REPORT TITLE

Evaluation of Bee friendly Yard and Garden Concentrate when applied as a Direct Spray Application Against Honey Bees *Apis mellifera*

STUDY

Honey Bee Direct 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.

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

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Submitter: _____ Date: _____

Typed Name of Signer: _____

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

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: Date: 07/21/22

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 direct 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 (*Apis mellifera*)	Field	Workers	Field Collected	Incubator at 77°F/80%RH with a 12:12 L:D photoperiod

MATERIALS AND METHODS:

311.1 **Materials:**

Test Arena Information:

311.1.1 Treatment Arenas: 2.25" CPVC mesh cartridge with Tulle mesh. The Test Arenas were used to contain the test systems during the test substance applications.

311.1.2 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).

311.1.3 Food/Moisture: 50:50 sucrose: water-soaked cotton ball.

Test Equipment:

311.1.4 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).

311.1.5 Digital Balance(s): Balances were used as needed in preparing and/or weighing the test substance canisters before and after applications.

311.1.6 CO_2 and Regulator: A standard CO_2 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.

311.1.7 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.

311.1.8 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:

311.1.9 Application Equipment: Stihl SR 200 Backpack Mister.

312.2**Methods:**

Test Design:

311.2.1 The evaluations of this study followed the photographs in the Appendix A: Photograph section of the report.

311.2.2 Each Treatment 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 Treatment and 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).

311.2.3 The test systems were sorted into the Treatment Arenas using the appropriate methods based on the species type.

311.2.4 All test systems were confirmed to be of "good vigor" (alive) prior to exposure to the test substance(s).

311.2.4.1 Only live test systems were selected for use in the study.

311.2.4.2 After all test systems were transferred into the test arenas, they were confirmed to be alive and exhibiting normal behavior before continuing with the study.

311.2.5 The number of replicates conducted per test substance and the number of test systems evaluated per replicate and trial were as follows:

# Reps per Substance	# Systems per Rep	# Systems per Substance	# Test Substances	Total # Systems
4	10	40	2	80

Test Substance Preparation & Applications:

311.2.6 The test substance was diluted with water and adequately shaken prior to applications.

311.2.7 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

Observation Methods:

311.2.8 The number of "Alive", "Knockdown (KD)", and "Dead" test systems per arena were recorded prior to applications (Pre-trt), and at 30 mins, 1 hr, 2 hrs, 4 hrs, 24 hrs, and 48 hrs after the applications.

311.2.9 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.

311.2.10 The test systems were transferred from the Treatment Arenas into the clean Post-Treatment Arenas 1-hour after the applications.

311.2.11 Definitions of "Alive", "Knockdown (KD)", and "Dead":

311.2.11.1 Alive – Test System exhibited normal forward motion and/or the ability to fly.

311.2.11.2 Knockdown (KD) – Test System exhibited some movement but could not crawl and/or fly.

311.2.11.3 Dead - Test System exhibited no movement when stimulated.

Environmental Conditions:

311.2.12 The test systems were tested under the following conditions.

311.2.13 Average environmental conditions recorded during the study:

311.2.13.1	Applications:	Temperature: 85°F	Humidity: 70%
311.2.13.2	Laboratory:	Temperature: 71°F	Humidity: 54%
311.2.13.3	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 applications.

Percent Mortality = Number Dead/Total Population X 100
Abbott's Percent Mortality = (1-(1-Treatment Mortality) / (1-Control Mortality))

The honey bees that were treated with Bee Friendly Yard and Garden formulation (1:64 dilution) recorded 3% mortality at 24 and 48 hours after the applications. 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.

Percent Mortality							
Test Substance:	**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%	0%	3%	3%
Abbott's Percent Mortality (1925.)							
Bee Friendly Yard and Garden (1:64 dilution rate)	0%	0%	0%	0%	0%	3%	3%

APPENDIX A: PHOTOGRAPHS

Photograph 1. Test Substance

Photograph 2. Mesh Cartridge with Honey Bees

Photograph 3. Application with Stihl SR 200 Sprayer

Photograph 4. Honey Bees in Post-Treatment Arena

APPENDIX B: RAW DATA

Sponsor: Bee Friendly Pest Control, LLC. **TM#:** 311 **Page** 1 of 1

Study: Honey Bee Direct 22 **Trial:** APISME

Test Arena Info:
Treatment Arena: 2.25" CPVC cartridge with tulle mesh.
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: 9:25AM 06/14/22
Exp. Duration: 1 hr

Test Substance Info:
Mix Time/Date: 9:19AM 06/14/22
Treatment Time/Date: 9:25AM 06/14/22
Dry Time/Evaluation: N/A

Asterisk Info:
* =
** =
*** =

Start Date/Environmental Conditions: **Application:** **Laboratory:**
Rep #'s: A-D Date(s): 06/14/22 Temp (F): 85 RH%: 70 Temp (F): 71 RH%: 54
Rep #'s: Date(s): Temp (F): RH%: Temp (F): RH%:

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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:								
Date:		06/14/22	06/14/22	06/14/22	06/14/22	06/14/22	06/15/22	06/16/22

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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 Stihl 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	9	9	9
	KD	0	0	0	0	1	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	10	10	10
	KD	0	0	0	0	0	0	0
	Dead	0	0	0	0	0	0	0
Initials:								
Date:		06/14/22	06/14/22	06/14/22	06/14/22	06/14/22	06/15/22	06/16/22

Researcher(s):
Name: Joshua Scott Signature: Joshua Scott Date(s): 06/14/22 Role: Princ. Inv.
Name: Signature: Date(s): Role: