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Toxicity and Efficacy of Two Emulsifiable Concentrates of 2-Tridecanone against Red Imported Fire Ants

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Abstract

2-tridecanone is a major constitute of defensive secretion in tawny crazy ants, *Nylanderia fulva* that is an ant species reported to be able to displace red imported fire ants, *Solenopsis invicta* in the field. 2-tridecanone was proven to be toxic to *S. invicta*. Although 2-tridecanone is commercially available, utilization of this naturally occurring compound in fire ant management has received little attention. In this study, toxicity and efficacy of two 2-tridecanone formulations were assessed against *S. invicta*. Two emulsifiable concentrates were prepared using 2-tridecanone as an active ingredient, vegetable oil as a solvent and polyoxyethylene tridecyl ether as a surfactant. Piperonyl butoxide (PBO) was used as a synergist in one formulation. Both formulations showed significant toxicity in laboratory bioassays. In a field trial, at application rate of 5.28 mL/L and 14 days after mound drench treatment, 100% control was achieved for formulation with PBO and 90% control for the formulation without PBO. In conclusion, both formulations had significant efficacy against *S. invicta*. Due to the low mammalian toxicity of 2-tridecanone, no involvement of hazardous synthetic organic solvents, no phytotoxicity at applied concentrations, and relatively low cost, both formulations are promising alternatives to commercial insecticide products for fire ant mound drench. The outstanding efficacy of both formulations observed in this study warrants further research on their efficacy against other pest insects.

Keywords

Defensive Chemical, Fire Ant Control, Mound Drench

1. Introduction

The red imported fire ant, *Solenopsis invicta* Buren, is a significant pest affecting humans, wildlife, crops, and livestock [1]. Synthetic insecticides are commonly used in fire ant management [2] [3]. Insecticide products exert toxic effect on fire ants through either contact or bait ingestion. Contact based products are applied using the following methods based on their formulations: 1) liquids for individual mound drench, 2) granules for mound surface treatment, which is followed by water application in order to facilitate the active ingredient to reach the target, 3) granules or dusts for mound surface treatment without applying water, 4) liquid or aerosol for mound injection, 5) liquids or granules for broadcast over the entire treatment area, and 6) liquid for Immersion/dip treatment to treat containerized nursery stock and balled-and-burlapped plants for quarantine purpose [4]. Baits are usually applied by broadcasting; however they can also be used to treat individual mounds [3]. Contact based products usually provide faster control than bait because baits require time for ants to trophallactically spread the toxicant within the colony. In some situations, contact based products are the only option if a quick control is desired.

Numerous synthetic insecticides have been registered for fire ant control, particularly in contact based products [3]. Due to the ever increasing concern to the negative impact of synthetic insecticides on human health and environment, the use of more toxicologically and environmentally benign chemicals is highly desirable. Chemicals from natural products are believed to be more desirable insecticides than conventional synthetic insecticides, due to their rapid environmental biodegradable property and lower toxicity to natural enemies, humans and other mammals [5] [6].

Plants have been a major source of natural insecticides [5] [6]. Well-known examples include pyrethrins from *Chrysanthemum cinerariifolium* in Asteraceae family, nicotine and related alkaloids from *Nicotiana* spp. in Solanaceae family, rotenone from *Lonchocarpus* spp. and *Derris* spp. in Fabaceae family, sabadilla from *Schoenocaulon* spp. in Melanthiaceae family, azadirachtin from *Azadirachta indica* in Meliaceae family (Neem), and essential oils and their derivatives in plants from numerous families, such as Myrtaceae, Lauraceae, Rutaceae, Lamisceae, Asteraceae, Apiaceae, Cupressaceae, Poaceae, Zingiberaceae and Piperaceae [6]. Several plant-derived compounds have been tested against red imported fire ants, such as d-limonene, pyrethrins, rotenone, pine oil and turpentine [3] [7].

Another potential source of natural toxins against fire ants is the defensive chemicals in other ants. Chemical defense is common in ants [8] [9]. As one of primary defense mechanisms, defensive secretions often determine the outcome of interspecific competition between ants [10]-[13]. Many ants compete with *S. invicta* [14]-[24] with one prominent example being the tawny crazy ant, *Nylanderia fulva* (Mayr). Tawny crazy ant is an ant species reported to be able to displace *S. invicta* in the field [13]. Tawny crazy ant workers produce formic acid in their poison glands and methylketones and alkanes in Dufour glands. Of these compounds, 2-tridecanone has the smallest LD_{50} value against *S. invicta* (18.51 to 24.67 μg/ant) [25], and may be a promising naturally occurring toxin that can be used in fire ant management. In this study, two emulsifiable concentrates were prepared using 2-tridecanone as an active ingredient, soybean oil as a solvent and polyoxyethylene tridecyl ether as an emulsifier. Since piperonyl butoxide (PBO) was found to improve the toxicity of 2-tridecanone against imported fire ants (Chen, unpublished data), PBO was used as a synergist in one formulation. For both formulations, contact and immersion toxicity were determined in the laboratory and efficacy as mound drench treatment was assessed in the field.

2. Materials and Methods

2.1. Insects

Red imported fire ant colonies were collected from Washington County, Mississippi. Colonies were separated from soil using water dripping method [26] and reared in a plastic tray (44.5 × 60.0 × 13.0 cm). The social form of *S. invicta* colonies was determined using PCR on Gp-9 alleles [27]. All ants used in laboratory bioassays were from monogyne colonies; however, social form of colonies in field mound drench treatment was not determined. The same diet (10% sugar water and house crickets) was used for rearing all laboratory colonies. Colonies were maintained in a rearing room at 25°C, 80% R. H. with a 12:12 (L:D) photoperiod.

2.2. Formulations

Two emulsifiable concentrates were prepared using 2-tridecanone as an active ingredient, vegetable oil as a sol-

vent and polyoxyethylene tridecyl ether as an emulsifier. Surfactants are commonly used in insecticide formulations as adjuvants to enhance the performance of the insecticidal active ingredients. However, surfactants may have their own intrinsic detrimental effect on insects. The responses of the red imported fire ant to polyoxyethylene tridecyl ether surfactants (Ethox Chemical, LLC, Greenville, SC, USA) and their relationships to the hydrophilic-lipophilic balance (HLB) numbers have been investigated. Polyoxyethylene tridecyl ether surfactants have intrinsic toxicity against *S. invicta*. Their contact toxicity was found to be inversely correlated with HLB numbers (Chen, unpublished data). Polyoxyethylene tridecyl ether surfactant with HLB number 7.9 (the smallest HLB number available for this particular type of surfactant) was used in both formulations. The compositions of two formulations are shown in Table 1. Since piperonyl butoxide (PBO) was found to improve the toxicity of 2-tridecanone against imported fire ants in the laboratory (Chen, unpublished data), PBO was used as a synergist in one formulation.

2.3. Laboratory Bioassays

Toxicity in soil treatment. About 1 kg of soil was collected from a fire ant mound, dried in oven under 100˚C for 24 h, and then sieved through a #35 sieve to remove debris and large particles. Ten grams of dry soil was placed in a 20-mL glass scintillation vial (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) and mixed with 0.8 ml water. Soil in vial was then pressed firmly using a wood stick. Twenty workers of various sizes (0.4 to 4.3 mg) were placed in the vial. Ants were allowed to settle down and dig for 12 h before 1.0 ml water solution of a test formulation was added into the vial. Glass vials were loosely capped and then placed in a 30 ®C water bath. Dead ants were counted 24 h after soil was treated. Five concentrations were tested, including 0.50%, 0.25%, 0.14%, 0.06% and 0.03% (emulsifiable concentrate dissolved in water w/v). Water was used as a control. Three colonies were used. There were 4 replicates for each concentration. The LC_{50} value was reported as mg test material per gram soil.

Toxicity in immersion treatment. Mortalities of worker ants after being immersed in water solutions of a tested formulation were determined. Twenty workers of various sizes were treated by immersing ants in 5 ml solution in a 20-ml glass vial. The glass vials were placed in a 30˚C water bath. Treatment times included 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 h. After treatment, ants were transferred onto a filter paper and then into a 20-ml glass vial with a piece of moisten filter paper. The vial was loosely capped and kept at room temperature (~23˚C). Dead ants were counted 24 h after ants were treated. Two concentrations, 0.01% and 0.1% (w/v), were tested for both formulations. Three colonies were used and there were 4 replicates for each concentration. Immersion in water was used as a control. Since ants can float on the water surface, the immersion procedure was slightly modified. After 20 ants were placed in the vial, the vial was completely filled with distilled water and capped with a petri dish. After all ants in the vial were immobilized, the petri dish was removed.

2.4. Field Experiment—Mound Drench

Both formulations were tested as mound drench along with water-only as a negative control and registered insecticide, Entrust® SC Naturalyte® Insect Control (22.5% spinosad, Dow AgroSciences LLC, Indianapolis, Indiana), as a positive control. Two applications rates, 5.28 mL/L and 2.64 mL/L, were tested. For mounds less than 20.32 cm in diameter, 3785 ml of emulsion was used for each mound. For mounds 20.32 cm or larger, 7570

Table 1. Composition of two emulsifiable concentrates of 2-tridecanone.

Formulation	Component	Percentage (w/w)
A	2-tridecanone	30
	Soybean oil	30
	Ethal TDA	40
B	2-tridecanone	20
	Soybean oil	20
	Ethal TDA	40
	PBO	20

ml of emulsion was used. The Entrust® SC was applied per label instruction (0.39 mL/L). The final spinosad concentration in the suspension was 0.0088%. The study was conducted in Leroy Percy Wildlife Management Area Hollandale, MS in August and September 2014. Colonies on the road side (highway 12) were used. A colony was randomly assigned to a treatment or control. Each treatment or control had 15 to 16 replicates (mounds). Mounds were measured one week before the experiment. The widest aspect of a mound was recorded as its size. All mounds had a minimum size of 10 cm and all mounds were minimum 4 m apart. One day before treatment, ant activity was evaluated for each mound between 7:00 am and 9:00 am. A wood rod was inserted into the center of the mound to a depth of 10 cm. After 10 seconds, the rod was removed from the mound and ants on the rod were dislodged into a container and counted. Treatments or controls were applied between 7:00 am and 10:00 am, when ants were in the upper part of the mound. All treatment or controls were applied using a 10-L plastic container. About 10% of the dilution or water was first applied around the perimeter of the mound and the remaining volume was directly applied on the mound. Ant activities were then measured 1, 3, 8, and 14 days after treatment. Relocated mounds that were formed after treatment and within a 1.5-m radius of the original mound were inspected, their sizes and ant activity measured. Mounds were scored according to a system (**Table 2**) adapted from Vogt *et al*. [7].

2.5. Data Analysis

PoloPlus (Version 2.0, LeOra Software, Petaluma, California, USA) was used to estimate LC_{50} and LT_{50} with 95% confidence interval (CIs). The relative toxicity ratio with their upper and lower 95% confidence limits was used to evaluate the significance of difference between LC_{50} values. The significance was set at P = 0.05 probability level. If the 95% confidence interval of the ratio between two LC_{50} values include 1, they were not considered significantly different [28]. For field experiment, analysis of variance (PROC GLM; SAS Institute 2008) was performed on mound score and the percentage reduction in ant activity. Means were separated using Tukey's Multiple Comparison Test (P < 0.05). Percentage reduction in ant activity was calculated using the following equation: percentage reduction = ((pre-treatment ant count − post-treatment ant count)/pre-treatment ant count) × 100. If there were relocated colonies, the sum of ant count for all colonies was used as the post-treatment ant count.

3. Results

3.1. Laboratory Bioassay

Toxicity in soil treatment. The LC_{50} values for both formulations are presented in **Table 3**. LC_{50} value ranged from 0.066 to 0.102 mg/g for formulation A and 0.039 to 0.070 mg/g for formulation B. For both formulations, LC_{50} values were significantly different among three colonies. For formulation A, it needed 0.214 mg/g in soil to cause 100% mortality for colony 1 and 2 and 0.430 mg/g for colony 3. For formulation B, it needed 0.107 mg/g to cause 100% mortality for colony 2 and 0.214 mg/g for colonies 1 and 3.

Toxicity in immersion treatment. The LT_{50} values for both formulations are shown in **Table 4**. Formulation B had higher immersion toxicity than formulation A. Three data sets were not adequate for calculating LT_{50} values: formulation A at 0.1 mL/L for colony 1 and formulation B at 0.1 mL/L for colony 1 and at 1.0 mL/L for colony 2. Within a colony, all available pairwise comparison indicated that formulation B always had smaller LT_{50} value than formulation A at the same concentration level. For each formulation, the higher its concentration, the

Table 2. Score system used to evaluate the effect of mound drench treatment[*].

Score	Treated mound alive?	Number of new mounds <1.5 m away
1	no	0
2	no	1
3	no	2
4	yes	0
5	yes	1
6	yes	2

[*]: Adapted from vogt *et al*. [7].

Table 3. Toxicity of two formulations in soil treatment bioassay.

Colony	Formulation	LC$_{50}$ (mg/g)	95% CI	Slope (±SE)	X^2
1	A	0.066	0.056 - 0.074	5.072 ± 0.73	8.79
	B	0.047	0.040 - 0.054	4.64 ± 0.62	16.18
2	A	0.089	0.055 - 0.110	4.47 ± 0.89	28.64
	B	0.039	0.034 - 0.043	6.16 ± 0.89	3.88
3	A	0.102	0.077 - 0.123	3.52 ± 0.46	24.24
	B	0.07	0.061 - 0.078	5.18 ± 0.65	17.78

Table 4. Toxicity of two formulations in immersion treatment bioassay.

Colony	Formulation	Conc (%, w/v)	LT$_{50}$ (h)	95% CI	Slope (±SE)	X_2
1	A	0.1	1.32	1.18 - 1.47	6.33 ± 0.55	35.45
		0.01	——[*]	——	——	——
	B	0.1	1.17	0.91 - 1.40	7.34 ± 0.75	81.22
		0.01	——	——	——	——
2	A	0.1	0.88	0.79 - 0.96	4.72 ± 0.43	15.66
		0.01	1.28	1.15 - 1.41	5.01 ± 0.39	26.25
	B	0.1	——	——	——	——
		0.01	0.85	0.78 - 0.92	6.04 ± 0.63	16.45
3	A	0.1	0.92	0.77 - 1.08	4.23 ± 0.36	52.44
		0.01	1.07	0.98 - 1.17	4.94 ± 0.40	17.66
	B	0.1	0.6	0.54 - 0.65	6.44 ± 0.82	2.34
		0.01	0.95	0.86 - 1.04	4.93 ± 0.43	19.6

[*]: LT$_{50}$ value could not be determined using PoloPlus.

smaller its LT$_{50}$ value. Mean mortalities of workers in immersion bioassay are shown in **Figure 1**. For formulation A, except colony 3 at the concentration of 0.01% that needed 2 h to cause 100% mortality, all other treatments need 3 h, whereas, for formulation B, colony 2 at 0.1% only needed 1h, other three treatments needed 2 h and only two treatments at 0.01% need 3 h.

3.2. Field Experiment—Mound Drench

Both formulations caused significant ant activity reduction. Fourteen days after treatment, all treatments including two formulations and the positive control all had significant reduction in ant activity (**Table 5**) and smaller mound scores (**Table 6**) than the water control. Although the difference among those treatments were not statistically significant, only formulation B at 5.28 mL/L achieved a complete control (100% activity reduction and mound score of 1.0). The positive control (Entrust® SC) had 88.06% activity reduction and 1.40 mound score and the negative control, water drench, had 25.50% activity reduction and 3.27 mound score. All treatment caused satellite mounds; however, only formulation B at 5.28 mL/L, all satellite mounds died out within 14 days.

4. Discussion

Due to the concern about the negative environmental impact of traditional synthetic insecticides, homeowners have an ever-increasing interest in the use of less toxic or "organic" products for fire ant control. Numerous naturally occurring compounds and materials have been investigated for their toxicity and repellency against fire ants [29]-[33]. Mortality was observed when red imported fire ants were exposed to plant essential oils, such as
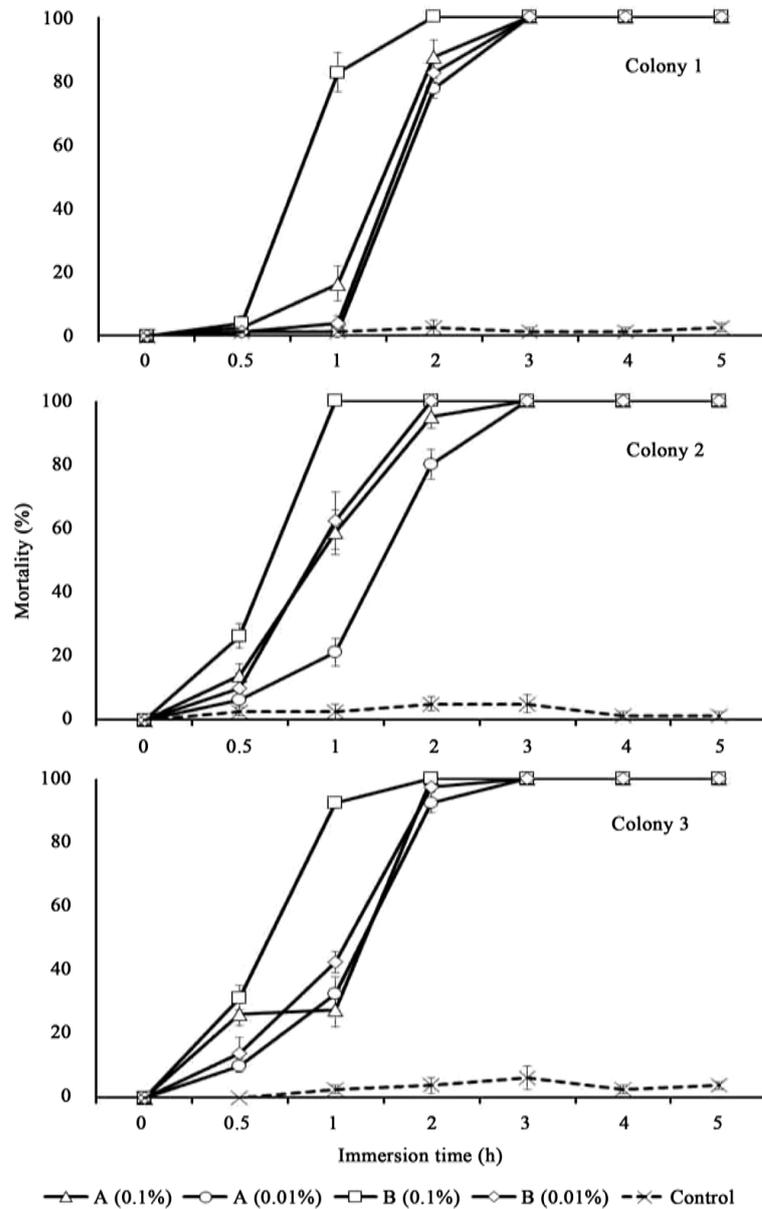
Figure 1. Mean mortality (%) of *S. invicta* workers in immersion bioassay.

Table 5. Ant activity reduction after mound drench treatment.

Formulation	Ant activity reduction (%, mean ± SE)[*]			
	1 day	**3 day**	**8 day**	**14 day**
A (5.28 mL/L)	88.98 ± 5.25 a	77.20 ± 8.88 a	73.06 ± 10.13 a	88.03 ± 8.98 a
A (2.64 mL/L)	79.39 ± 6.50 a	73.71 ± 11.55 a	71.38 ± 16.73 a	78.05 ± 8.49 a
Entrust® SC[**]	80.03 ± 6.42 a	89.24 ± 5.89 a	90.31 ± 6.61 a	88.06 ± 8.79 a
B (5.28 mL/L)	98.81 ± 1.19 a	81.62 ± 12.93 a	96.00 ± 4.00 a	100.00 ± 0.00 a
B (2.64 mL/L)	88.85 ± 5.33 a	75.16 ± 9.03 a	79.54 ± 11.13 a	96.28 ± 2.53 a
Water	31.14 ± 17.99 b	30.08 ± 12.57 b	12.84 ± 19.62 b	25.50 ± 16.19 b

[*]: Under the same day, means followed by the different letter are significantly different ($P < 0.05$). [**]: The Entrust® SC was applied per label instruction (0.39 mL/L).

Table 6. Mean mound score after mounds were drenched with two formulations, Entrust SC and water.

Formulation	Mound score (mean ± SE)[*]			
	1 day	3 day	8 day	14 day
A (5.28 mL/L)	2.13 ± 0.36 bc	2.53 ± 0.42 ab	2.13 ± 0.38 b	1.40 ± 0.21 b
A (2.64 mL/L)	3.27 ± 0.33 ab	2.73 ± 0.45 ab	1.60 ± 0.29 b	2.07 ± 0.37 b
Entrust® SC[**]	3.40 ± 0.32 a	2.07 ± 0.42 ab	1.20 ± 0.15 b	1.40 ± 0.34 b
B (5.28 mL/L)	1.31 ± 0.20 c	1.44 ± 0.16 b	1.06 ± 0.06 b	1.00 ± 0.00 b
B (2.64 mL/L)	2.07 ± 0.37 bc	2.13 ± 0.41 ab	1.60 ± 0.27 b	1.27 ± 0.21 b
Water	4.07 ± 0.07 a	3.40 ± 0.35 a	3.33 ± 0.36 a	3.27 ± 0.37 a

[*]: Under the same day, means followed by the different letter are significantly different ($P < 0.05$). [**]: The Entrust® SC was applied per label instruction (0.39 mL/L).

citrus oil [7], essential oil from *Cinnamomum osmophloeum* leaf [32] and mint oil [31]. Efficacy of several plant oil-containing products was demonstrated in mound drench treatment, including Garden-Ville Soil Conditioner (30% citrus oil) [7], Citrex™ (78.2% d-limonene), Concern® (5.8% d-limonene), Exxant (14.2% turpentine plus 0.2% ammonia) and TFA Super-Kill™ (89% pine oil) [33]. Unfortunately field efficacy data are available for only few products, such as Garden-Ville Soil Conditioner (Gardenville Fertilizer Co., San Antonio, TX) [7] and Citrex® Fire Ant Killer (EnviroSafe Labs, Conroe, TX) [34]. For Citrus oil products (d-limonene as the active ingredient), significantly high application rate was recommended, such as 62.42 ml/L for Citrex® Fire Ant Killer and 47 ml/L for Garden-Ville Soil Conditioner. Citrex® Fire Ant Killer was observed to cause discoloration and death of common turf grasses like Bermuda and St. Augustine grass. In this study, both 2-Tridecanoneformulations have achieved significant fire ant control at a very low application rate (5.28 mL/L). At this application rate, no phytotoxicity to the grasses was observed in the field experiment. Based on the price of each component at time of purchase, the cost per mound is $0.58 for formulation A and $0.57 for formulation B, which are less expensive than many commercially available mound drench products [7] [34]-[36].

Like d-limonene, 2-tridecanone is also widely used as a flavor/fragrance additive in cosmetics and foods, indicating its low human toxicity. Although, d-Limonene has been designated as a chemical with low toxicity based upon lethal dose (LD_{50}) and repeated-dose toxicity studies when administered orally to animals, skin irritation or sensitizing potential was reported following widespread use of this agent in various consumer products [37]. Vapor pressure for 2-tridecanone and d-limonene is 0.0164 and 1.96 mmHg at 25°C respectively, indicating that 2-tridecanone is much less volatile than d-limonene, indicating that 2-tridecanone will cause less potential vapor hazard in work places. Concerns about pesticide safety usually involved two aspects, the environment and end-user. To protect the environment the general trend is to use reduced levels of active ingredients. This trend creates a need for pesticide formulation with improved efficacy at low application rate. To protect the end-user, environmentally safe formulations that eliminate organic solvent-based formulations are needed. The two new formulations reported here do not use any synthetic organic solvents.

Insecticidal property of 2-tridecanone has been demonstrated in many arthropod pests [38]-[44]. Naturally occurring 2-tridecanone was first identified in the wild tomato *Lycopersicon hirsutum* f. *glabratum* [41]. It is possible to obtain about 2.4 mg of 2-tridecanone from one gram of wild tomato leaflets [45]. 2-tridecanone was found in many other plants, such as coconut, palm, American cranberry, rabbiteye blurberry, raspberry [46]. Another potential significant natural source of 2-tridecanone is *Eclipta indica* L. (Asteraceae). 2-Tridecanone is the most abundant constituent in the essential oil from *E. indica* (89.7%) [47]. Formulations have been prepared using the leaves of wild tomato plants and the persistence of -2-tridecanone was evaluated on the leaves of seven vegetables [48]. Half-life ($T_{1/2}$) of 2-tridecanone was 1.5 h on squash leave and 4.0 h on broccoli leaves, indicating a short persistence 2-tridecanone on the vegetable leaves [48].

Like synthetic insecticides, naturally occurring toxins may also have potential undesired effects on non-target organisms. For example, pyrethrins, well known natural insecticides derived from *Chrysanthemum cinerariifolium*, are extremely toxic to fish and beneficial insects such as bees [49]. D-limonene, active ingredient of Citrus oil products for fire ant control, shows high acute toxicity to fish and Daphnia [50]. The material safety data sheet (MSDS) shows that 2-tridecanone is also very toxic to aquatic life with long lasting effects. So every effort

must be made to avoid contaminating water in the application of these two formulations.

Acknowledgements

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