	AK	AK	AK	AK	AK	AK	
Date:		06/14/22	06/14/22	06/14/22	06/14/22	06/14/22	06/15/22	06/14/22	

Researcher(s):  
 Name: Joshua Smith Signature: [Signature] Date(s): 06/14/22 Role: Princ. Inv.  
 Name: \_\_\_\_\_ Signature: \_\_\_\_\_ Date(s): \_\_\_\_\_ Role: \_\_\_\_\_