



Aedes albopictus / aegypti
Aedes sollicitans
Culex quinquefasciatus
Culex tarsalis
Ochlerotatus triseriatus
Anopheles

10/02/2019

CGCI Environmental Laboratory
Phase I to 3 evaluation Vector Control Research project number #01012019
Botanical FIFRA (25b) BigShot Maxim Concentrate – made with organic ingredients.
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- MONDAY, Aug. 20, 2018 (HealthDay News) -- A new class of natural-based mosquito repellents appears to be effective; researchers report.
- Each year, nearly 700 million people worldwide contract mosquito-borne diseases, such as malaria, West Nile, Zika and dengue fever, resulting in more than 1 million deaths.
- Many mosquito species have become resistant to commonly used pyrethroid-based insecticides, so an urgent search is on for alternatives.

Name	Preferred Habitat	Hours of Activity	Range
Aedes	Temporary floodwater pools, fresh and brackish marshes, containers.	All hours	Worldwide distribution, including extreme northern latitudes
Anopheles	Fresh- or salt-water marshes, swamps, grassy ditches, the edges of streams and rivers, and small, temporary rain pools.	Dawn, dusk, at night	Temperate, subtropical and tropical areas worldwide
Culex	Freshwater pools, ditches, ponds, and sewage treatment plants.	Dusk, daytime	Tropics to cool temperate regions worldwide
Mansonia	Aquatic plants, water lettuce, and cattails.	Sunset	Tropics worldwide
Psorophora	Temporary floodwaters, woodland pools, roadside ditches, and pastures.	Early evening, daytime in shade	Tropics and warmer temperate regions of North and South America
Wyeomyia	Bromeliad habitats and pitcher plants.	Daytime	Central and South America, the Caribbean and Florida

- Text book-Industrial Toxicology (safety and Health Applications in the Workplace) Ga tech University, Williams and Burson– page 128 3.) “Pyrethrin’s” (the most prolific pesticide used globally) “ These are similar in action to DDT.

COMMON PESTICIDES

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3 PYRETHROID INSECTICIDES. Examples: (active ingredient: Resmethrin) is a synthetic pyrethroid insecticide. Pyrethroids affect the nervous system. They have been linked with liver and thyroid problems and they can also interfere with the immune and endocrine systems. Also contains the synergist (a chemical that increases the effectiveness of the active ingredient), pipernyl butoxide, which is classified by the EPA as a possible human carcinogen. Example: (active ingredient: Sumithrin) is a synthetic pyrethroid insecticide, which may affect the central nervous system. these products contain 10% pipernyl butoxide. Sumithrin was shown to demonstrate significant estrogenicity in a 1999 study.¹ at the Mt. Sinai School of Medicine. This means it may promote tumor growth in cancers of the reproductive organs including breast cancer and prostate cancer. 1. Estrogenic and Antiprogestagenic Activities of Pyrethroid Insecticides. Biochemical and Biophysical Research Communications, October 1998, vol.251, no.3, p.855-859. PERMETHRIN is a synthetic pyrethroid insecticide and neurotoxin. It is more acutely toxic to children than to adults. The US Environmental Protection Agency (EPA) has classified it as a human carcinogen and it has been shown to cause immune system damage as well as birth defects. Note: Pyrethroids are highly toxic to fish, crustaceans, and bees. For that reason, EPA has established restrictions that prohibit their direct application to open water within 100 feet of lakes, streams, rivers, or bays.

MALATHION is an organophosphate insecticide that can cause acute and long-term neurological health problems. Malathion is being reviewed by the EPA for its potential as a low-level carcinogen. It is toxic to fish and highly toxic to aquatic invertebrates and amphibians.

A solution

The 'next-gen' spatial repellents are new tools that could provide safe and novel protection against mosquitoes in treated yards, parks, campgrounds, horse stables and livestock facilities. The study herein provides an efficacy study of a Botanical organic Product called; BigShot Maxim Concentrate. A FIFRA (25b) made from organic Pesticide.

The Evaluation

Phase I to 3 evaluation Vector Control Research project number #01012019
Botanical FIFRA(25b) BigShot Maxim Concentrate

Phase 1) The effectiveness of the pesticide Bigshot Maxim Botanical Mosquito control product .

Phase I is a laboratory evaluation and reflects the actual efficacy of the Pesticide in the actual application. Chamber method (18"x9"). The inner surface is smooth and impervious to insecticides and free from cracks and projections. The Peet-Grady Chamber testing method was used for adult flying mosquitos and a glass jar method was used for the evaluation of the larvicidal phase testing.

Phase 2) is an evaluation which consisted of several small-scale field trial inclusive of outdoor assessments of area spray formulations including residual assessments of impregnated typical building materials shrubs and trees

Phase 3) to provide the actual control outcome from the use of the Botanical FIFRA(25b) BigShot Maxim pesticide such as reduction in the density of the target species.

Phase 1

The Apparatus, procedure and conditions for official valuation are described. The mosquitos were collected from low lying wooded areas near Oakwood Georgia in CDC mini light traps and were kept at a temperature of 80-85°F. at a relative humidity of 40-70 per cent., and the testing room during tests at 75-35°F. These were natural site collected as larvae for testing and the adult mosquitos collected from the rearing chambers aged 2 to 5 days for adult mosquito testing. The adult mosquitos were captured via a CO₂-baited light trap.

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Adult mosquitos

The test chamber consisted of a tight-fitting port opening for the introduction of the insecticide of uniform distribution. The atomizer was a commercially available 32 oz sprayer operated at a pressure of 12.5+.0.5 lb. per sq. in. A discharge rate of (2 each .803 gm/ 1 sec-spray) was discharged into the Peet-Grady Chamber of 20 oz per gallon from concentrate or 15.7% from concentrate solution. The spray was applied through an introduction port of the chamber. Knockdown of mosquitoes was observed at the indicated intervals of 5 minutes, 10 minutes, 20 minutes, 60 minutes and, 15 days and 31 days. When *the natural site adult mosquitos* were exposed to the box after treatment over the first hour BigShot produced 100% mortality. At a 60-minute post exposure on day 15, mosquitoes exposed to the treated box and had a 98% mortality and at day 31 a 48% mortality was observed at 60-minute post exposure.

larvae

The test chamber consisted of a tight-fitting port opening for the introduction of the insecticide of uniform distribution. The atomizer was a commercially available 32 oz trigger sprayer operated at a pressure of 12.5+.0.5 lb. per sq. in. A discharge rate of (.803 gm/ 1 sec-spray) was discharged into the Peet-Grady Chamber of 20 oz per gallon from concentrate or 15.7% from concentrate solution with a glass jar containing 100 larvae. The spray was applied through introduction ports of the chamber. Knockdown of mosquito larvae was observed at the indicated intervals.

The Artificial Breeding Containers.

A natural location was chosen in a low very shaded wooded area near a slow-moving water source outside to raise the mosquito larvae. Water was collected from the local water source. The mosquito larvae were scooped out of the containers with a small fish net. Two containers were used in order to maintain a continual supply of larvae uncovered in the field location. Approximately every nine days larvae were collected from the containers along with a portion of the water then placed in a separate container covered with a cheese cloth lid. The larvae were stored in the laboratory and maintained at a temperature of (80F to 85F) and relative humidity (81- 85%). The larval diet. mainly carbohydrate products used were: bread, powdered rice and wheat bran flour.

Natural Site Common Species

Standing Water Species

Aedes albopictus / aegypti

Aedes sollicitans

Culex quinquefasciatus

Culex tarsalis

Ochlerotatus triseriatus, Anopheles'

Using natural untreated water as control. Three sets of experiments were carried out with the residual activities being monitored. The study was conducted using different sized containers. The water was allowed to stand for at least 48 hours prior to the experiment. 100 mosquito larvae (late third or early fourth instars) each of the mosquito species from field collected colonies were introduced separately into jars at specific times (24 h, (then tested) day 3(then tested), day 7(then tested), week 4(then tested), week 5(then tested), residual periods). Larval mortality was recorded at 5 min., 10 min. 20 min, 40 min, 60 min and 48 hours. post-treatment at each introduction period.

A treatment regimen was adopted: 5 sets of 3 jars (treatment at 15.8 % from concentrate solution or 19.7 oz per gallon from concentrate dosage including 5 controls) One set was not subjected to any replenishment of water, one set received replacement water from natural source (the water is only topped up to the water-level mark when evaporation occurred); the other set (also 5 jars received no dosage (control) is given a daily replenishment of about 15% of water from the total tested volume so that there was a weekly turnover of the whole volume.) The daily replenishment was to simulate daily usage of water in the container.

Active ingredient botanicals	Associated product and/or mixture	Mosquito species evaluated <i>Aedes albopictus / aegypti</i> <i>Aedes sollicitans</i> <i>Culex quinquefasciatus</i> <i>Culex tarsalis</i> <i>Ochlerotatus triseriatus, Anopheles</i>	Method of application	Method of assessment Peet-Grady Chamber testing	Finding Laboratory phase 1
BIGSHOT MAXIM	15.7% from concentrate	Adult Larvae	Compression sprayer	Landing count	

100 mosquito larvae-field captured placed in each of the 5 sets of 3 jars - test were Male, female representative of common species in Georgia.

Jar Control - mortality						
Day Exposure Interval check time.	Day 1	Day 3,	Day 7,	4 weeks	5 weeks	
	<u>Larvae</u>					
5-10 min	0%	0%	2%	1%	3%	
20 min	0%	0%	0%	0%	0%	
40 min completed in lab	0%	2%	1%	2%	0%	
60 min completed in lab	1%	1%	1%	0%	0%	
48 hrs completed in lab	2%	0%	2%	0%	1%	

Jar Treated with field water replenish to evaporated fill- mortality

Day Exposure Interval check time	<u>Larvae</u>					
5-10 min	100%	99%	98%	89%	68%	
20 min	100%	100%	100%	91%	71%	
40 min completed in lab	100%	100%	100%	89%	78%	
60 min completed in lab	100%	100%	100%	91%	81%	
48 hrs completed in lab	100%	100%	100%	100%	89%	

Jar Un-Treated with natural field water replenish - mortality

Day Exposure Interval check time.	<u>Larvae</u>					
5-10 min	100%	99%	98%	98%	99%	
20 min	100%	100%	99%	98%	100%	

Jar Control - mortality

Day Exposure Interval check time. Day 1 Day 3, Day 7, 4 weeks 5 weeks

40 min completed in lab 100% 100% 100% 100% 100%

60 min completed in lab 100% 100% 100% 100% 100%

6 48 hrs completed in lab 100% 100% 100% 100% 100%

Phase 2

Outdoor Assessment of Spray Formulations. The test site for outdoor fogging trial was an open space measuring more than 43560 sf approx..1 acre. The following environmental parameters were recorded for the trial: time of spraying (30 minutes), temperature (80F to 85F), relative humidity (81%) (no rainfall when spraying). In addition, wind direction and velocity (0.5 – 3.0 m/s) was also recorded in the outdoor trial. For the outdoor trial, the back pack fogger was sprayed at ounces per gallon from concentrate or 15.7% solution from concentrate, with the head nozzle pointing upwards at an angle of 30 degrees to the horizontal plane. The sprayer traveled around the area perpendicular to the spray angle at a speed of 3-5 mph.

The efficacy of the insecticide was assessed at 20 and 75 meters (a total of 2 checkpoints) downwind of the Backpack sprayer. Water sensitive papers were set at each check point as indicators of the presence of the insecticides at each of the check points. The adulticidal effect was assessed by keeping twenty adult mosquitoes of the respective mosquito species in a cylindrical cage constructed of fine mesh fabric (nylon) with wire frame support (diameter 10 cm x height 15 cm x tapping cover 10 cm). One mesh size, 0.5 mm was used. For Aedes , Culex quinquefasciatus and Aedes albopictus. The respective caged mosquitoes are placed approximately 1.5 meters above the ground at each checkpoint. Knockdown of mosquitoes are read at 0, 10, 20 30, 40- and 60-minutes post-spraying. After field exposure of 30 minutes, the mosquitoes were brought back to the laboratory at a temperature of 80-85 F and relative humidity of 80-85% rH% and transferred into clean polyethylene cups with 10% sucrose pad. The 0, 10, 20- and 30-minute knockdown readings were read in the field, while the 40- and 60-minute knockdown readings were read in the laboratory. The transportation time between field and laboratory was about 15 minutes. Mortality of mosquitoes is recorded at 24 h post treatment. The same protocol was followed for the control using water. The Larvicidal effect was assessed by placing twenty larvae of the respective mosquito species in separate paper cups on the ground below the adult cages. After field exposures of approx. 32 minutes, the larvae were brought back to the laboratory and kept in a laboratory environment at a temperature of 83 to 85F and relative humidity of 65 - 85%. Mortality of larvae were recorded at 24 h post treatment. The droplet size of the STIHL fogger is listed as 5-25-microns droplet size in the equipment literature.

Summary of active ingredients and products evaluated in laboratory, semi-field, field barrier spray studies. Mosquito species, method of application, mosquito surveillance method, and results are shown below:

Active ingredient	Associated product and/or mixture	Mosquito species evaluated	Method of application	Method of assessment	Finding Field phase 2
BIGSHOT	15.7% from concentrate	<i>Aedes albopictus</i> / <i>aegypti</i> <i>Aedes sollicitans</i> <i>Culex quinquefasciatus</i> <i>Culex tarsalis</i> <i>Ochlerotatus triseriatus</i> , <i>Anopheles</i>	Compression sprayer	Peet-Grady Chamber testing	Landing count
MAXIM		Adult Larvae			

100 mosquitos-field captured placed in each 10cmx 15cm (4x 6-inch mesh fabric nylon trap with 4-inch trap cover). Male, female representative of common species in Georgia.

Trap A - mortality

20 meters from spray downwind, (85/cm²) Water sensitive paper

Interval check time -post spray.	<u>Adult</u>
0 min	0%
20 min	48%
40 min completed in lab	87%
60 min completed in lab	91.8%
48 hrs completed in lab	99.8%

Trap B- mortality

75 meters from spray downwind, (60/cm²) Water sensitive paper

Interval check post spray.	<u>Adult</u>
0 min	0%
20 min	32%
40 min completed in lab	58%
60 min completed in lab	71%
48 hrs completed in lab	99.7%

(87%–99.7% reduction) were greatest in the 48 to 72 hours

Water sensitive Paper utilized at each field test location to determine coverage - Syngnta Teejet water sensitive paper 52x76mm.

Research and experience suggest that 85 discrete fine/medium-sized droplets per square centimeter and a total coverage of 15% should be sufficient for most foliar insecticides and fungicides. The papers won't show the finest droplets (<50 µm), so there may be a greater coverage of spray even though it was not observed.

The machines used to apply the barrier application appeared to be critical to the effectiveness of the application against mosquitoes. The vegetation Leaves treated with the STIHL backpack sprayer showed deposition on the top versus the bottom of leaves and peak deposition occurred 1.2 m into the vegetation. Droplet coverage was

significantly affected by sprayer type, card depth, and vegetation height. The BigShot product had none to minimal effect on beneficial insect populations, no reported effect on Apis, Bombus, Nymphalidae or Vespidae.

Classification of adult mosquitoes as alive, knocked down or dead in bioassays is summarized below:

ALIVE	KNOCKED DOWN OR DEAD AFTER EXPOSURE	
	MORIBUND	DEAD
Can both stand and fly in a coordinated manner	<ul style="list-style-type: none"> • Cannot stand (e.g. has only one or two legs) • Cannot fly in a coordinated manner • Lies on its back, moving legs and wings but unable to take off • Can stand and take off briefly but rapidly falls down 	<ul style="list-style-type: none"> • No sign of life • Immobile • Cannot stand

Phase 3 Trial

After evaluations the 10-19.7 ounces (per 3 gallons clean water formulation from concentrate) of Bigshot Maxim was carried out. The trial was conducted in a residential area of Florida, Georgia and Kansas. This field trial was conducted in collaboration with a national RESIDENTIAL mosquito control company. A total of 100-150 houses were chosen for the Big Shot Botanical mosquito control evaluation.

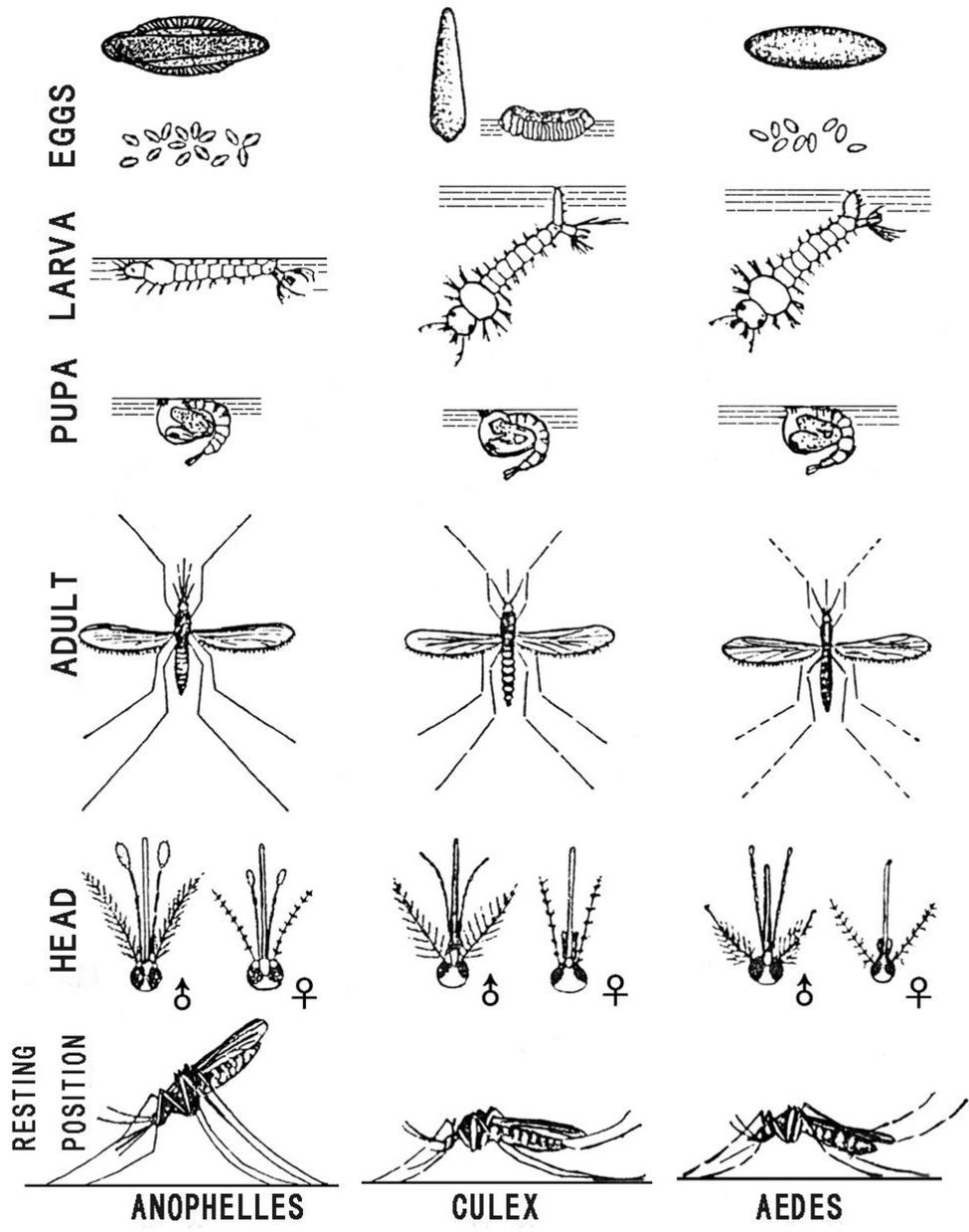
The Botanical organic Bigshot Maxim Concentrate mosquito control product was field evaluated on blocks of properties in several neighborhoods over 4 weeks from 8/01/2019 to 9/02/2019. The Properties were treated by commercial operators using backpack mist blowers.

In south Florida the results reported by the company as reported.” The technicians using the product preferred it over the current bifenthrin and pyrethrin based products.” No resprays were needed over the 30 days. Control of the mosquito populations were improved and the public preferred the BIGSHOT Botanical mosquito control product as a safe and friendly natural mosquito control product.

The first 30-day trial used 19.5 ounces per 3 gallons of water. During this period no resprays were needed to control the residentially applied areas and rain fall during the trial was less than 1 inch.

A second 30-day trial using 10 ounces per 3-gallons of water was completed. During this period no resprays were needed to control the residentially applied areas for 25 days and rain fall during this period was 2.5 inches.

Morphology:



GEORGIA MOSQUITOS FOUND IN NATURAL SITE BRACKISH WOODED AREA FLOWERY BRANCH GEORGIA

Culex spp

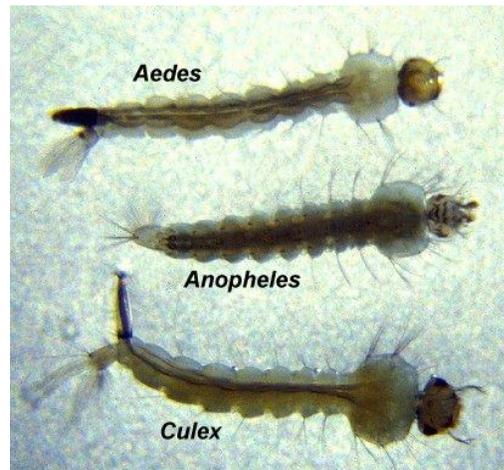


vectors of WNV

Anopheles quadrimaculatus



Aedes aegypti





Field Trial glass and wood application Post 31 days 19.5oz per gallon dilution into 3 gallons clean water



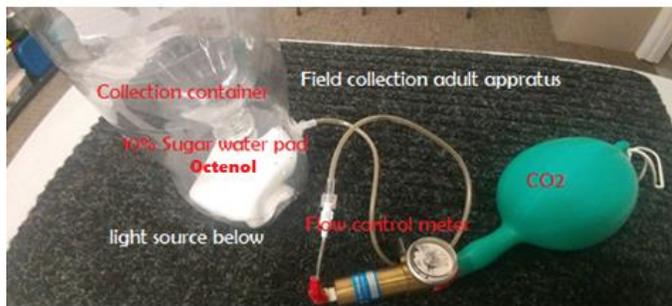
Live larvae before bigShot in natural water source



Mortality larvae 98% (5 min after post application
Natural water source)



Test Chamber



Attractant Test



CDC Mini Light Trap



Transport containers adult



Fogger



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