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Full Length Research Paper

Evaluation of larvicidal properties of the latex of *Euphorbia tirucalli* L. (Euphorbiaceae) against larvae of *Anopheles* mosquitoes

Julius Mwine[1]*, Patrick Van Damme[2] and Francis Jumba[1]

[1]Faculty of Agriculture, Uganda Martyrs University, Nkozi, P. O. Box 5498, Kampala, Uganda.
[2]Faculty of Bioscience Engineering, Tropical and subtropical laboratory of Agronomy and Ethnobotany, University of Ghent, Coupure links 653, 9000 Ghent, Belgium.

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Malaria is the most important vector-borne disease in tropical and sub-tropical countries. Although, different control measures like use of insecticide-impregnated mosquito nets and curtains, chemotherapy and others are in place, so far, malaria eradication has proved impossible in affected areas. Therefore, any measure that attempts to fight the parasite or its vector (*Anopheles* spp.) would be of help. In this experiment, we assessed the efficacy of *Euphorbia tirucalli* latex both as a herbal mosquito remedy and larvicide against *Anopheles fenestus* Giles and *Anopheles gambae* Giles in a semi-natural environment. Our results indicate that *E. tirucalli* latex can bring about total mortality of *Anopheles* species larvae at the highest dilution used of 1: 250 in 5 days. LT 50 and LT 90 for the same dilution were attained at 12 and 36 h respectively. Latex was active only for eight days which is typical for herbal biocides, whose advantage is that they do not accumulate in the environment. It is concluded that *E. tirucalli* latex has a high efficacy against *Anopheles* mosquito larvae and could eventually be considered for adoption as a plant based mosquito larvicide, after further research.

Key words: *Euphorbia tirucalli*, *Anopheles* spp., latex, larvicide, malaria, efficacy.

INTRODUCTION

Malaria is the most important vector-borne disease in the tropics and sub-tropics (Komisar, 2007; Stratton, 2008). WHO (www.who.int/whosis) reports that malaria affects over 100 countries and approximately 40% of the world's population, killing about one million people annually. Africa is reported to be the most affected continent and every one of five childhood deaths is due to the disease. Those who survive usually suffer from malarial after-effects like slow growth, learning impairment and sometimes general disability (Carter et al., 2005; Urbach, 2008). In Uganda alone, malaria accounts for 25 to 40% of all visits at health care facilities and over 20% of all hospital admissions. The disease is responsible for death of 70,000 to 100,000 children under 5 years annually (www.go.ug/malaria.htm). According to the latter source, average households spend about 25% of their income on malaria while sub-Saharan governments spend about 40% of their health budgets on malarial-related activities.

*Corresponding author. E-mail: mwinej@yahoo.com.

The international community has attempted to provide help by setting up such assistance facilities as the Global Fund for HIV- AIDS, tuberculosis and malaria to check incidences of these diseases (Wendo, 2003; Atun and Kazatchkine, 2010).

A number of measures to control malaria have been put in place since the discovery of quinine from the cinchona tree (*Cinchona officinalis* L., Rubiaceae). Although, chemotherapy remains the most important method to combat malaria, frantic efforts have been devised to fight it at different levels, for example at vector level by killing mosquitoes or preventing them from reaching their prey (Goodman et al., 1999). In Uganda, this has been done using different methodologies such as environmental management, spraying walls that act as mosquito resting places, use of insecticide- impregnated mosquito nets and curtains among others (Kilian et al., 2008; Rubaihayo et al., 2009; Pullan et al., 2010). To enhance present control measures, indoor spraying with DDT (which was banned in many countries) has been co-opted by the Uganda government possibly for lack of better alternatives (Bimenya et al., 2010). Elimination or killing of mosquitoes is one of the most commonly used methods of fighting malaria (Killeen et al., 2002b). Mosquitoes can be attacked at different developmental stages including egg, larval and adult stages. During larval stages, mosquitoes are active and aquatic. This puts them at a disadvantage as their mobility is limited to water bodies where both food and air for gaseous exchange are obtained, making them susceptible to any changes that occur in the water body (Killeen et al., 2002a). This weakness can be used against larvae, which can be attacked by blocking food supply, breathing systems or both.

The use of larvicides is one of the oldest methods of controlling malaria (Killeen et al., 2002b). Among other advantages, use of larvicides controls mosquitoes before they are able to spread and transmit diseases (Fillinger et al., 2003; Killeen et al., 2002a). While other methods like adult spraying may have direct effects like visible protection of populations and may show quick results, larval control has yielded several success stories where malaria has been brought under control, including in countries such as Brazil, Egypt and Zambia (Killeen et al., 2002b). According to these authors, use of larval control methods was abandoned after the discovery of DDT, which was used as an adult spray and was seen as a panacea to all insect problems.

Due to the disadvantages associated with such synthetic pesticides, including development of pesticide resistant strains, ecological imbalances and harm to non-target organisms, there is a renewed effort to develop substances of plant origin which are considered to be more environmentally friendly due to their innate bio-degradability and lower toxicity to most organisms (Frederich et al., 2002).

Several researchers have investigated the application of plant extracts to fight malaria vectors. Plant species cited in literature for this purpose include *Achyranthus aspera* (Bagavan et al., 2008) *Azadirachta indica* (Aliero, 2003), *Jatropha curcas*, *Euphorbia tirucalli*, *Euphorbia hirta*, *Phyllanthus amarus* and *Pedilanthus tithymaloides* (Rahuman et al., 2008a), *Piper nigrum* (Rasheed et al., 2005), *Chenopodium album* (Sharma et al., 2006), *Solanum xanthocarpum* (Mohan et al., 2005), *Ajuga remota* (Sharma et al., 2004), *Thymus capitatus* (Mansour et al., 2000), *Tagetes erectes*, *Cleome icosandra*, *Ageratum conyzoides*, *Eichhornia crassipes* (Saxena et al., 1992) among others. These authors have validated and independently reported on chemical substances like flavonoids, diterpenoids, triterpenoids, esters and alkaloids among others, in respective plant tissues and their degree of anti-malarial efficacy.

In this paper, we focused on assessing the larvicidal properties of *E. tirucalli* L (Euphorbiaceae) against *Anopheles* mosquitoes*. E. tirucalli* is a small tree that grows 3 -6 m tall and has pencil-like branches. Therefore, it is commonly referred to as the pencil tree (English), or Utupa or mtupa (Swahili), and in some Ugandan dialects, *Oruyenje* (Runyankole) and *Kakoni* (Luganda). The tree is typically used as a fence plant and for boundary demarcation since it is not braised by domestic animals, has few pests and is not easily destroyed by hardier conditions like drought and salt stress (Van, 2001). Like many other Euphorbiaceae, *E. tirucalli* is a well known medicinal plant both locally and internationally. It is reported to have curative features for a number of diseases.

In Africa, it is said to cure snakebites, warts, sexual impotence, and syphilis and to extract skin parasites. In Asia, it is popular for treating broken bones, hemorrhoids, pains, warts, swellings and ulcerations. In Brazil, the list is even longer including the above in addition to scorpion bites, asthma, cancer, spasms and others (Cataluna and Rates, 1997; Van, 2001). Available literature also shows that *E. tirucalli* has larvicidal (Rahuman et al., 2008a; Yadav et al., 2002), anti-fungal (Mohamed et al., 1996), piscicidal (Neuwinger, 2004), anti-viral (Betancur-Galvis et al., 2002) as well as anti-bacterial (Lirio et al., 1998) features. Petroleum extracts of *E. tirucalli* have been tested against *Aedes aegypti* and *Curex quinquefasciatus* and were found potent enough to cause larval mortality for *A. aegypti* and *C. quinquefasciatus* (LC 50 = 4.25 and 5.52 ppm, respectively; Rahuman et al., 2008a). Having shown a high larvicidal efficacy against *Aedes* and *Curex* spp, this study hypothesized that the same potency could be shown against *Anopheles* species. The present study was therefore designed to investigate whether latex of *E. tirucalli* has enough potency to cause harm or kill *Anopheles* mosquito larvae in their semi-natural environment so as to be adopted for use as a mosquito control measure.

MATERIALS AND METHODS

Experimental site

The experiment was set up in a swampy area at Kajansi (0°13'09 58'' N; 32°32'03 18'' E) about 10 km from Kampala city, central Uganda. A neglected fish pond belonging to National Agricultural Research Organization originally used by Kajansi Fisheries Research Development Center to breed fish was used. The pond was 28 by 19 m in size and 1.2 m deep when it was active. At experiment time, it contained shallow rainwater, which was about 0.2 m in depth.

Experimental design

The present experiment was designed following Fillinger et al. (2003) with a few modifications. Sixteen plastic tubs, each with a diameter of 0.5 m, were buried in a neglected pond to a depth of 0.4 m. Tubs were arranged in the pond in four rows and four columns with a distance of 2 m in between. All tubs were provided with approximately 6 kg of top soil drawn from the experimental area to provide normal abiotic and biotic conditions to mosquitoes. They were then filled with tap water and maintained at a depth of about 0.4 m with a provision of overflow holes to allow excess water leave the tubs in case of rain. A nylon net meshing was provided at overflow holes to stop mosquito larvae from escaping with excess water. Tubs were left open to allow oviposition. Water temperatures during the experiments ranged between a minimum of 16°C and a maximum of 38°C. The experiment was carried out during November 2008 (rainy season) and repeated in March 2009 (dry season).

Euphorbia tirucalli extract

E. tirucalli latex used was locally obtained by making incisions on mature branches of trees from which latex oozed into small sample bottles. Sample bottles were corked and wrapped with aluminum foil to stop latex solarization that would make it deteriorate (Oliveira-Filho EC and Paumgartten, 1997). Bottles were put in a cooler (icebox) and kept at about 4°C to stop coagulation until experimental time.

Experimental mosquitoes

Our preliminary studies (unpublished) revealed that dominant *Anopheles* species at the experimental site (neglected fish pond) were *Anopheles funestus* and *A gambae* in a ratio of 1:3 for *A. gambae* and *A. funestus* respectively. During this particular study, experimental mosquitoes were identified with the help of senior entomologist at vector control division, Kampala. Mosquitoes assessed were those found naturally breeding in the area in order to approximate to a semi-natural environment.

Experimental assay

Following our preliminary experiments carried out at Department of Vector Control in Wandegeya, Kampala on lethal dose (Mwine, unpublished), the following latex dilutions were used: 1:250, 1:200 and 1:150 of fresh latex to water (volume by volume) whereas the control was treated with tap water. These initial dilutions were obtained by mixing appropriate quantities of latex with 1 L of water drawn from respective experimental tubs. A randomized complete block design was used in experimental setup and each treatment was replicated four times. Before treatment with each respective dilution, the set up was left to stand for eight days to allow oviposition and emergence of a good number of larvae to ease the sampling process (Fillinger et al., 2003). Following the methodology of the same author, sampling for mosquito larvae to establish their presence, taxonomic characteristics and stage of development (before treatment) was carried out using a 350 ml dipper at different sides of tubs. Four dips per tub were taken for this initial test.

Sampling and assessment

Sampling of larvae was carried out at 0, 12, 24 and 36 h and then on a daily basis for 15 days using the same technique as for the initial test. Sampling was sequenced in such a way that replicates of similar dilution were sampled consecutively with the same dipper. One dip was taken from each tub at a time, returning to it later, on completion of the cycle for that particular treatment until all four samples were taken. This was done to allow diving larvae return to the surface for equal chances of being sampled (Fillinger et al., 2003). Larvae sampled were observed for livelihood by touching them with a seeker. Both dead and living larvae were recorded and returned to their respective tubs but pupae were removed and disposed of to minimize emergence of adults. Tubs were checked twice a day for this purpose (Fillinger et al., 2003).

Data analysis

Treatment means of larvae and pupae found alive after treatment were calculated and subjected to Kruskal-Wallis one-way analysis of variance by ranks test (H test) to determine if there was a significant difference in population trends. Individual means were compared between one another using Mann-Whitney U test to establish significant differences between treatments and the control. Results with $P \leq 0.05$ % were considered statistically significant. Percentage mortality was calculated using the formula of Mulla et al.; (1971) which takes into account natural factors of population change in an ecosystem. Mortality = 100 – (C1/T1 xT2/C2) x100, where C1 and C2 represent average numbers of larvae in control tubs pre- and post-treatment respectively, and T1 and T2 for tubs treated with experimental formulations, pre- and post-treatment respectively. Mortalities less than zero were corrected to zero. Microsoft Excel for Windows 2007 was used for performing calculations.

RESULTS

A high efficacy against *Anopheles* mosquito larvae was returned by all *E. tirucalli* treatments unlike the control as shown on Figure 1. A statistical comparison of treatments means after mortality peak (day 10 after treatment) reveals that there is a significant difference between effectiveness of different treatments ($H = 12.17$, $df = 3$, $P = 0.007$). A post-test comparison of treatment means (compared with control) using a Mann-Whitney U test reveals that latex treatment samples were significantly different from the control (T1: $U = 11.27$, $P = 0.0008$; T2:

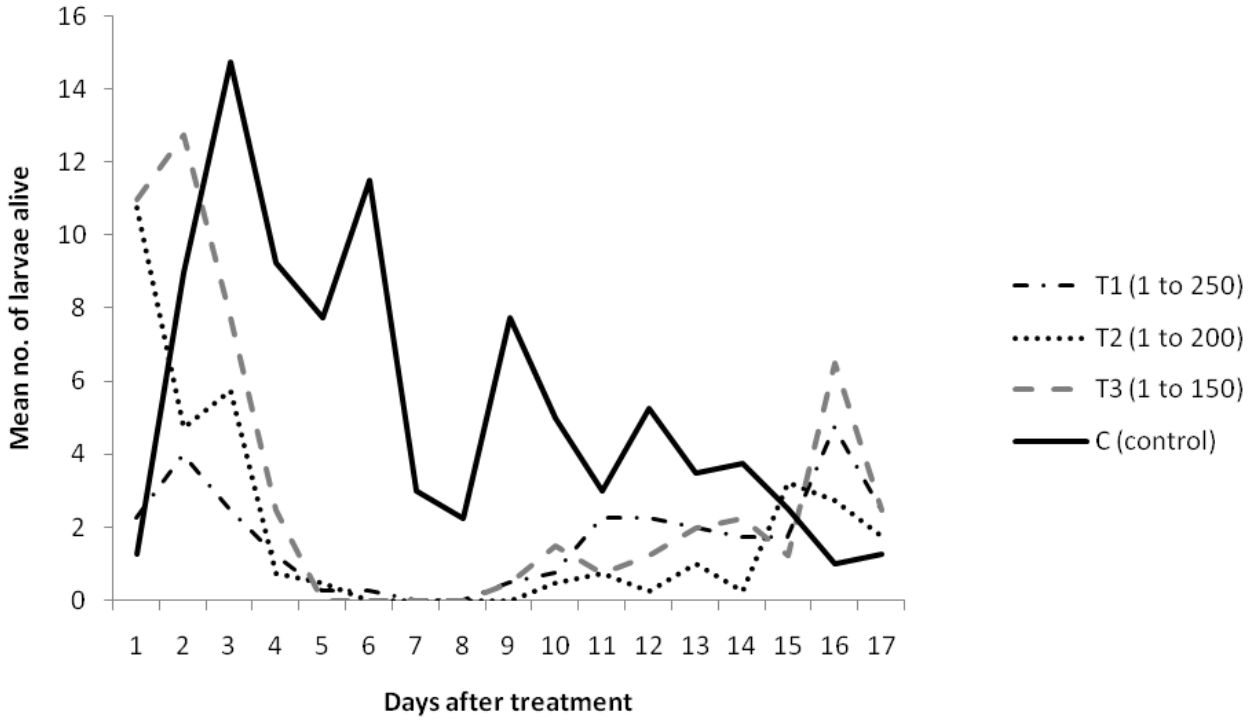

Figure 1. Means of *Anopheles* spp larvae alive after treatment with *E. tirucalli* latex.

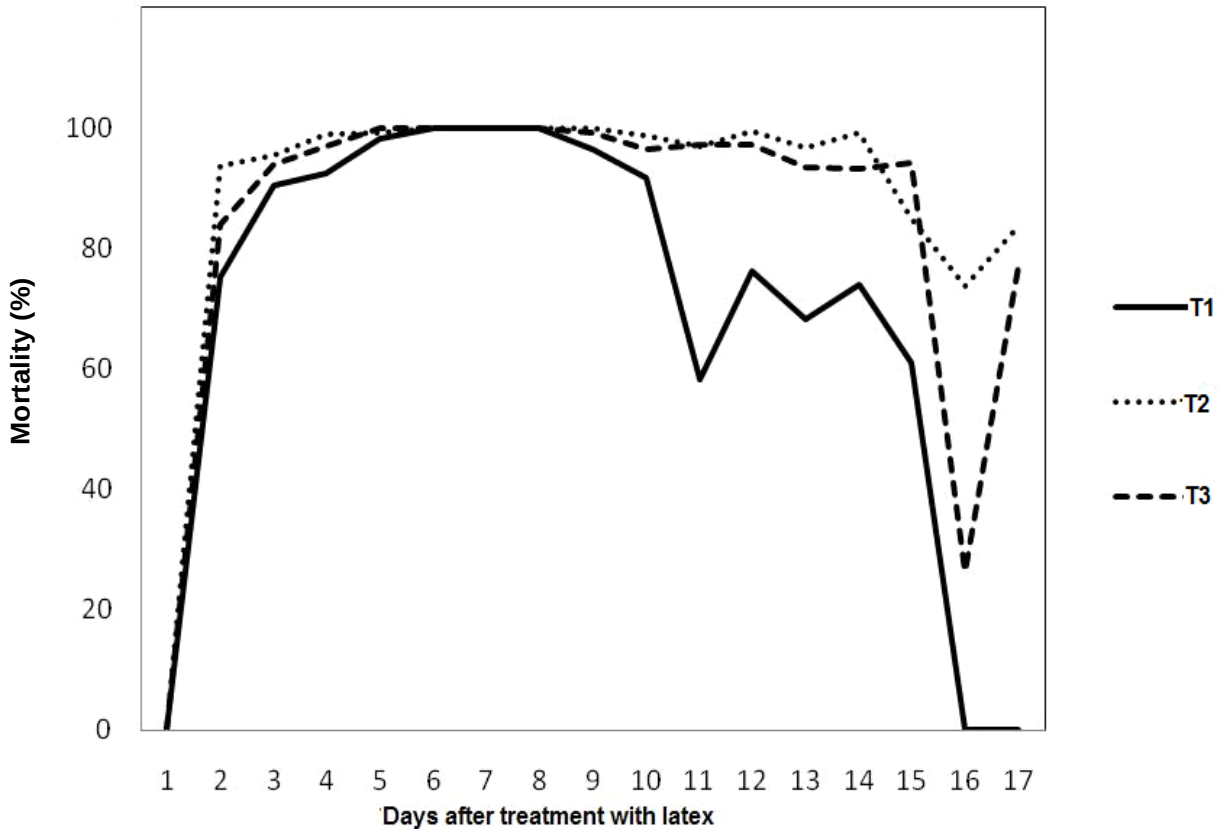

Figure 2. Larval mean mortality in the treatments.

U = 7.85, P = 0.005; T3: U = 4.59, P = 0.032). Our assumption (null hypothesis) of uniform efficacy for the treatments and control is rejected. Calculated larval mortality also revealed a high efficacy for all three latex treatments. LT 50 and LT 90 were reached within 12 and 36 h of application respectively, for the highest dilution (T1 = 1:250) attaining total mortality in 5 days (Figure 2). Latex appeared effective between day two and day eight after treatment, as numbers of mosquito larvae alive were diminishing during this period but started rising again after the eighth day in all treatments.

DISCUSSION

Use of larvicidals against mosquitoes is an old method of malarial control (Fillinger et al., 2003) and has of late been brought back on the market due to need of alternatives from harmful sprays (Bagavan et al., 2009; Kamaraj et al., 2009). Along the same line, the revival of research on plant-based pesticides over the last few decades responds to recognition of a need to replace harmful, non-selective and environmentally unfriendly synthetic pesticides some of which have already been internationally banned. Our results indicate that *E. tirucalli* latex has a high efficacy against *Anopheles* mosquito larvae. Figure 2 shows that at a dilution as low as 1: 200, fresh latex was able to cause 80% larval mortality in only 12 h rising to 100% in 4 - 5 days. Of the dilutions tested (Figure 1) all three attained LT50 within 12 h and LT 90 in 36 h. This indicates that, in analogy with most crude plant extracts (Mullai and Jebanesan, 2007; Rahuman et al., 2009; Yenesew et al., 2003), *E. tirucalli* latex does not have a knockdown effect but displays a steady killing rate and high efficacy against *Anopheles* mosquito larvae. Results also show that fresh *E. tirucalli* latex is active for a short time. Figures 1 and 2 indicate that larval mortality peaks between day five and eight after application and starts decreasing thereafter. This is a typical response of plant based pesticides which generally do not persist in the environment. However, the disadvantage is that one has to apply them frequently in order to bring down pest levels. Since all three dilutions tested have sufficient potency to kill mosquito larvae within 4 days, the lowest dilution can be recommended for application so as to avoid usage of heavy doses which may bring about problems. After all, *E-tirucalli* is known to be toxic (Furstenberger and Hecker, 1977; Shlamovitz et al., 2009) and has been pointed out to be co-carcinogenic (Furstenberger and Hecker, 1985; Liu et al., 1998; MacNeil et al., 2003). Therefore, lower dilutions are to be preferred. However, there are indications that much lower doses may not have similar efficacy as we found out in our preliminary trials that dilutions lower than 1:250 do not return good results. Results from the present study are not a surprise since Euphorbiaceae plants are known to possess chemical substances like triterpenes (Khan et al., 1988; Rahuman et al., 2008b; Rasool et al., 1989), diterpenes (Khan and Malik, 1990; Marco et al., 1997) rotenoides (Yenesew et al., 2003), saponins (Bagavan et al., 2008), tannins (Yoshida et al., 1991) flavonoids and alkaloids, among others, which have been found to have reasonable efficacy against a range of mosquito species. Rahuman (2008a) and Yadav et al. (2002) tested *E. tirucalli* latex against *Culex* spp and *Aedes* spp*,* albeit in extracted form and established similarly high efficacies as in the present experiment. To the best of our knowledge, this is the first report on evaluation of *E. tirucalli* latex against *Anopheles* spp. Results of extraction and purification of active compounds in *E. tirucalli* latex will be published elsewhere.

Conclusions

The present study has demonstrated that fresh *E. tirucalli* latex has a reasonably high efficacy to cause mortality in *Anopheles* spp larvae. Since all tested dilutions can return LT 90 within 36 h, our interest should focus on the highest dilution (1: 250) to minimize excess latex usage and thus avoid spoilage but also minimize problems and risks associated with overdosing. According to our results, latex is most active in only five days. It is therefore logical, to apply it twice weekly to maintain an active dose in the environment during the period of high larval incidence. During periods of low larval incidence, a single application once a week should suffice. It should be noted that while we recommend local people to continue spraying *E. tirucalli* latex as a larvicide, extended use and commercialization should await further research, which may include extraction and purification of active ingredients.

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