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*Full Length Research Paper*

# Oral acute toxicity study of selected botanical pesticide plants used by subsistence farmers around the Lake Victoria Basin

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**A survey carried out around the Lake Victoria region showed evidence that people around this region use plant extracts, parts and powders to protect stored food commodities from insect pests. The widely used plants were identified and selected for biosafety assessments namely: *Ocimum gratissimum, Tithonia diversifolia*, *Eucalyptus saligna, Eucalyptus globulus* and *Cupressus lusitanica.* Wistar mice were acclimatized and divided into groups of six. Each mice group was administered with one extract at different concentrations. The extracts were administered orally and the animals were observed for 24 h. A control group was kept which received only the carrier substance orally. The $LD_{50}$ values were determined by the use of the graphical method and regression analysis. Oral acute toxicity studies established the $LD_{50}$ values for essential oils of *O. gratissimum*, *E. saligna* and *C. lusitanica* as 4,570, 2,290, and 3,311 mg/kg, respectively. For ethanol extracts, $LD_{50}$ values were 12,882, 12,302, 14,996 and 11,481 mg/kg for *O. gratissimum, E. globulus*, *C. lusitanica* and *T. diversifolia*, respectively. For the aqueous extracts, the $LD_{50}$ of *T. diversifolia* was found to be 12,302 mg/kg. For *E. globulus* and *C. lusitanica,* their aqueous $LD_{50s}$ were beyond 15,000 mg/kg. The oral acute toxicity tests showed weak toxicities for all the plant extracts investigated in the study. The low toxicity levels exhibited by these extracts may be the reason why these plant products have been used by local communities for long without adverse effects. Chronic studies should be carried out to assess whether these extracts have serious effects on experimental animals exposed to them at small doses for a long period of time.**

**Key words:** Oral acute toxicity, biopesticide, plant extracts, Lake Victoria Basin.

## INTRODUCTION

There is a very long history of use of botanical extracts for human and veterinary medicine, as well as for the protection of field and stored crops (Berger, 1994). In the recent decades, however, due to the introduction of synthetic pesticides, the adoption of these traditional approaches of crop and post harvest protection has not been improved (Berger 1994). Today, the use of plant extracts for controlling pests has been limited to subsistence farmers, who in most cases are supported by various Non Government Organizations (NGOs) and women groups (Kamatenesi-Mugisha et al., 2008). The use of synthetic pesticides has undoubtedly increased crop production. This has been possible through reduced

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losses caused by crop pests but many of these chemicals are hazardous to both humans and the environment.

According to Blackman and Eastop (1999), it has been estimated that hardly 0.1% of the agrochemicals used for crop protection reach the target pest leaving the remaining 99.9% to enter the environment and cause hazards to non-target organisms including humans. The situation is alarming especially in Africa and Asia where it has been reported that there is a lot of misuse of conventional pesticides due to, lack of suitable application equipment, and lack of technical training to ensure effective and safe use of pesticides (Attila, 1990).

Botanical pesticides are extracted from various plant parts (stems, seeds, roots, leaves and flower heads) of different plant species. These pesticides are known for having a broad spectrum of activity, being easy to process and use/apply, having a short residual activity and for not accumulating in the environment or in fatty tissues of warm blooded animals because they are highly biodegradable (Philip and Robert, 1998). It is, however, important to note that botanical pesticides, despite being derived from plants, may not necessarily be safe to humans and the environment. Some may be quite toxic. Toxicological studies aimed at assessing their safety should be done before they are used to avoid possible dangers to non target organisms and the environment (Belmain et al., 2001). Some plants have been scientifically tested and have been found to have good pesticidal properties. There is a concern, however, as most of the studied plants are of western origin (Jaya and Dubey, 2005). Botanical pesticides, if sufficiently exploited, can surely play a big role in reducing pollution, health risks and crop losses to pests.

## Problem statement

The steadily increasing problems emanating from the use of synthetic pesticides including pest resistance, pollution of the environment and the side effects to beneficial flora and fauna demand for an urgent and intensive search for safer pesticides (Attila, 1990). On the other hand, synthetic pesticides are expensive and are in most cases not affordable by many farmers especially those with small farms around the Lake Victoria region (Mathenge, 2001). Subsistence farmers around the Lake Victoria region use botanical pesticides to protect their stored produce from storage pests; however, they have no standard dosage rates and are ignorant about the toxicity levels of these pesticides. It is important to note that, most botanical pesticides are naturally developed, some may not be safe for use by humans and may be potential pollutants of the environment. Biosafety assessments should be done before these botanicals are used (Belmain et al., 2001). Thus, this study was conducted mainly to assess the safety of plant extracts used as pesticides by the local communities around the Lake Victoria region. This study specifically determined the acute oral toxicity of the essential oils, ethanol and aqueous extracts of the selected plants using mice. In addition, the determination of the phytochemical profile of the crude ethanol and aqueous extracts of the selected plants was done.

## A review of the selected plants for biosafety assessments

### *Ocimum gratissimum* (L)

According to Katende et al. (1995), *O. gratissimum,* formerly known as *Ocimum suave* is an aromatic, perennial herb, 1 to 3 m tall. The stem is erect, round-quadrangular, much branched, glabrous or pubescent, woody at the base, often with epidermis peeling in strips. According to Hassanali et al. (1990), *O. gratissimum* is generally found growing across Africa and tropical Asia in the upland forest areas and open waste areas. It is used as a traditional medicine against stomachache, cough and influenza. The essential oil of *O. gratissimum* has antibacterial activity. Leaf extracts of *O. gratissimum* has antidiabetic properties in streptozocin-induced in diabetic rats. Other studies have shown that the oils obtained from the leaves of *O. gratissimum* contain phenols and recent studies have reported that the major components of the oil are p-cyme (59%), alpha-cyme 10%, myrcene 7% and thymol 7% (Keita et al., 2000).

### *Eucalyptus saligna* Sm

According to Cremer (1990), *E. saligna* (Myrtaceae) is a fast growing hardwood tall tree, 30 to 55 m in height; with diameter at breast height over bark up to 2 m. Exceptional specimens may attain 60 and 2.5 m. The trunk is generally of excellent form, straight and clear of branches for half to two-thirds of tree height. Upper bark is smooth, grey-green, bluish-green or white, with a stocking of persistent, rough, brown or grey bark 1 to 4 m from ground level. The flowers are small, white in clusters of 7 to 11. Fruits are woody capsules, obconical to slightly pear-shaped, with 3 or 4 valves with thin, pointed tips which are exerted (protruding) and erect or out-curved. Seeds are brown, 1 to 2 mm long, cuboid or ovoid, with the dorsal surface pitted. It is predominantly found in moist forests on better soils of the east coast of Australia. *E. saligna* is moderately drought and frost tolerant and is widely planted in Australia and overseas (Cremer, 1990). The dry leaves of *Eucalyptus* species are frequently used in grain stores across Africa to deter feeding by *Sitophilus zeamais* and *Rhyzopertha dominica* to protect stored grains against insect attack (Hermann, 2010).

***Eucalyptus globulus* Labill**.

According to Katende et al. (1995), *E. globulus* (Myrtaceae) is usually a fast growing tall tree, grows up to 55 m high, the crown is rounded and open with straight main stems. The bark is blue-grey, smooth peeling in long strips and rough at the base. Young leaves are opposite, oval, blue-grey without stalks. The mature leaves are deep blue-green, thin and long to 30 cm, slightly curved with sharp tips, stalked and smell like camphor when crushed. The flowers have grey-green buds, wrinkled up to 2.5 cm, usually one, rarely 2 or 3 white flowers up to 4 cm across. The fruits are woody, half spheres, rough, 3 cm across with no stalks. The wood is hard, heavy and strong. The wood is usually used for poles and construction. The tree can be planted in pure stands, as an ornamental or as an avenue tree. The young leaves of this species are used to produce an essential oil used in pharmaceutical products. *E. globulus* grows naturally in the cool wet parts of S. W Australia. It prefers good-quality loams with adequate but not excessive moisture. It is suitable for areas over 2,000 m above sea level. This species does well in several uplands of Uganda. Its oil can be used for cleaning and as a natural insecticide.

***Cupressus lusitanica* (Mill)**

According to Katende et al. (1995), *C. lusitanica* (Cupressaceae) is an evergreen fast-growing tree. It has a straight trunk, generally conical but irregular in shape, branches hang down with branchlets in all directions and grow up to 35 m high. The bark is red-brown with vertical grooves which turn grey with age. The leaves are tiny, dull blue-green, in 4 ranks with spreading pointed tips. The fruit has male cones which appear like fat tips on branchlets, producing clouds of yellow pollen dust. Female cones are rounded, 1.5 cm across, brown with central pointed projections. About 75 winged seeds are released from beneath the cone scales. *C. lusitanica* grows fast on good sites and tends to be invasive at high altitudes. It is used for firewood, poles, posts, timber, ornamental purposes, shade and windbreaks and for live fencing. The leaves of this plant are used to cure some skin diseases caused by dermatophytes and have also been used to ward off insects from stored grain. In Costa Rica, a drink made by steeping a branch in alcohol is taken to alleviate coughs and cold symptoms Kuiate et al. (2006). The essential oil of *C. lusitanica* is extracted from the needles and twigs of young branches by steam distillation. This clear and fresh smelling oil is used for aromatherapy and massage to correct several body disorders. For example it restores calmness, sooths anger, improves circulation, sorts out coughs and bronchitis. It helps in varicose veins and female menstrual problem. The essential oil was screened for antimicrobial activity, and it showed antibacterial activity against *Bacillus cereus* and antifungal activity against *Aspergillus niger* (Hassanzadeh et al., 2010).

***Tithonia diversifolia* (Hemsl.)**

*T. diversifolia* family Asteraceae, is believed to have originated from Mexico and is commonly known as the Mexican sunflower. It is now widely spread throughout the humid and sub humid tropic regions. *T. diversifolia* is thought to have been introduced to several parts of Africa as an ornamental plant. According to Akanbi et al. (2007), it is reported to be growing in several African countries including Kenya, Nigeria, Rwanda, Zimbabwe, Cameroon, Uganda and Zambia. It is a perennial aggressive weed, a shrub, grows to about 2.5 m of height and grows well in most soils. Sun flowers are up to 10 cm across; petals are orange to yellow and 4 to 5 cm long and produce many seeds which can remain dormant for up to 4 months (Muoghalu and Chuba, 2005). It is commonly found growing in waste or abandoned lands, along roads, railway lines and on cultivated farmlands. It is considered to be an invasive plant in many parts of southern and eastern Africa, Australia and many pacific Islands. It is reported to be allelopathic where by the dense shading below the plant inhibits growth of other seedlings. Rapid vegetation reproduction and the production of many light seeds allow the plant to invade a disturbed habitat very quickly. It also competes with crops (Muoghalu and Chuba, 2005, Akanbi et al., 2007). *T. diversifolia* is known to be used for various purposes worldwide among which include the following: poultry feed, fuel, manure, fodders and land demarcation. The leaf extract is used to protect crops from termites and other insect attacks. The green biomass has been reported to be an effective nutrient source for low land rice, maize, okra, water melon and pumpkins (Akanbi et al., 2007).

## MATERIALS AND METHODS

### Collection of plant materials

Leaves of the selected pesticide plants (Figure 1) were collected from Mukono District, Uganda for aqueous and ethanol extraction. The essential oils were sent from the Department of Biological Sciences, Egerton University, Egerton, Kenya. Leaves were collected because the local communities use them to prepare the insecticides. The plants were identified by taxonomists in the Herbarium Department of Botany, Makerere University. This was done to ensure that the plants are rightly identified.

### Preparation, extraction and concentration of plant materials

The collected plant materials (leaves) were separately exposed under shade until they became dry. Leaves of *C. lusitanica, O. gratissimum, T. diversifolia* and *E. globulus* were pounded using an

*Ocimum gratissimum*

Source: Floridata.com

*Eucalyptus saligna*

Photo taken by P.K. Bett (2011)

*Eucalyptus globulus*

Source: Floridata.com

*Cupressus lusitanica*

Source: Floridata.com

*Tithonia diversifolia*

Source: Floridata.com

**Figure 1.** Plants used in the study.

electric motor. 200 g of the pounded material were separately soaked in ethanol (95%) followed by shaking periodically for three days and then filtered. This was repeated three times to allow ethanol extract substantial quantities of the chemical constituents from the pounded plant materials. The solutions were filtered by the use of cotton wool and the filtrates were concentrated using a rotary evaporator and water bath maintained at 70°C. The concentrated ethanol extracts were put in a hot air oven maintained at 50°C and subsequently air dried. The dried extracts were kept in well labeled, dry, bijous bottles. Ethanol was used as a solvent because of its long and widespread use as a solvent for substances intended for human contact and consumption. Such substances include medicines, colorings, flavorings and scents among others.

In order to obtain the water extract, 200 g of the pounded materials were separately soaked in distilled water followed by shaking periodically for three days. The solutions were then filtered through a cheese cloth before further filtration using whatman A-1 filter paper. The filtrates were concentrated in a hot air oven

maintained at 50°C for two days and were subsequently air-dried to thick residues. The obtained extracts were labeled and kept in a fridge at 4°C till when needed.

Water was used as a solvent due to its good solvent properties brought about by its polarity. Because of this, a wide range of substances are known to be soluble in water. In addition to this, the study intended to use the same or similar methods local people use to obtain or prepare botanical insecticides. A high percentage of local communities are known to use water as solvent to prepare herbal medicines and insecticides.

**Extraction of the essential oils**

The extraction of the essential oils of *O. gratissimum*, *C. lusitanica* and *E. saligna* was done in the Department of Biological Sciences, Egerton University, Egerton, Kenya. The essential oils were extracted by steam distillation from fresh leaves. The leaves were cut into small pieces and put into a distillation flask. Steam was allowed to pass through each batch of leaves for two hours. Essential oils were trapped in collecting tubes and put in clean, dry, well labeled bottles. The essential oils were then kept in a fridge maintained at 0°C to freeze the water which had been trapped together with the oil. The oils were decanted off since they do not freeze like water. The essential oils were kept in a fridge so as to minimize their volatile behavior which is catalyzed by relatively high temperature including room temperature.

**Preparation of the test animals and determination of the acute toxicity of the plant extracts**

Test animals (Wistar mice) were purchased at 4 weeks of age from the School of Veterinary Medicine, Makerere University. They were randomly group housed in stainless wire cages living enough space for clear observation of each animal. The animals were fed on conventional laboratory food with unlimited supply of drinking water (*ad libitium*). This was done for 5 days prior to dosing so as to get them acclimatized to laboratory conditions. During this period, the mice were observed to assess their health conditions based on their external appearance, nutritional conditions and general behavior.

In order to determine the preliminary acute toxicity of the different plant extracts, 4 dose levels were prepared for each plant extract. For essential oils, the following dose levels were administered: 2,500, 5,000, 7,500 and 10,000 mg/kg. Each dose level was assigned 2 test animals (mice). The animals were randomly selected and marked to permit individual identification. The animals were only given drinking water and not food for 4 h prior to dosing. This was done to avoid food-drug interactions so as to aid optimum absorption of the plant extracts from the gut. The mice were weighed and the freshly prepared test substances administered base on their weights as single doses orally (gavage method). Concentrations of the extracts were prepared in such a way that no test animal received more than 2 ml/100 g body weight for the water extracts and 1 ml/100 g body weights for the rest of the extracts. The administration of the test substances was done by using intragastric plastic tubes for the different groups of test animals. For each extract, a control group was kept and given equal amounts of the vehicle substances (distilled water for ethanol and water extracts and sunflower seed oil for the essential oils). One group was kept, it neither received plant extracts nor carrier substance throughout the experiment. The mice were observed for 24 h. The dose level that killed 50% of the members of the test group was taken as the approximate $LD_{50}$. The results were recorded. The approximate $LD_{50}$ was then used to determine the correct $LD_{50}$.

For the correct $LD_{50}$, five dose levels were set within the range of the approximate $LD_{50}$ for each extract. Different dose levels were set for the different extracts as shown in Tables 1, 2 and 3. Each group was assigned 6 members (mice). Administration of the extracts was done as explained above. The results were recorded in Tables 1, 2 and 3 for each group. Probits were determined by use of Fisher and Yates statistical tables. The $LD_{50}$ values were determined by the use of the graphical method, plotting probits against the log dose, and thereafter running a regression analysis using a general formula:

$y = mx + c.$

## RESULTS

The acute toxicity tests showed weak toxicities for all the plant extracts investigated in the study. However, when compared with each other, essential oils showed lower lethal doses (more toxic) followed by ethanol extracts (slightly toxic) and lastly by the aqueous extracts (relatively harmless). *E. saligna* essential oil had the lowest $LD_{50}$ value (2,290 mg/kg) followed by *C. lustanica* (3,311 mg/kg) and then *O. gratissimum* (4,570 mg/kg). For ethanol extracts, *T. diversifolia* showed the lowest $LD_{50}$ value (11,481 mg/kg), followed by *E. globulus* (12,302 mg/kg), *O. gratissimum* (12,882 mg/kg), and lastly *C. lusitanica* (14,996 mg/kg). For the aqueous extracts, *T. diversifolia* was found to have the lowest $LD_{50}$ value (12,302 mg/kg). For *O. gratissimum, C. lusitanica* and *E. globulus,* their extracts did not show toxicity signs up to 15,000 mg/kg concentrations. The results are shown in the Table 4.

During the acute toxicity studies, the following general observations were made after the administration of the different essential oils to mice: Abnormal diminished body activity (hypo-activity), dizziness, rapid and deep breathing (hyperventilation), excess loss of saliva (salivation) and death of some group members. Deaths were recorded within 4 to 5 h after the administration for all essential oils. Similar observations were made for the ethanol extracts except that the dose levels at which such effects were observed were higher than those of the essential oils. It was also noted that the essential oils caused toxic signs and death within short periods as compared to the ethanol extracts. For the aqueous extracts, deaths were recorded for only *T. diversifolia*, although dizziness was observed for all extracts for sometime after which the mice recovered.

### Qualitative phytochemistry

Phytochemical screening was determined asdescribed by Parekh and Chanda (2007). There were more classes of phytochemical constituents found in the aqueous extracts as compared to those found in the ethanol extracts (Table 5). Tannins, alkaloids and steroid glycosides were found to be most abundant for both the ethanol and water extracts of all the plants. Starch was not found in any of the extracts, while flavonosides were only found in the ethanol extract of *E. saligna*.

**Table 1.** The dose levels used in the determination of $LD_{50s}$ of essential oils for different extracts.

| Group | *Ocimum gratissimum* | | | | | *Cupressus lusitanica* | | | | | *Eucalyptus saligna* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 |
| Dose level (mg/kg) | 4,500 | 4,800 | 5,000 | 5,500 | C | 2,500 | 3,000 | 4,000 | 4,500 | C | 1,500 | 2,000 | 2,500 | 3,000 | C |
| Log dose | 3.65 | 3.68 | 3.69 | 3.74 | | 3.39 | 3.47 | 3.60 | 3.65 | | 3.17 | 3.30 | 3.39 | 3.47 | |
| Dead/total | 1/4 | 3/4 | 3/4 | 4/4 | 0/6 | 2/6 | 3/6 | 2/6 | 5/6 | 0/6 | 0/6 | 1/6 | 2/6 | 6/6 | 0/6 |
| Dead (%) | 25 | 75 | 75 | 100 | | 33 | 50 | 33 | 83 | | 0 | 16 | 33 | 100 | |
| Corrected (%) | 25 | 75 | 75 | 93.75 | | 33 | 50 | 33 | 83 | | 4.16 | 16 | 33 | 95.8 | |
| Probits | 4.33 | 5.67 | 5.67 | 6.52 | | 4.56 | 5.00 | 4.56 | 5.95 | | 3.30 | 4.05 | 4.56 | 6.64 | |

C =control.

**Table 2.** The dose levels used in the determination of $LD_{50s}$ of different ethanol extracts.

| Group | *Eucalyptus globulus* | | | | | *Cupressus lusitanica* | | | | | *Ocimum gratissimum* | | | | | *Tithonia diversifolia* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 |
| Dose level (mg/kg) | C | 10,0.00 | 12,000 | 14,000 | 15,000 | C | 10,000 | 12,000 | 14,000 | 15,000 | C | 10,000 | 12,000 | 14,000 | 15,000 | C | 10,000 | 12,000 | 14,000 | 15,000 |
| Log dose | | 4 | 4.07 | 4.14 | 4.17 | | 4 | 4.07 | 4.14 | 4.17 | | 4 | 4.07 | 4.14 | 4.17 | | 4 | 4.07 | 4.14 | 4.17 |
| Dead/Total | 0/6 | 1/6 | 2/6 | 4/6 | 5/6 | | 0/6 | 1/6 | 2/6 | 3/6 | 0/6 | 0/6 | 1/6 | 3/6 | 6/6 | 0/6 | 1/6 | 3/6 | 6/6 | 6/6 |
| Dead (%) | | 16 | 33 | 66 | 83 | | 0 | 16 | 33 | 50 | | 0 | 16 | 50 | 95.83 | | 16 | 50 | 100 | 100 |
| Corrected (%) | | 16 | 33 | 66 | 83 | | 4.16 | 16 | 33 | 50 | | 4.16 | 16 | 50 | 95.83 | | 16 | 50 | 95.83 | 95.83 |
| Probits | | 4.01 | 4.56 | 5.41 | 5.95 | | 3.30 | 4.01 | 4.56 | 5.00 | | 3.30 | 4.01 | 5.00 | 6.64 | | 4.01 | 5.00 | 6.64 | 6064 |

C=control.

**Table 3.** The dose levels used in the determination of $LD_{50s}$ of aqueous extract of *T. diversifolia.*

| Group | Dose level mg/kg | Log dose | Dead/total | Dead (%) | Corrected (%) | Probits |
|---|---|---|---|---|---|---|
| 1. | Control | | 0/6 | | | |
| 2. | 12,000 | 4.08 | 3/6 | 50 | 50 | 5.00 |
| 3. | 10,000 | 4.00 | 2/6 | 33 | 33 | 4.56 |
| 4. | 8,000 | 3.90 | 0/6 | 4.16 | 4.16 | 3.30 |
| 5. | 6,000 | 3.77 | 0/6 | 4.16 | 4.16 | 3.30 |

C=control.

## DISCUSSION

According to the toxicity classification scale of Hodge and Sterner (1956), acute toxicity tests showed weak toxicities for all the plant extracts investigated in the study. Essential oils however, showed lower lethal doses (more toxic) followed by ethanol extracts (slightly toxic) and lastly the aqueous extracts (relatively harmless). The weak

**Table 4.** Showing the $LD_{50s}$ of the different plant extracts tested in the study.

| S/N | Plant name | Extract | $LD_{50}$ Value (mg/kg) |
|---|---|---|---|
| 1 | *Ocimum gratissimum* (L) | Essential oil | 4,570 |
| | | Ethanol | 12,882 |
| | | Aqueous | >15,000 |
| 2 | *Cupressus lusitanica* (Mill) | Essential oil | 3,311 |
| | | Ethanol | 14,996 |
| | | Aqueous | >15,000 |
| 3 | *Eucalyptus saligna* (Smith) | Essential oil | 2,290 |
| 4 | *Eucalyptus globulus* (Labill) | Ethanol | 12,302 |
| | | Aqueous | >15,000 |
| 5 | *Tithonia diversifolia* (Hemsl) | Ethanol | 11,481 |
| | | Aqueous | 12,302 |

**Table 5.** Phytochemical analysis of plant extracts.

| Parameter | *Ocimum gratissimum* | | *Eucalyptus globulus* | | *Tithonia diversifolia* | | *Cupressus lusitanica* | |
|---|---|---|---|---|---|---|---|---|
| | Aqueous extract | Ethanol extract | Aqueous extract | Ethanol extract | Aqueous extract | Ethanol extract | Aqueous extract | Ethanol extract |
| Tannins | + + + | - | + + + | + + + | + + | - | + + + | + + + |
| Alkaloid salts | + + + | + + | + + | + + + | + + + | + + | + + + | + + |
| Anthracenosides | + + | - | + + + | | - | - | + + + | - |
| Coumarin derivatives | + + + | + + | + + + | + + + | - | - | + + | + + + |
| Steroid glycosides | + + + | + + | + + | + + + | + + | + + + | + + + | + + + |
| Flavonosides | - | - | - | + + | - | - | - | - |
| Anthocyanosides | + + | - | + + | - | + + | + + | + + | + + |
| Reducing compounds | + + + | - | + + + | + + + | - | - | + + + | + + + |
| Starch | - | × | - | × | - | × | - | × |
| Polyuronides | + + + | × | - | × | + + + | × | - | × |
| Glucides | + + + | × | + + | × | + + + | × | + + + | × |
| Saponins | - | × | + + + | × | - | × | + + + | × |

The presence and qualitative abundance of phytochemical compounds IS shown: Very abundant (+++), moderate (++) , little (+), not available (-) test not done (×).

acute toxicity properties could be the reason why these plants have for long been used by local communities around the Lake Victoria region as storage pesticides.

Essential oils showed low $LD_{50}$ values (more toxic) as compared to ethanol and aqueous extracts of the same plants. This could be attributed to their complex nature since they are normally a mixture of various compounds. Hassanzadeh et al. (2010) reported that the major components of *C. lusitanica* leaf oil are α-pinene (40 to 82%), limonene (4 to 18%), isobornyl acetate (up to 10%) and *cis*-muurola-4 (14%), 5-diene (up to 7%). For *E. saligna*, the major components are, 1, 8-cineol (eucalyptol), α-pinene (45.1%), limonene (1.5%), terpinene (8.6%), p-cymene (cymol) (22.5%), terpenene-4-ol (8.6%), terpineol (9.9%) and carvocrol (Hermann, 2010; Patricia et al., 2007). The essential oil of *O. gratissimum* has components including thymol (31.79%), euginol, myrcene (6.94%), ɣ-terpinene (12.34%), α-thujene (6.11%) and p-cymene (15.57) (Hermann, 2010; Koffi et al., 2009). Many components of essential oils are thought to be toxic when taken orally (Shaaya et al., 1991).

More phytochemical constituents were found in the aqueous extracts as compared to those found in the ethanol extracts. However, ethanol extracts showed lower $LD_{50}$ values as compared to aqueous extracts. This could be due to the presence of toxic substances in the ethanol extracts which were not extracted by water due to their polarity characters. The results obtained from this study may be used to develop safer alternatives to fight crop pests. Based on the available literature, it is evident that plant extracts are biodegradable and thus will not cause similar environmental risks as many of the widely used synthetic agrochemicals. Biodegradability of botanicals may be an important factor which will increase demand for plant-based chemical products (Kari et al., 2011). On the other hand, it has also been found that the rapid biodegradability of these botanicals may hinder the registration process with the authorities. It is very difficult to obtain data indicating the spread of botanicals in the environment, because all the measurable components break down in soil within a few days. There is no single active ingredient or decomposition product which can be used as an indicator of the leaching risk (Kari et al., 2011). Other challenges include: the fact that most botanicals are slow in action and lack the rapid knockdown affect, brings about doubt as to whether they are as effective as the synthetic products. The issue of genetic variability of plant species in different localities is instability of the active ingredients when exposed to direct sunlight. Competition with synthetic pesticides through aggressive advertisement by commercial pesticides dealers and commercially formulated botanicals are more expensive than synthetic insecticides and are not as widely available (Yallappa et al., 2012).

## Conclusions

All the extracts assessed in this study were found to have weak acute toxicity properties. The weak acute toxicity properties could be the reason why these plants have for long been used by local communities around the Lake Victoria region as storage pesticides.

## RECOMMENDATIONS

The essential oils assessed in this study should be used with caution to avoid individuals from applying them for long term exposure at relatively high doses.

From this study, chronic toxicity studies should be carried out using several routes of exposure such as the oral, inhalation and skin absorption (dermal toxicity) since they are relevant occupational exposure routes.

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