EFFICACY OF VARIOUS PLANTS AS LARVICIDES AGAINST *AEDES ALBOPICTUS*

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Abstract. Yearly estimates from the World Health Organization report that over 2 million people die worldwide due to mosquito borne illnesses (WHO 2017). Methods for managing mosquitoes include repellants or common larvicide *Bt*. Alternative strategies should be investigated however, since repellents do not serve as population controls and *Bt* is often inaccessible to the general public. Population controls at larval stages are most effective at managing new adult populations. Given that plant compounds are often derivatives for contemporary pesticides; this study investigates the larvicidal efficacy of plants widespread in North America. Common plants with known insecticidal components were tested against *Aedes albopictus*, a problematic disease vector. First, instar *Ae. albopictus* larvae were placed in C*hrysanthemum indicum* (chrysanthemums), *Erigeron pulchellus* (Robin's plantain), *Nepeta cataria* (catnip) and *Cichorium intybus* (chicory) plant teas, and mortality was recorded over time. All *Ae. albopictus* reared in *Chrysanthemum intybus* and *Erigeron pulchellus* had 100% mortality within 24 hours, reaching the same effectiveness as *Bti*. Relying on plants replaces the need for chemical and plastic manufacturing, eliminates the chance for synthetic chemical ingestion, and provides an option for individuals from all socioeconomic backgrounds.

INTRODUCTION

The expected projection of mosquito related deaths in 2017 is approximately 2.7 million, making mosquitoes the most dangerous organisms on the planet (WHO 2017). Despite a projected increase in mortalities with changing climate, there have been few developments humans can employ at the individual level aside from the repellent spray, a method that has been the most utilized since the mid-20th century. Repellant sprays however such as DEET (N, N-diethyl-m-toluamide or N, N-diethyl-3-methyl-benzamide) or Off! do not eliminate mosquitoes but merely temporarily deter them from landing on human skin. While there is value in finding ways to avoid mosquito-human contact, this strategy has had no significant difference on mosquito populations. Refocusing our energy into investigating the potential for a new and effective larvicide will reduce the population of emerging female mosquitoes, and in turn reduce the transmission of vector pathogens. Larvicides are capable of reducing a mosquito population by attacking the mosquito before its emergence, while it is still in its larval aquatic phase. The most common larvicide sold to the public is *Bacillus thuringiensis*, also known as Bt, a type of ground dwelling bacteria that destroys the stomach lining of the larvae. While Bt is 100% effective, this larvicide is not always available, it is often out of price range, and warning labels indicate the product is not safe to touch. Bt is not an option for everyone, but it is the only method for reducing local mosquito populations without the necessary training required for other toxic insecticides. Before Bt and commercially sold repellants, individuals turned to folkloric remedies or ethnobotanical knowledge to kill troublesome pests. Relying on ethnobotany allowed individuals to utilize plants as accessible, effective, economical tools for warding off insects.

The ultimate goal with this study is to review plants with rich ethnobotanical histories and known insecticidal components to determine which plants common or native to North America exhibit a potential to behave as larvicides. It should be noted that there are multiple species spanning various plant families that possess insecticidal components found in North American yards and roadsides. However, the purpose of this study is to focus on widespread and common species. Preliminary searches indicated that chrysanthemum (*Chrysanthemum indicum*), catnip (*Nepeta cataria*), chicory (*Cichorium intybus*), and Robin's plantain (*Erigeron pulchellus)* were both widely accessible and had reported insecticidal properties. Below is an overview of each plant, its distribution, availability, and potential as a larvicide.

Chrysanthemum indicum

Chrysanthemum is a member of the *Asteraceae* family. The flower is known for its antifungal and antimicrobial properties, and it is a commonly planted ornamental in patios and gardens (Lin 2009). Although not native to the Americas, chrysanthemums have been widely available since the 1800's, and can now be found in most garden centers throughout the summer months. The pesticide pyrethrin is derived from pyrethroid, an active compound found in the chrysanthemum flower head. Lastly, a recent study indicated a high larval mosquito mortality in the presence of the plant's flower heads (Alverson 2014).

Nepeta cataria

Catnip belongs to the mint family, *Lamiaceae*. The species is native to Eastern Europe, Middle East, Central Asia, and northern China and it has since spread throughout the entire United States and grows as a perennial weed. It is resistant to cooler temperatures, and it is capable of thriving in poor soil conditions. *N. cataria* is also marketed in its dry form and can be found in most pet supply stores since it is a psychotropic plant for the domestic cat. Its potential as an insecticide was evaluated when the active compound nepetalactone found in the trichomes of its leaves was shown to be 10x more effective at deterring mosquitoes in the *Culex* genus than DEET (ACA 2001).

Erigeron pulchellus

Robins plantain is another member of the *Asteraceae* family, and a close relative of chamomile. Though seemingly benign, chamomile has demonstrated toxicity against ticks. In one study, pregnant ticks submerged in a steam-distilled preparation of chamomile had increased mortality within 24 hours and when dissected, 50% of the eggs were also dead (Pirali-Kheirabadi 2007). Robin's plantain was chosen in place of chamomile due to its abundance as a weed, and close familial relation to chamomile. Robin's plantain occurs in the entire Eastern half of the United States, and its weak shallow roots make the plant easy to remove and harvest.

Cichorium intybus

Chicory is also a common flowering plant in the *Asteraceae* family and found along the roadsides of the United States and in Southern Canada. Due to its abundance, chicory is often sprayed or mowed over since it crowds into other more desirable plants. Medicinally, chicory has been brewed into teas as an aid for diabetics, and to treat constipation. Recent testing however indicates that chicory has insecticidal properties, and has been shown to be equitoxic towards *Anopheles pharoensis* and the domestic housefly (Mansour et al. 2014).

In this study, we examine mortality rates of *Aedes albopictus* larvae reared in the presence of these four plants. *Aedes albopictus*, also known as the tiger mosquito is an invasive species introduced to the United States circa 1985 via imported tires (Hartman 2001). In its native home range in Asia, *A. albopictus* larvae develop in small stagnant pools of water, a behavior trait that has allowed it to be extremely successful in colonizing the urban landscape. The eggs are desiccation resistant, a trait allowing the eggs to hatch only when conditions are favorable. Since the mosquito's first discovery in Texas and later

Florida, *A. albopictus* has continued to spread north and can now be found far into New York, New York, and Baltimore, Maryland (TISI 2014). *Ae albopictus* has is a potentially dangerous pest since it is a competent vector for dengue, chikungunya, and Zika virus (ECDC 2016).

METHODS

Plants were obtained in Millbrook, NY from roadsides (*Cichorium intybus*, *Erigeron pulchellus*) or from Local greenhouses (*Chrysanthemum indicums*, *Nepeta cataria). Fresh flower heads* (*Chrysanthemum indicums, E. pulchellus*) or leaf and stem materials (*N. cataria, C. intybus*) were added to 500 ml well water in 3 amounts: 50g, 25g, and 12.5g. These four 'plant teas' were then incubated in liter-sized glass mason jars at 28.8 degrees centigrade for 48 hours. All plant material was filtered out and the liquid teas were used to rear *A. albopictus* larvae. In addition to the four plant treatments, two additional treatments included an untreated well water control, and a known larvicidal control. The larvicidal control was 500ml well water with 1 tsp of *Bacillus thuringiensis*. *Aedes albopictus* larva were obtained from a Maryland colony (F3-F5) managed by P. Leisnham at UMD. Replicate plastic containers (5 per treatment) were filled with 50ml of the specific treatment 'tea' and ten first instar larva (within 48 hours of hatching) were added to each replicate. Larval mortality was recorded every 24 hours for four days. Methods were then repeated for the plant treatments that had high mortality. Plant leachates for *Chrysanthemum indicum* and *Erigeron pulchellus* (plantain) were diluted twice in concentration in order to determine their threshold for effectiveness. First dilution included a 50% reduction, 1 part plant per 20 parts water (25g/500ml), the second dilution included another 50% reduction, 1 part plant per 40 parts water (12.g/500ml). Statistical analyses were performed using the R Statistical Program and significance was evaluated at alpha <0.05.

RESULTS

Larval mortalities differed significantly across the treatments (F=81.20, df=4, $p < 0.05$) and were greatest at 100% in the chrysanthemum (*C. indicum*), plantain (*E. pulchellus*) and Bt treatments. The catnip (*N. cataria)* and chicory (*C. intybus)* treatments resulted in 34% (±26%) and 4% (±8%), respectively but only the catnip treatment was significantly greater than the control at 24 hours (t=2.9, p<0.05). While no additional larval mortality occurred after the first 24 hours in most treatments, mortality in the catnip treatment continued over 4 days, reaching a final mortality of 76% (mean=58% (±6%), t=9.09, p<.05). Given that catnip and chicory were not as effective, these plants were not diluted in concentration for additional rounds of testing. Secondary testing with chrysanthemum and plantain involved a 50% reduction (25g plant/500ml water), and larval mortality was again 100% within 24 hours in both treatments and significantly different from the well-water control (F= 245.4, df=14, p<0.05). Finally, concentrations were reduced an additional 50% (12.5/500ml). At this lower concentration, larval mortality was still significantly greater in the chrysanthemum treatment (mean=78% (±10%), t=9.2, p<0.05). However, the plantain treatment at this concentration was no longer significantly different from the control.

DISCUSSION

Chrysanthemums and Robin's plantain are effective mosquito larvicides in dilutions as low as 1 part plant per 20 parts water (25g/500ml). This is equivalent to approximately 12 flower heads per half a liter of water for chrysanthemums, and 50 flower heads for Robin's plantain. These plants could either be established in a garden near water or collected through the summer time and added to areas where mosquitoes might oviposit, such as birdbaths, storm drains, empty flowerpots, or any area that might fill with stagnant water. While chrysanthemums do not grow natively, they are one of the most commonly grown garden plants and are accessible and inexpensive. Further, some variants of chrysanthemum are perennials, making that initial investment a financially sustainable option over time. Robin's plantain grows commonly as a weed throughout the eastern half of the United States, as does catnip--occurring throughout the entire United States. These plants may be acting as natural larvicides in areas where they already grow. Using plants to create 'sink' habitats for larvae has been demonstrated as an effective option for controlling mosquitoes on a larger scale in Champaign, Urbana, where blackberry leaf infusions were added to storm drains and producing statistically a significant decline in adult populations over time (Gardener 2014). Collecting or adding plants to a garden avoids the production of unnecessary plastics, and eliminates the probability of ingesting potentially harmful synthetics that might be unfamiliar to individuals. Further, the addition of these plants could attract a variety of pollinators such as bees, wasps, butterflies, moths, beetles, birds, or even small mammals to a garden as well as reduce a local mosquito population (Ingram et al. 1996).

It is important to note however, that the majority of fatalities attributed to mosquito related illnesses are not in North America, but in tropical regions of the world such as sub-Saharan Africa, South or Central America, and Southeast Asia. While the variations of chrysanthemum, Robin's plantain, or Catnip one might use to fight mosquitoes in North America will not be found in these regions, it is known that chrysanthemum and Robin's plantain are both members of the daisy family, *Asteraceae*. Further study should sample the breadth of common asters occurring in these mosquito hotspots, and test the leachate of these flowers to determine which plants might have insecticidal components. The use of plants in these particular areas of the world is of the utmost importance, given that these mosquito hotspots lie in some of the most impoverished areas of the world, where individuals have little or no access to effective repellants or larvicides in their community. Common plants offer a sense of familiarity and trust that bottled synthetic chemicals to not offer individuals in stranded or isolated communities, where chemically based Western products are often distrusted. Instead, plants are an important and accessible strategy for reducing mosquito populations that individuals can easily access. The Kosodo village in the Kenya has served as a case study for demonstrate how crucial plants have been since the beginning of the NEEM agroecology project, an attempt to reduce mosquito populations by using neem tree bark leachate (Omuku 2007). Pointing a finger in the direction of the *Asteraceae* family will likely provide more options, particularly if a specific method already being employed has been exhausted. Given that there are 23,000 known species in the aster family, the likelihood of coming into contact with a flower with insecticidal components native to a mosquito hotspot is high. As for North Americans or Europeans who have access to Chrysanthemum, Robin's plantain or Chamomile and Catnip, these plants will aid in maintaining low incidences of mosquito deaths, and have the potential to bring these casualties closer and closer zero. Lastly, plants offer a way for the individual or community to combat disease in the face of the escalating effects of climate change. Mosquito populations can be reduced without the unnecessary packaging and fabrication of the chemically based compounds that are in turn potentially wreaking havoc on the environment around us.

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APPENDIX

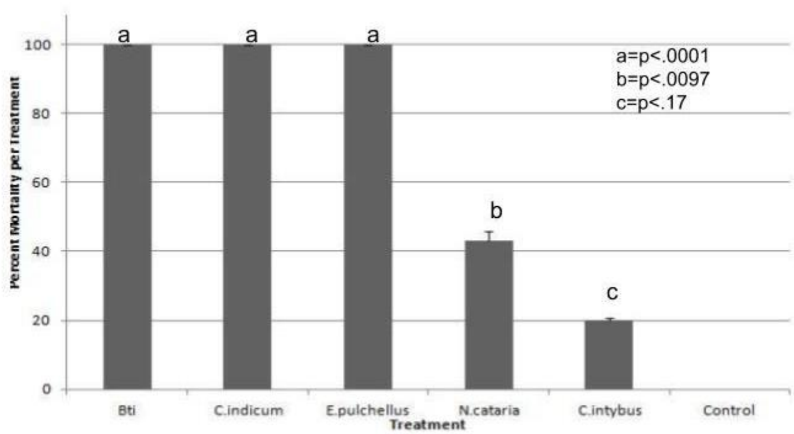

FIGURE 1. Percent of *Aedes albopictus la*rvae found dead after a 24-hour waiting period per treatment in a concentration of 1 part plant per 10 parts water (50g/500ml).

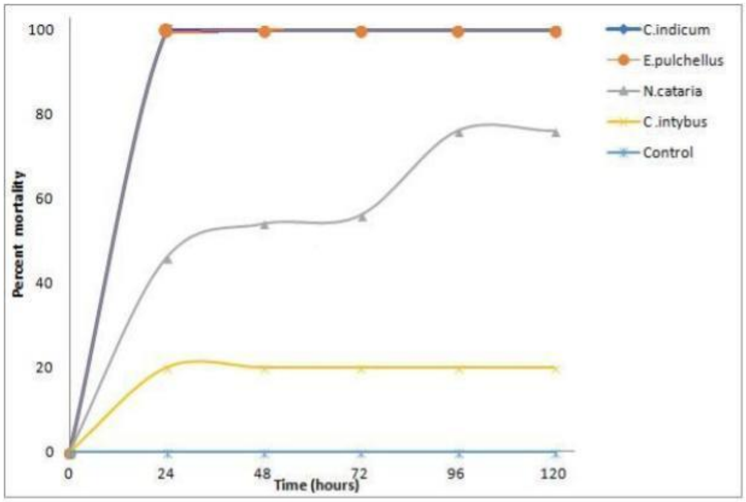

FIGURE 2. Rate of *Aedes albopictus* larvae dead over a 120-hour period in a concentration of 1 part plant per 10 parts water (50g/500ml).