Evaluation of Pesticidal Properties of *Euphorbia tirucalli* L. (Euphorbiaceae) against Selected Pests

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EVALUATION OF PESTICIDAL PROPERTIES OF *EUPHORBIA TIRUCALLI* L. (EUPHORBIACEAE) AGAINST SELECTED PESTS

Thesis submitted in fulfillment of the requirements of the degree of Doctor (**Ph.D.)**

in Applied Biological Sciences

Dutch translation:

EVALUATIE VAN PESTICIDE-EIGENSCHAPPEN VAN *EUPHORBIA TIRUCALLI* L. (EUPHORBIACEAE) OP GESELECTEERDE PLAGEN

Front and back cover photographs by **Julius Mwine**

Front cover photo: ***Euphorbia tirucalli* young pencil-like branches**

Back cover photo: **Cabbage (*Brassica oleracea* L.) plants treated (R) and untreated (L) with *E. tirucalli* latex**

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Chapter one

Introduction

1.1 Background and justification of the study

During the last few decades, environmental concerns have become an inevitable part of human livelihood. As a result, the search for safer and environmentally-friendlier implements for both agricultural and human use has become an important branch of science. More and more scientists are today involved in research trying to establish why there are now more catastrophes than ever before in terms of tsunamis, landslides, torrential rains, hurricanes, wild fires, severe pest attacks and more virulent and incurable diseases in plants and animals, wild and domestic, just as in humans. For example, Kundzewicz (2003) reports an increase of world floods frequency and severity in the last decade, while Van Aalst (2006) says that hurricanes have become harsher in the last two decades. Advanced technology i.e. computerized high precision implements and state of the art biotechnology, expected to solve these problems and ease man's work, appears to be causing more problems than it is solving. Consequently, one generation after another, more and more unexpected happenings are inherited to the chagrin of not only man but the whole biosphere.

The billion dollar question is: has man interfered with natural systems so much that there is a danger of complete breakdown of natural orders? Are calamities becoming the new order of events?

1.1.1 Climate change, environment and agriculture

Probably, the most daunting problem for everyone today and among the most widely discussed issues as likely causes for prevailing environmental dilemmas, are global warming and climate change. The latter two are attributed to numerous causes including gaps created in the ozone layer by Ozone Depleting Substances (ODSs) such as organochlorides/chlorofluorocarbons (CFCs) and methylbromide, as well as an abnormal accumulation of Green House Gasses (GHGs), like carbon dioxide, methane and others that increase atmospheric thermal retention (Guo *et al.*, 2001; Joos *et al.*, 2001; Weatherly and Arblaster, 2001; Zillman, 2001). For example, Crowley (2000) observed that a 21st-century global warming projection far exceeds the natural variability of the past 1,000 years and is greater than the best global temperature change estimate for the last interglacial period. He continues to say that natural variability only plays a subsidiary role in this global warming and that the most parsimonious explanation for most of the warming is an anthropogenic increase in GHGs. Evidence in support of his views has been provided in form of numerous models that have been developed (Gabric *et al.*, 2005; He *et al.*, 2005; Lo Monaco *et al.*, 2005; Schultz and Lebon, 2005) and through direct observations of abnormal environmental occurrences (Allan *et al.*, 2010; Thomas *et al.*, 2004; Walther *et al.*, 2002; Wolf and Woolf, 2005). Some specific observations include: changes in coral reef composition and rate of formation (Baker *et al.*, 2004); decline in number and breeding behaviour alterations of migratory birds (Both *et al.*, 2006); changes in outbreak ranges of insects

(Williams and Liebhold, 2002) as well as change in precipitation trends (Kasei *et al.*, 2010; Zhu and Meng, 2010) among others.

CFCs and methylbromide which are said to be the main ODSs (Butchart and Scaife, 2001; Fraser, 1997; Tokioka, 1990) are mainly anthropogenic (Dameris *et al.*, 2007; Manning *et al.*, 1996; Tanhua *et al.*, 2006). Main sources have been identified as: industrial, agricultural and domestic (Cartwright, 1993; Fraser, 1997; Gros *et al*., 2003). Mitigation measures should therefore focus on the reduction of emissions of ODSs as recommended by the Montreal and Kyoto protocols (Velders *et al.*, 2007; Wittneben *et al*., 2006).

The climate change story may be vast and mind-boggling but the sober truth is that each of us has in a way contributed to the problem and should therefore play his role of whatever magnitude in order to mitigate its ugly impact.

As agronomists, our cardinal function is to produce food. We should ensure that safe food is available in required quantity and quality not only for today but also for tomorrow. It is our obligation to ensure that production means used do not overexploit or adulterate (pollute) the environment for the benefit of the next generation. As a Kashmiri proverb puts it '*we have not inherited the world from our forefathers, we are borrowing it from our children*'. The implication is that resources ought to be used rationally for purposes of sustainability.

Therefore, agronomists should be environmentalists. They should bear in mind how the environment may be affected by agricultural activities. Zinn and Blodgett (1989) lament, that there is a gap between agronomists and environmentalists. They state that numerous agronomists focus on maximizing production, regardless of environmental impact. This implies that they over/misuse resources in such a way that what matters to them is just the harvest. In the same perspective, Goldburg (1992) reports that some pesticide-producing companies promote environment-unfriendly chemicals and block research into alternative technologies that are environment-friendly to improve their sales. This is likely to be dangerous to the environment.

It should be remembered that most resources used in agricultural production i.e. soil, water, air and vegetation are consumables. They can be exhausted whereas their productivity depends upon how they are used. There are numerous examples where soil exhaustion has caused low harvests (Gold *et al.*, 1999) and desertification has occurred due to poor methods of farming such as deforestation prior to cultivation (Anjum *et al*., 2010; Mulitza *et al*., 2010). Similarly, cases of diversity reduction and organism poisoning as a result of pesticide over/misuse have also been reported (Brittain *et al*., 2010; Rico *et al*., 2010). All these scenarios reflect the precarious balance of natural resources and remind us of how agriculture in particular and life in general can be affected by environmental degradation.

1.1.2 Pesticides and the environment

Casida and Quistad (1998) predict that because of their unequalled and invincible characteristics, synthetic pesticides are bound to be ubiquitous and here to stay. Indeed, the invention of DDT was hailed by everyone and was seen as a panacea to all pest problems, while its inventor, Paul Müller, won a Nobel Prize in 1948. But as Palumbi (2001) comments, before the Nobel ceremony, resistance had been reported in house flies whereas resistance in mosquitoes followed by 1960s. These cases were shortly followed by *The Silent Spring* (Carson, 1962) where she states ".. *chemicals have the power to kill every insect, the good and the bad, to still the song of birds and the leaping of fish in the streams, to coat the leaves with a deadly film, and to linger on in the soil…"* which became a wakeup call for pesticide users. Following *The Silent Spring's* publication, research on synthetic pesticides to establish their environmental impact grew in leaps and bounds. In agreement with Carson's observations, synthetic pesticides have been reported to accumulate in the environment, i.e. seep or leach into ground water systems (Albanis and Hela, 1995; Cerejeira *et al*., 2003; Hung and Thiemann, 2002; Matin *et al*., 1998), enter into food webs and accumulate in body fat of fish, reptiles, birds and humans (Campbell and Campbell, 2001; Dong *et al*., 2004; Hoshi *et al*., 1998; Minh *et al.*, 2004; Tanabe *et al*., 1998), where they disrupt physiological body functions and cause diseases like breast cancer (Alavanja *et al*., 2004; Hunter and Kelsey, 1993; Mathur *et al*., 2002). Pesticide accumulation has also been reported in the air where it can cause acid rain (Gong *et al*., 2010; Graveland, 1998; Millet *et al*., 1997) whereas methylbromine and organochlorides have been reported to contribute to ozone layer depletion and global warming (McConnell *et al.*, 1992; Ristaino and Thomas, 1997; Thomas *et al*., 2004). These disadvantages in mind, environmentalists have tried to lobby for policies that tend towards restricting pesticide use or even make farmers abandon them completely. But in agreement with Casida and Quistad (1998), Wilson and Tisdell (2001) affirm that farmers will continue using pesticides in spite of knowing the calamities associated with them, because they are 'locked in' the technology and cannot 'move out' for fear of economic losses.

Indeed, agriculture is a business and like all businesses, farmers aim at make a living otherwise they would have to abandon it. The environmentalists' call to abandon synthetic pesticides has been heard far and wide but some farmers have failed to visualize how to translate the call into cash. Instead, pesticide advocates like Avery (1996), were quick to prophesy doom for agriculture without synthetic pesticides. He contends that reduction of synthetic pesticides would cut yields, increase cost of production, increase size of farmland and therefore spell doom to wildlife and increase soil erosion and pest infestation, all resulting into famine. He also condemns organic farming as a gimmick for the rich to murder the poor concluding that synthetic pesticide must stay. In related cases, Zilberman *et al.* (1991) and Falconer and Hodge (2001) share the same views but advocate for a milder way of controlling pesticides by introducing a user fee/tax, so that farmers find them expensive and abandon them. Yet, Pimentel *et al*. (1991) considering an increase in production cost (due to increased cost of alternative pest controls) found out that a 50% reduction in synthetic pesticide use can increase food cost only by a meager 0.6%. Similar findings have been reported by Brethour and Weersink (2001) and Pimentel (2009) among others.

It is probably from such considerations that new principles like sustainable agriculture and integrated pest management (IPM) were born, to bring about a possible win-win situation between environmentalists and pesticide advocates. IPM, for example, seeks to integrate all applicable pest management techniques such as cultural means, use of resistant cultivars, botanicals, biological control and application of minimal quantities of synthetic pesticides, in effect reducing environmental pollution. On the extreme end of the no pesticide usage story is organic farming, which among other principles advocates for zero tolerance for chemical pesticides and fertilizer application.

These ecological and environment-friendly techniques are sometimes condemned by pesticide advocates like Avery (1996) in terms of cost effectiveness and pest management capabilities but their advantages/benefits have been documented. For example, Letourneau and Goldstein (2001) found no significant increase in production cost of tomatoes grown organically compared to those grown on conventional farms. Instead, they found that organic farms had an advantage of having high natural enemy abundance and greater species richness of all functional groups of arthropods i.e. herbivores, predators and parasitoids. Related findings showing economic benefits in organic farms have been reported (Brethour and Weersink, 2001; Edwards, 2009) while others like Bengtsson *et al.* (2005) and Hole *et al.* (2005) independently found an increase in biodiversity in organic systems. A related but more interesting finding is that organic food contains more essential nutrients than conventionally produced food (Brandt and Mølgaard, 2001). Citing better soil management practices in organic farming as the main cause of the difference, Worthington (2001) reports higher levels of vitamin C, iron, magnesium and phosphorus in organically produced food although Trewavas (2001) and Rosen (2010) deny it saying that it is a marketing gimmick. As Vasilikiotis (2001) asks ‘Can we afford not to go organic?’

In summary, advantages of reducing synthetic pesticides to a bare minimum are numerous as discussed above. Since pesticides have been associated with health problems like cancer and are reported to contribute to ozone layer depletion and global warming, as agronomists, we should play our part. We should try to reduce or completely abandon the use of harmful chemicals in favour of more ecological means of pest management such as cultural techniques, use of plant-based alternatives, biological control, use of resistant cultivars and others in order to produce safer food and save the planet for us and the next generations.

1.1.3 Application of plant-based pesticides (botanicals)

According to Benner (1993) and Isman (2005), botanical insecticides have long been touted as attractive alternatives to synthetic chemical insecticides for pest management because they reputedly pose little threat to the environment and human health. But as Isman (1997, 2005) says, only a handful is in commercial use in crop protection today. He points out that there are four major types of botanical products used for insect control. They include: pyrethrum, rotenone, neem and essential oils while the rest such as ryania, nicotine, sabadilla, garlic and others are still used traditionally on a small scale. While success stories of these plant-based pesticides do exist, e.g. (Cloyd *et al*., 2009; Gahukar, 2000; Gupta, 2007), there are still numerous commercialization barriers for these substances including: scarcity of their natural sources, standardization and quality control as well as their registration (Isman, 1997). In addition to these barriers, it is possible that more pesticidal plants and their active ingredients are yet to be discovered in addition to their mode of action. A number of them are still at valuation stages (Cloyd *et al.*, 2009) and will take time to reach the end user, i.e. the farmer. This is likely to be one of the reasons why many farmers mostly in developing countries, apply them in their crude form. Trial and error is often used in application which may bring about disadvantages of overdosing and eventual food poisoning. Luckily, as Shaalan *et al.* (2005) state, most botanicals have low human toxicity and low half-life, and can disappear from the environment in a short time.

In developing countries where crude extracts are mainly used (Isman, 2008), traditional knowledge concerning their application is of paramount importance, so that the correct concoctions are formulated and applied to pests on the right crop. This calls for concerted efforts between scientists and traditional farmers/healers to share ideas and come up with well-researched user packages - the reason why Indigenous Knowledge (IK) is important in sustainable agriculture. It should be noted that most IK in developing world is only scantly documented and is held by only a few people some of whom are aging. Much of such knowledge is passed on to the next generation by word of mouth or by observation of elders' practices (Gradé, 2008).

Conclusively, as organic farmers await research and registration of botanicals, (but they are acceptable without certification and registration in Uganda), agricultural scientists should endeavour to compile IK regarding botanicals, evaluate and package them appropriately for the benefit of the pest management fraternity.

1.2 Problem statement

Uganda is basically an agricultural country and about 85% of its population is involved in agriculturally-related activities. Most farmers practice subsistence agriculture on peasantry farms ranging from 0.5 to 5 ha (UBOS, 2007). Because of small farm size and low incomes involved, farm resources are meager and therefore, farmers mostly use local implements. For example, family labour is used, home-grown seed is a common feature and agro-chemicals such as fertilizers and pesticides are commonly applied only on commercial crops like cotton and tea because subsistence farmers cannot afford them and they are uneconomical in such a setting (Abate *et al*., 2000). Those who attempt to use them do so erratically due to affordability problems creating yet a worse problem of pesticide resistance.

Yet, farmers continuously face big problems of pests and diseases. Additionally, the country has of late embraced organic agriculture, since organic products are likely to fetch a better price on the international market. By principle, organic agriculture does not allow the use of synthetic agro-chemicals, Genetically Modified Organisms (GMOs) and heavy agricultural machinery (Rigby and Cáceres, 2001). As a result, even those who could afford synthetic pesticides cannot use them in an organic setting. Farmers are therefore faced with a pest and disease dilemma. Confronted with shortage of better technologies like biological control techniques and refined organically acceptable alternatives, farmers have been compelled to turn to traditional means like application of plant extracts which are unfortunately often not researched upon. Indeed, some native farmers are knowledgeable about some pesticidal plants but most do not know the particular pests to use them against, the dose to apply nor how often to apply them (Mwine, 2009). The implication is that even when the extracts would be effective, if they are wrongly applied, they would not perform to expected efficacy. Many Ugandan farmers, especially those practicing organic farming are faced with this dilemma. But agricultural extension system in Uganda encourages farmers to seek researched advice from National Agricultural Research Institutions (NARIs) of which Universities are part. This research is an attempt to answer a farmers' call for the need of researched information on plant-based/pesticidal extracts.

1.3 Objectives of the study

1.3.1 General objective

To evaluate pesticidal properties of *Euphorbia tirucalli* L. (Euphorbiaceae) against selected pests.

1.3.2 Specific objectives

- Develop an inventory of pesticidal plants used in southern Uganda in order to establish use and utility of *E. tirucalli* as a pesticidal plant.
- Evaluate efficacy of *E. tirucalli* fresh latex on major cabbage pests in the field.
- Assess *E. tirucalli* fresh latex efficacy on *Anopheles* mosquitoes in the field.
- Establish nematode host status of *E. tirucalli* in order to find out if it is nematicidal or a nematode victim.

1.4 Thesis outline

This thesis is presented in 7 chapters. Chapter one introduces the study and covers its background in terms of human influence to the environment and how application of technologies such as synthetic pesticides has resulted into catastrophes like depletion of the ozone layer, global warming and climate change. A call to minimize or completely abandon such technologies is made, echoed in the problem statement and objectives of the study. Chapter two reviews available literature related to the study subject. Euphorbiaceae family in general and *E. tirucalli* species in particular are reviewed in terms of their wide chemical composition, pointing out why they are important medicinal plants. Chapter three deals with an ethnobotanical survey of pesticidal plants in Southern Uganda, to establish their use and utility in general and that of *E. tirucalli* in particular. In the next three chapters, *E. tirucalli* is experimentally analyzed for pesticidal properties. Chapter four analyses its insecticidal properties in the crop fields, chapter five deals with its larvicidal features against *Anopheles* mosquitoes while chapter six reports on screening of *E. tirucalli* against nematodes for evidence of nematicidal properties. Chapter seven is a summary of findings with comments about different efficacy levels against the selected pests, ending with final conclusions and suggestions for further research work.

Chapter Two

Literature Review

2.1 Euphorbiaceae Family – A Source of Plant-based Medicinal Compounds

Adapted from:

J. Mwine and P. Van Damme Why do Euphorbiaceae Tick as Medicinal Plants? A review of Euphorbiaceae family and its medicinal features *Journal of medicinal plants research*, accepted December 21, 2010

Abstract

Euphorbiaceae is among the large flowering plant families (consisting of a wide variety of vegetative forms) some of which are plants of great importance. Its classification and chemistry have of late been subjects of interest possibly because of the wide variety of chemical composition of its members, many of which are poisonous but useful. In this review, we have tried to demonstrate why Euphorbiaceae are important medicinal plants. Two important issues have come up. The worldwide distribution of the family exposes its members to all sorts of habitats to which they must adapt, therefore inducing a large variety of chemicals (secondary substances) that are employed for survival/defense. Succulence and the CAM pathway that characterize a good number of its members were quoted as some of the adaptations that aid colonization and survival to achieve this induction. We have also found out that medicinal properties of some of its species may be due to stress factors that characterize most habitats of the family. Varying stress factors like temperature, salinity, drought and others were seen to operate in tandem with genetic factors such as gene expression and mutation loads to bring about synthesis of a wide assemblage of secondary substances that may probably be responsible for the family's medicinal nature. It was concluded that the family is a good starting point for the search for plant-based medicines.

Key words: bio-synthesis, ethnomedicine, secondary metabolites, stress physiology.

2.1.1 Introduction

Family Euphorbiaceae as traditionally delimited (Euphorbiaceae *s.l.*, Webster 1994) is one of the largest families of flowering plants, composed of over 300 genera and 8,000 species. According to the latter author, the family is very diverse in range, composed of all sorts of plants ranging from large woody trees through climbing lianas to simple weeds that grow prostrate to the ground. Members are widely distributed all around the world constituting both old world and new world plants some of which are yet to be identified. Many family members are inhabitants of tropical climates surviving hot dry desert conditions while others are rainforest trees and herbs. The family consists of species of great economic importance like *Ricinus communis* L. (castor oil plant), *Manihot esculenta* Crantz (cassava) and *Hevea brasiliensis* Willd. Ex. A. Juss (rubber tree) among others but also noxious weeds like *Euphorbia esula* L. and *E. maculata* L. (Schultes, 1987). The implication of this is that Euphorbiaceae is a complex family with a lot of research potential.

2.1.2 Euphorbiaceae classification

Complexity in habitat range and variability in morphology and genetics has made Euphorbiaceae classification difficult. Homogenous families have their classification based on simple, unique characteristics that cut across the family, e.g. monocotyledony and parallel venation for family Poaceae. In the case of Euphorbiaceae, there appears to be no particular and easily observable feature that can be used for its classification. In agreement with this, Webster (1994) states that no single feature characterizes the Euphorbiaceae. Instead, he enumerates several anatomic features like wood structure, laticifer type, trichomes and nature of stomata as being important for family classification, while others like pollen nuclear numbers, exine structures, type of pollination and inflorescence types are important for classifying genera, tribes and subfamilies.

According to Webster (1975), there have been several Euphorbiaceae classifications dating as far back as 1824 by taxonomists like Adrien Jussieu who identified the family's genera and Jean Mueller who provided a first detailed classification of the family into subfamilies, tribes and sub-tribes. Webster (1975) believes that Mueller's classification of 1866 was a milestone in Euphorbiaceae classification. He argues that Mueller was the first to use coherent phylogenetic characteristics that for a long period withstood the test of time.

Using Mueller's classification as a skeleton, Webster employed phylogenetic structures such as pollen morphology and anatomy to come up with his own classification. He divided the family into five subfamilies i.e. Acalyphoideae, Crotonoideae, Euphorbioideae, Phyllanthoideae and Oldfieldoiideae (Webster, 1975). According to this classification, the first three subfamilies are characterized by one ovule per locule (uni-ovulate) while the last two have two ovules (bi-ovulate). For several decades, this was the (traditional and) generally accepted form of classification.

As is common with such large and diverse families like Euphorbiaceae, there was constant pressure and proposals to re-define the family boundaries, to exclude genera that appear il-fitting and include those that appear left out, but also to carry out internal re-organization of subfamilies, tribes and sub-tribes. Phytochemical and molecular phylogenetic studies eventually accumulated evidence pointing to non-monophyly of Euphorbiaceae (Seigler, 1994b; Tokuoka and Tobe, 1995). This culminated into partitioning of the traditional Euphorbiaceae into five families, where only uni-ovulate subfamilies constituted family Euphorbiaceae *sensu lato*, others being upgraded with additions or subtractions into their own families (Webster, 1994) and was validated by the APG II group (Wurdack *et al.,* 2005).

The new classification left family Euphorbiaceae *s.l.* with five subfamilies, 49 tribes, 317 genera and about 8,000 species (Webster, 1994). However, little experimental support met this classification and more studies both in support and against or to further re-organize the family have since been in progress (Bruyns *et al*., 2006; Davis *et al*., 2007; Henderson, 1992; Hoffmann and McPherson, 2007; Seigler, 1994; Tokuoka, 2007; Wurdack *et al.*, 2005).

Using recent molecular study results based on DNA sequencing with molecular markers *rbcL*, *atpB*, *matK* and 18S *r*DNA; plastids *RBCL* and *TRNL-F* and a nuclear gene *PHYC* (Tokuoka, 2007; Wurdack *et al*., 2005), Euphorbiaceae *s. l.* family has recently been split again into five families namely Euphorbiaceae *sensu stricto*, Pandaceae, Phyllanthaceae, Picrodendraceae, and Putranjivaceae (Tokuoka, 2007). Work on re-organization and proof of monophyly within these groups has been going on (see Riina *et al*., 2010; Sierra *et al.*, 2010; Vorontsova and Hoffmann, 2008; Vorontsova *et al*., 2007; Wurdack and Davis, 2009). According to Stevens (2010), Euphorbiaceae Jussieu *s.s* is now made up of 218 genera and 5,735 species and has been subdivided into four (supported) clades namely Chelosiodeae K. Wurdack and Petra Hoffmann, Acalyphoideae Beilscmeid *s.s*, Crotonoideae Beilschmeid *s.s*, and Euphorbiodeae Beilscmeid *s.s*. The latter author contends that reorganization of the family still continues.

2.1.3 A note on Euphorbiaceae ethnomedicine

Just like the complexity in classification, ethnomedicine of Euphorbiaceae is very diverse. According to Seigler (1994), this diversity is due to the presence of a wide range of unusual secondary metabolites that makes most of the members poisonous. The family hosts one of the most poisonous substances of plant origin i.e. ricin, which is a protein found in *R. communis* (Palatnick and Tenenbein, 2000), whereas other species like *Jatropha curcas* L. are reported to be comparatively poisonous (Mampane *et al.*, 1987).

In an attempt to reveal the wide diversity of poisons of the family, Abdel-Fattah (1987) lists examples of species with following features: fish poisons e.g. *E. scheffleri* Pax, *E. tirucalli* L., and *E. inaequilatera* Sond; human poisons: *E. ledienii* A. Berger, *E. heterophylla* L., *E. cooperi* N.E.Br. ex A. Berger, *E. candelabrum* Kotschy, *E. virosa* Willd., *E. poissonii* Pax, *E. unispina* N.E.Br. and *E. venenifica* Tremaux ex Kotschy; poisons of domestic animals: *E. caput-medusae* L., *E. silenifolia* (Haworth) Sweet, *E. ingens* E. Mey. Ex Boiss; as well as irritating ones: *E. tirucalli*, *E. poissonii, E. unispina* and *E. venenifica*. In addition, some members are said to cause or influence susceptibility to certain body ailments. For example *E. tirucalli, E. leuconeura*, *J. curcas* and others are known to be co-carcinogenic and can influence/promote excessive cell division resulting in tumour growth (Hirota and Suttajit, 1988; Van Damme, 2001; Vogg *et al*., 1999). Also latex of *E. tirucalli* and *E. royleana* is known to cause conjunctivitis on contact with eyes (Shlamovitz *et al.*, 2009; Van Damme, 1989).

However, some members are very useful substances. Since time immemorial, many Euphorbiaceae have been popular traditional medicinal herbs. Genus *Euphorbia* and indeed family Euphorbiaceae were named in honour of a Greek physician to King Juba II of Mauritania called Euphorbus believed to have used *E. resinifera* latex to cure ailments for example, when the King had a swollen belly (Lovell, 1998; Van Damme, 2001). As early as 2 BC, Euphorbiaceae such as *Croton oblongifolius* Roxb. and *C. tiglium* Willd. were used to cure liver diseases, sprains, snake bites, and as a purgative for the first as well as insanity, convulsions, asthma, tumors, rheumatism for the latter, as documented in the Indian Ayurveda medicine system (Kapoor, 1989). Hooper (2002) also reports the use of *E. polycarpa*, *E. hirta*, and *Acalypha indica* L. for treatment of different ailments in the ancient Ayurveda system. In ancient Chinese medicine, Lai *et al.* (2004) report 33 species belonging to 17 genera of Euphorbiaceae used in herbal medicine. Similar reports have been cited for the ancient Yucatan herbal system applying different Euphorbiaceae like *E. ptercineura* for asthma and cough; *C. peraeruginosus* for pimples and *Phyllanthus micrandrus* Müll. Arg. for wounds, inflammations and infections among others (Ankli *et al*., 1999).

Even today, many Euphorbiaceae plant concoctions, fresh latex and teas are used in alternative medicine. For example, *E. tirucalli* is known for its curative features against diseases like warts, cancer, gonorrhea, arthritis, asthma, cough, earache, neuralgia, rheumatism, toothache, excrescences, tumours and others (Cataluna and Rates, 1999; Duke, 1983; Van Damme, 1989). *E. thymifolia* is used as an anti-viral against simplex virus-2 (Gupta, 2007) whereas *E. maculata* is said to cure cholera, diarrhea and dysentery (www.botanical.com.). The latter website lists a number of Euphorbiaceae with varying curative features including: *E. peplus* L., *E. peploides*, *E. pilosa*, *E. palustris* being remedies for hydrophobia; *E. peplus, E. helioscopia*, *E. humistrata*, *E. hypericifolia, E. portulacoides* L., *E. iata* Engelm, *E. marginata* Pursh, *E. drummondii* and *E. heterodoxa* for general home ailments. Most of the species, however, are cited in folk medicine where their dosage and efficacy are not clear hence the need for medical research to establish their safety.

Research has shown that some Euphorbiaceae are actually potent as medicinal plants and their extracts have been isolated and patented as modern drugs. A table of some US patents is shown in table 2.1.

Table 2.1 Examples of US patents of medicinal Euphorbiaceae extracts

Patent no.	Inventor	Claim/Ailments	Species involved	Patent date
US 5707631	Advanced plant Pharm. Inc.	therapeutic herbal composition	*E. lathyris*	January 1998
US 6844013	Peplin Biotech Pyt.	immuno-stimulation	*E. peplus, E. hirta, E. drummondii*	March 2001
US 2003/0165579 A1	LaRiviere Grubman & Payne LLP.	tumour inhibition	*E. antiquorum*	February 2002
US 6432452	Peplin Biotech Pty.	anti-cancer compound	*E. peplus, E. hirta, E. drummondii*	August 2002
US 2003/0171334 A1	Peter Gordon Parsons	prostate cancer	*E. aaron-rossii, E. tirucalli, E. tomentella, E. tomentosa*	September 2003
US 6923993	Nicholas Dodato	anti-cancer components	*E. obesa*	August 2005
US 2007/0248694 A1	PhytoMyco Research Corp.	anti-inflammatory properties	*E. hirta*	October 2007
US 2006/0198905 A1	Rajesh Jain & others	ano-rectal and colonal diseases	*E. prostrata*	May 2008

Source: United States Patent and trademark office (seen at http://patft.uspto.gov.)

Table 2.2 Examples of medicinal species in Euphorbiaceae and their remedies

Pesticidal feature	Species	Chemical compound(s)	Cited reference(s)
Anti- bacterial	*E. guyoniana* Boiss. & Reut.	diterpenes	El-Bassuony (2007)
	E. sororia Schrenk	ceramides and ellagic acid derivatives	Zhang *et al*. (2008)
	E. hirta	tannins, alkaloids and flavonoids	Ogbulie *et al*. (2007)
	E. pubescens Vahl.	diterpenes and ent-abietanes	Valente *et al*. (2004)
	M. esculenta	glycocide	Zakaria *et al*. (2006)
	E. sessiliflora Roxb.	triterpenes and ellagic acid derivatives	Sutthivaiyakit *et al*. (2000)
	E. segetalis L.	coumarins and steroids	Madureiira *et al*. (2002)
	J. podagrica Hook.	diterpenoids	Alyelaagbe *et al*. (2007)
	E. ebracteolata Hayata	casbane diterpenoids	Xu *et al*. (1998)
	E. heterophylla	saponins, flavonoids and tannins	Falodun *et al*. (2008)
	Drypetes inaequalis Hutch.	triterpenoid esters and saponins	Awanchiri *et al*. (2009)
Anti-viral	*E. kansui*	triterpenes, sterols and diterpenes	Zheng *et al*. (1998)
	E. hyberna L.	diterpenes	Bedoya *et al*. (2009)
	E. cotinifolia L. , *E. tirucalli*	diterpenes	Bentacur-Galvis *et al.* (2002)
	E. thymifolia	alkaloids	Jabbar and Khan (1965)
	E. thymifolia	triterpenes and alkaloids	Lin *et al*. (2002)
Anti-fungal	*Macaranga monandra* Müll.Arg.	diterpenes	Salah *et al*. (2003)
	E. nivulia Buch.-Ham.	-	Annapurna *et al*. (2004)
	E. hirta, E. tirucalli	diterpenes and triterpenes	Mohamed *et al*. (1996)
	R. communis	fatty acids	Maria Fatima *et al.* (2004)
	J. curcas	glucanase protein	Wei *et al*. (2005)
Nematicidal	*E. tirucalli*, *E. helioscopia*, *E. splendens* Bojer. Ex Hooke. *E. pulcherrima* Willd. Ex Klotzsch	diterpenes	Devi and Gupta (2000)
	E. pulcherrima	-	Cox *et al.* (2006)
	Phyllanthus niruri L.	flavanones	Shakil *et al.* (2006); Shakil *et al.* (2008)
	E. kansui	diterpenes and ingenane	Shi *et al*. (2007); Shi *et al.* (2008)
	E. hirta	phenols	Adedapo *et al*. (2005)
	J. podagrica	peptides	Dahiya (2008)
Moluscicidal	*E. tirucalli*	-	Jurberg *et al*. (1985); Vassiliades (1984)
	E. conspicua N.E. Br.	diterpenes and triterpenes	Dos Santos *et al*. (2007)
	E. splendens	-	de Vasconcellos and de Amorim (2003)
	J. elliptica Müll. Arg.	diterpenes	dos Santos and Sant'Ana (1999)
	J. curcas	phorbal esters	Gubitz *et al*. (1999)
	E. paralias L.	diterpenes	Abdelgaleil *et al*. (2002)
Insecticidal	*E. hirta*	flavonol glycosides	Liu *et al*. (2007)
	J. curcas	sterols, triterpenes alcohols and acids	Adebowale and Adedire (2006)
	R. communis	flavonoids	Shripad (2003)
	R. communis	ricinine	Maria Fatima *et al.* (2004)
	J. curcas	diterpenoids	Goel *et al*. (2007)
	C. pseudoniveus, C. suberosus	essential oils	Perez-Amador *et al*. (2003)
Anti-leishmanial	*D. chevalieri*	furansesquiterpene and triterpenoids	Wansi *et al*. (2007)
	J. grossidentata, J. isabellii	diterpenes	Schmeda-Hirschmann *et al*. (1996)
	J. grossidentata	diterpenes	Akendengue *et al.* (1999)
	P. cajucara	essential oils	Ahmed *et al*. (2006)

Some of the extracts are registered drugs and as such available on the market. Examples include Euphorbium (resiniferatoxin), from latex of *E. resinifera* (Appendino and Szallasi, 1997) marketed as 'Complexe Lehning Euphorbium N 88' and used as a nasal spray or compositum against viral infections, rhinitis of various origins, sinusitis, chronic nasal discharge, dry and inflamed nasal membranes as well as flu symptoms. *E. pilulifera* (the asthma weed) extract has been cited in Steadman's drugs list and can be applied against asthma, coryza and other respiratory infections and as an anti-spasmodic (www.drugs.com). Dysenteral® is an extract from *E. hirta* and is used in the treatment of diarrheal diseases (Elujoba *et al*., 2005) while Radix is an extract from *E. kansui* roots used as a purgative. Many other Euphorbiaceae are prospective important veterinary and agricultural biocides as demonstrated in literature (see table 2.2).

The main constituents of these pharmaceutical substances are volatile compounds generally referred to as essential oils. According to Masada (1976) and Boyom *et al*. (2003), essential oil is an 'amorphous' term used generally to describe a complexity of bioactive chemical substances with different molecular formulae. They maintain that individual oils may have hundreds of constituents, the principle components being terpenes (monoterpenes and sesquiterpenes) and their oxygenated derivatives. Other compounds may include phenylpropenes and specific compounds containing sulfur or nitrogen depending upon the plant species. Essential oils have a wide pharmaceutical range including anti-plasmodial (Boyom *et al*., 2003), anti-leishmanial (Ahmed *et al*., 2006), anti-cancer (Sylvestre *et al*., 2009), insecticidal (Perez-Amador *et al*., 2003) and others as also shown in table 2.2 and 2.3. Essential oils are not limited to Euphorbiaceae but are widely spread in many plant families including Annonaceae (Nguemtchouin *et al.,* 2009), Rutaceae (Rajkumar, and Jebanesan, 2010) and others.

Other uses of Euphorbiaceae include biodiesel production e.g. *E. tirucalli* (Duke, 1983; Van Damme, 2001), *E. lathyris* (Duke, 1983); *J. curcas* (Achten *et al*., 2008; de Oliveira *et al*., 2009; Kaushik *et al*., 2007; Kumar and Sharma, 2005); *M. esculenta* (Adeniyi *et al*., 2007); *R. communis* (Benavides *et al*., 2007; Meneghetti *et al.*, 2007) among others. Others are sources of food e.g. *M. esculenta* (Aloys and Ming, 2006), starch e.g. *M. esculenta* (Sanchez *et al*., 2009; Srinivas, 2007), while others are ornamental due to their attractiveness such as *E. milli*, *E. tirucalli* (Van Damme, 1989 and 2001), *E. obesa* and *E. pulcherrima.* Other minor uses include production of fuel wood, curving of wooden crafts, use as hedge/fence plants, timber production, use in re-forestation programs and others.

The above account indicates that in addition to other uses mentioned, Euphorbiaceae is an important source of herbal medicine of human, veterinary and agricultural importance. The outstanding question is 'Why is this family significant as a medicinal taxon?' The objective of this review is to attempt to provide an explanation why this may be so.

2.1.4 Why is Euphorbiaceae rich in medicinal compounds?

Euphorbiaceae *s.l.* is composed of five subfamilies, 49 tribes, 317 genera and about 8,000 species (Webster, 1994). This makes it one of the biggest plant families with probably the highest species richness in many habitats. The implication is that in absolute terms, there is a higher probability of having more species that are medicinal in that one family as compared to other families. Although such comparison (as to which family has the highest number of medicinal species) may not have been done, it appears that Euphorbiaceae may not compare badly to other families. Cited inventories in different parts of the world reveal that in Kenya, of 900 medicinal species recorded, about 60 belong to Euphorbiaceae (Leakey, 2006), in Loja province (southern Ecuador) they are 11 species of 214 (Bussmann and Sharon, 2006), in Jinja district (eastern Uganda) they are 5 out of 88 (Bukenya-Ziraba and Kamoga, 2007), in Sango bay area (southern Uganda) they are 14 out of 186 (Ssegawa and Kasenene, 2007), in Riau province, Sumatra, Indonesia, they are 11 out of 114 (Grosvenor *et al*., 1995). This approximates to about 7% of the species cited. Bearing in mind that there are about 350 families in the plant Kingdom, this is not a bad score. This, however, depends upon the region in question since Euphorbiaceae is most prevalent in tropical and subtropical areas.

According to Oldfield (1997), a good number of Euphorbiaceae species especially of genus *Euphorbia* (650 species), are succulent. The author describes succulence as a plant characteristic mainly tropical or subtropical that has to conserve water due to habitat aridity. Von Willert *et al*. (1990) describe it as a characteristic that makes a plant temporarily independent from external water supply when soil water conditions have deteriorated such that the roots are no longer able to provide the necessary water from the soil. They argue that this is only a temporary adaptation to aridity and unless conditions allow the refilling of the plant's succulent tissues, it will not survive. This implies that such plants tend to avoid mechanisms that result into water loss (including thin leaf surface, broad leaves and a large number of leaves), whereas they invest in features that conserve water such as thick waxy leaves, scaly leaves or thorns, fewer/sunken stomata, use of stems for photosynthesis, use of Crassulacean Acid Metabolism (CAM) and others. It is therefore a survival strategy for plants in arid and semi-arid areas.

According to Griffiths *et al*. (2008), leaf succulence is a key morphological correlate of the capacity for CAM, since succulence increases the plants' commitment to the use of CAM pathway during carbon dioxide fixation. In their experiments with two succulent plants, the latter authors found out that the magnitude of CAM was higher for the more succulent leaves of *Kalanchoe daigremontiana*

Raym. Hamet & H. Perrier (Crassulaceae) compared to the less succulent leaves of *K. pinnata* (Lam.) Pers. In the same spirit, Van Damme (1989) had earlier on pointed to the CAM-succulence syndrome stating that the two have to always go together, to which Lüttge (2004) concurs insisting that all CAM plants display some level of succulence.

The CAM pathway is known to be under circadian control and is subject to regulation by multiple oscillators, which modulate elements of the pathway in line with environmental conditions (Borland *et al*., 1999; Borland and Taybi, 2004). In line with this, Lüttge (2004 and 2008) enumerates a 'wealth of environmental factors' known to determine, or at least modulate the expression of CAM to include: carbon dioxide, water, absolute temperatures, day-night temperature regimes, irradiance and salinity among others. The latter author goes on to provide a model that relates these factors, drawing a conclusion that CAM-prone ecosystems are those that are governed by a network of interacting stress factors requiring versatile responses and not systems where a single stress factor strongly prevails. They point out a number of CAM domains or ecosystems that are likely to host CAM/succulent plants including submerged aquatic sites, deserts, salinas, savannahs, inselbergs, forests, and high latitudes such as tropical highlands and alpine regions. This implies that although the CAM pathway is an adaptation for succulent plants to balancing their carbon and water budgets (Lüttge, 2008; von Willert *et al*., 1990), it is also a survival mechanism for adverse conditions. Indeed, the extent of succulence has been positively correlated to both colonization of increasingly arid habitats and an increased contribution of CAM activity to total carbon gain (Herrera, 2009; Kluge *et al.*, 2001, Van Damme, 1989 and 2001), implying that succulent plants are better equipped for new habitat colonization than their non- or less succulent relatives, which gives them higher chances for survival in a wider habitat range.

As an extra adaptation, some succulent plants have been found to combine both C3 and CAM pathways. For example, Van Damme (1989 and 2001) reports that *E. tirucalli* utilizes both pathways. Its minute deciduous leaves utilize the C3 pathway while the stem uses CAM. He explains that the small leaves are preferentially used in normal situations whereas the green stem takes over when the leaves fall off in arid conditions. According to him, a combination of both pathways increases the plant's Water Use Efficiency (WUE) since the small leaves have a high affinity for carbon dioxide but tend to use water less efficiently. He predicts that there could be many other Euphorbiaceae using the same mechanism which he says improves productivity and ability to colonize a wide range of habitats.

Euphorbiaceae are very widely distributed in almost all habitats and occupy a wide range of climatic and soil disparities. Ahmad *et al*. (2006) and Bloomquist (2004) report that different habitat conditions e.g. soils, pH, temperatures and moisture tend to influence plant physiological processes hence the manufacture and accumulation of different chemical substances. In agreement, Melten *et al*. (2009) confirm that plants' responses can differ due to different factors. For example, physiological alteration of photosynthetic enzyme ratios to adjust to changing light conditions, suberin production to limit moisture loss from roots, genetic regulation of enzymes in response to resource limitation, or production of secondary metabolites in leaves in response to insect/microbial attack. In the same view, Veronese *et al*. (2003) found out that different herbivores (as found in different environments) tend to induce action of different defense systems. The implication of all this is that due to a wide range of conditions, which different Euphorbiaceae species are subjected to, the latter tend to manufacture a wide range of secondary plant substances to aid response to a disparity of stimuli in their particular habitats. For example, Zhang *et al*. (2000) reported manufacture of different lectins due to different environmental stress factors that occur in varying habitats. Lectins constitute part of a plant's defense system against herbivores (Van Damme, 2008). Similarly, Agrawal and Rutter (1998) found that changes in environmental cues can trigger modifications in a plant's defense strategy as was witnessed in ant plants (*Myrmecodia* spp.). Also, Taniguchi *et al*. (2002) established that production of secondary substances like pentagalloylglucose was remarkably enhanced under light irradiation compared to dark conditions while tannin production was greatly affected by changing the concentrations and composition of nitrogen sources. These and other findings support the view that Euphorbiaceae may have a variety of medicinal substances due to a disparity of environmental conditions (stress factors) accruing from a wide habitat range.

Biosynthesis of secondary metabolites is a complex process and is still poorly understood. Hadacek (2002) states that biosynthesis and accumulation of secondary metabolites arise from highly regulated processes requiring both genetic and environment-specific controls. In a related view, Cavalier-Smith (2007) says that secondary metabolites are produced from universally present precursors mostly acetyl-CoA, amino acids or shikimate (shikimic acid) by taxon specific enzymes – the reason why most secondary metabolites are restricted to a single major taxon on the universal phyllogenetic tree or evolutionally related taxa. This specificity has been shown and supported by modern molecular techniques, for example, using cytochrome P450 enzymes (CPY79A1 and CYP71E1) involved in biosynthesis of the cynogenic glucoside dhurrin in *Sorghum bicolor* L. (Kahn *et al.*, 1999). This phenomenon is not new in the plant kingdom because some secondary metabolites are considered to be so taxon specific as to be used in classification of certain taxa or acting as proof of monophylly (see Gershenzon and Mabry, 1983; Herbert, 1989; Seigler, 1994; Wink, 2003). What may be controversial, however, is that there is a growing volume of evidence to show that plants of the same genus or family may synthesize different or at least varying secondary metabolites when growing in different conditions (Figueiredo *et al*., 2008; Koricheva *et al.*, 1998; Wink, 2003). Such inconsistencies in secondary metabolite profiles have been attributed to among other factors, differential expression of corresponding genes (Wink, 2003) but also change in gene sequence and mutations (Theis and Lerdau, 2003). These genetically related factors act in the wake of varying environmental factors, to cause alterations in secondary metabolite assemblages reflecting adaptations and particular life strategies embedded in a given phylogenetic framework (Koricheva *et al*., 1998). As indicated by Ogunwenmo *et al*. (2007), this argument appears plausible for the case of widely distributed families like Euphorbiaceae. Varying secondary metabolites may be synthesized within the taxon, as a result of different gene expression and increasing mutation loads, accruing from stressful environments that characterize most Euphorbiaceae habitats. This may result into a richer assemblage of secondary metabolites within the family.

During their evolution, CAM plants have developed a number of anatomical, physiological and genetic changes/adaptations, which differentiate them from C3 plants. Cushman and Bohnert (1997) mention several of them such as thin-walled cells containing prominent vacuoles, varying degrees of succulence and capacity to handle high degrees of organic acid accumulation. The latter authors go on to say that just like evolution processes, CAM induction or process of shifting from C3 to CAM, involves the regulation of a variety of enzymes and metabolite transporters making it a very complex metabolic adaptation (to environmental stress). They give an example of increased activities of glycolytic, gluconeogenic and C4 acid metabolism enzymes including phosphoenolpyruvate carboxylase (PEPC), phosphoenolpyruvate carboxykinase (PEPCK) and pyruvate phosphate dikinase (PPDK) which increase 40 fold due to environmental stress. On the genetic side, the same authors, citing transcription essays with nuclei isolated from leaves of *Mesembryanthemum crystallinum* L. (Aizoaceae), show that CAM-specific genes increase two to six times when plants are exposed to high salinity (a stress factor). In support, Borland and Taybi (2004), note that although physiological processes associated with this high organic acid accumulation are energy-intensive, the potential for high productivity is not compromised. To substantiate this fact, they give examples of agronomically important CAM species including pineapple (*Ananas comosus* Mill.) and *Agave* spp. that show productivities rivaling that of C3 and C4 plants. Since organic acids and genes are responsible for physiological plant processes, this implies that CAM plants are likely to have higher productivity both in quantity and quality (variety) of chemical substances including enzymes, proteins, amino acid as well as secondary metabolites of various nature.

Possession of a variety of chemical substances may entail being rich in medicinal attributes. For example, over sixty jatrophane, modified jatrophane, segetane, pepluane and paraliane diterpenoids were extracted, purified and characterized from different Euphorbiaceae such as *E. dendroides*, *E. characias*, *E. peplus*, *E. amygdaloides*, and *E. paralias*. Based on jatrophane and modified jatrophane skeletons these were shown to be potent inhibitors of P-glycoprotein activity – a membrane protein that confers upon cells the ability to resist lethal doses of certain cytotoxic drugs by pumping them out of the cells, thus reducing cytotoxic effects (Barile *et al*., 2008). Similarly, Corea *et al*. (2005) report the discovery of two new diterpenes, pepluanone 1 and 2 from *E. peplus*, which act as anti-inflammatory agents. Also Falodun *et al*. (2008) identified secondary metabolites such as saponins, flavonoids and tannins from *E. heterophylla* which exhibited good activity against xanthine oxidase enzymes. Related findings are distributed in the Euphorbiaceae literature (see table 2.3).

2.1.5 Conclusion

Each plant family may have its own good reason for possession of medicinal properties. For Euphorbiaceae family members, it would appear that their diverse medicinal properties are associated with their wide distribution which is supported by their survival adaptations such as succulence and CAM pathway. The exposure to a wide range of habitats predisposes them to inevitably high mutation loads (accruing from stressful habitats) hence the necessity to develop a wide battery range of defensive secondary metabolites that may explain why the family is widely pharmaceutical.

These literature review findings compare well with reports of other workers like Ahmad *et al*. (2006) and Okgibo *et al*. (2009) who similarly but independently reviewed a variety of Euphorbiaceae-based phytochemicals including alkaloids, phenols, flavonoids, saponins, tannins and essential oils and described their origins, characteristics and therapeutic uses.

This review has revealed a rich variety of medicinal and potentially medicinal properties of Euphorbiaceae and attempted to elucidate why Euphorbiaceae tick as medicinal plants. Euphorbiaceae is therefore, a good starting point for a search for phytomedicines of human, veterinary or pesticidal nature.

Table 2.3 Chemical substances found in Euphorbiaceae and their pharmaceutical indications

Chemical substance	Medicinal indication	Cited reference(s)
Diterpenes	anti-tumor	Duarte *et al*. (2008); Konoshima *et al*. (2001); Krebs *et al*. (2004)
	anti-biotic	El-Bassuony (2007); Li *et al*. (2008); Mathabe *et al*. (2008)
	anti-fungal	Salah *et al*. (2003)
	anti-plasmodial	Attioua *et al*. (2007)
	anti-ulcergenic	Hiruma-Lima *et al*. (2002)
	trypanacidal	Schmeda-Hirschmann *et al.* (1996)
Triterpenes	anti-biotic	Awanchiri *et al*. (2009); Mathabe *et al*. (2008)
	vaso-depressor	Barla *et al*. (2006)
	anti-inflammatory	Canelon *et al*. (2008); Nkeh *et al.* (2008)
	analgesic	Nkeh *et al*. (2003)
	anti-fungi	Ekpo and Pretorius (2007)
Flavonoids	anti-malarial	Liu *et al*. (2007)
	anti-inflammatory	Ekpo and Pretorius (2007)
Saponins	cytotoxic	Kiem *et al*. (2009)
	anti-ulcer	Ukwe (1997)
Tannins	anti-septic	Ekpo and Pretorius (2007)
	anti-viral	Bessong *et al*. (2006); Liu *et al.* (1999)
	anti-mutagenic	Rossi *et al*. (2003)
	anti-fungal	Hwang *et al*. (2001)
Alkaloids	anti-microbial	Dias *et al*. (2007); Gressler *et al.* (2008)
	anti-tumor	Suarez *et al*. (2004)
Esters	anti-tumor	Blanco-Molina *et al.* (2001); Goel *et al*. (2007)
	anti-biotic	Goel *et al*. (2007)
	cytotoxic	Baloch *et al*. (2006)
	allergic reactions	Thumm *et al*. (2002)
	cancerous	Cataluña and Rates (1999)
Ricin	cytotoxic	Lombard *et al*. (2001)
	lipolytic	Lombard *et al*. (2001)
	invertase activation	Vattuone *et al*. (1991)
Phenols	anti-tumor	Yu *et al*. (2005)
	anti-oxidant	Yang *et al*. (2007)

2.2 *Euphorbia tirucalli* – A Multi-purpose Tree

Adapted from:

J. Mwine and P. Van Damme *Euphorbia tirucalli* L. (Euphorbiaceae) – The Miracle Tree: Current Status of Available Knowledge *Scientific research and essays,* submitted October 2010

Abstract

Euphorbia tirucalli is one of the most important tree Euphorbias, known worldwide for its many uses. Endemic to tropical Africa where it often grows wild, it is usually planted for boundary demarcation but also as a live fence around compounds, shrines and kraals due to its ability to withstand extreme aridity and possession of low herbivore pressure. *E. tirucalli* has white latex which is vesicant and rubifacient but also known to be a remedy against many ailments. However, most of its medicinal features are reported in folk medicine and there appears to be little medical/laboratory analysis to validate them. In this review, we attempt to explore the current knowledge status about *E. tirucalli* in relation to its classification, chemical content and functions, and the extent to which modern research has gone to validate them. It was found that although a great deal has been done to analyze its chemical composition (bark, roots and latex), and potential for biodiesel production, little is available on validation of its application for medicinal purposes, yet it continues to be used in traditional and alternative medicine on a daily basis. Empirical research is called for to achieve this.

Key words: Ethnobotany, uses, folklore, Ethnopharmacology

2.2.1 Introduction

Euphorbia tirucalli L. belongs to genus *Euphorbia*, one of the 8,000 species within family Euphorbiaceae. It is a shrub or a small tree endemic to tropical areas with pencil-like branches from which it derives its vernacular name, the pencil-tree. *E. tirucalli* is generally evergreen since its stems and branches remain green all year round and are rarely fed on by herbivores. It bears white poisonous latex, which may possibly account for the low herbivore pressure and medicinal features.

According to Agroforestry online data base (seen at www.worldagroforestrycentre.org.), its common names in different languages include:

Amharic: Kinchib;

Arabic: Knjil;

English: Finger euphorbia, Indian spurge tree, Milk bush, Naked lady, Pencil-tree, Rubber euphorbia;

Filipin: Bali bali;

French: Arbre de Saint Sebastien, Euphorbe effile euphorbe, Garde maison, Tirucalli;

Malay: Kayu patah, Tentulang, Tulang, Tulang-tulang;

Somali: Dana;

Spanish: Alfabeto chino, Antena, Esqueleto, Palito, Aveloz;

Swahili: Mtupa mwitu, Mwasi, Utupa;

Thai: Khia cheen, Khia thian;

Vietnamese: San h(oo) xanh, X(uw) (ow)ng c(as); and in Ugandan languages: Kakoni (luganda), Oruyenje (runyankole).

In chinese, it is known as. 绿玉树 lü yu shu (www.efloras.org).

2.2.2 Classification

In the binomial system (USDA plants data base seen at www.plans.usda.gov), *Euphorbia tirucalli* L. belongs to:

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Malpighiales

Family: Euphorbiaceae

Genus*:* *Euphorbia*

Species: *E. tirucalli*

Binomial name: *Euphorbia tirucalli* L.

According to the APG II system (Stevens, 2001), *Euphorbia tirucalli* L. belongs to:

Cladus: Eukaryota
Regnum: Plantae
Cladus: Angiospermae
Cladus: Eudicots
Cladus: Core eudicots
Cladus: Rosids
Cladus: Eurosids I
Ordo: Malpighiales
Familia: Euphorbiaceae
Subfamilia: Euphorbioideae
Tribus: Euphorbieae
Subtribus: Euphorbiinae
Genus: *Euphorbia*
Species: *Euphorbia tirucalli.*

While classification from Regnum to Species is clear, no literature has been cited for the lower subdivisions such as subspecies, varieties and others. But referring to his 18 accessions/samples of *E. tirucalli* collected from different countries, Van Damme (2001) reports that there are some differences in vegetative structure in terms of relative dimensions of stems, leaves, general plant habit and growth rate, although no conclusive bio-chemical/molecular tests have been made to evaluate their genetic differences that would be a basis of classification. According to Van Damme (pers. comm.), differences are minor in most specimens except for the specimens from US, New York State, whose pencils/branches are yellow-red in colour while the rest are green. Our observation on these accessions at the Laboratory of Tropical and Subtropical Agriculture and Ethnobotany, University of Ghent, Belgium, revealed that some differences do exist. For example, specimens from Morocco and Senegal form thick, short and stocky whorls at branching which remain relatively closed, giving them a closed broom appearance even after opening up. On the other hand, specimens from Burundi, Kenya and Rwanda tend to form thin, elongate and more-open-at-base whorls. The former specimens show a higher canopy formation tendency than the latter. Ugandan specimens have similar appearances as those from Burundi, Kenya and Rwanda (see plates 2.2a and b). Also, as communicated by Van Damme above, young pencils of samples obtained from US (New York State), are bright yellow-red in colour and stand out from others which are green (see plate 2.1). It was not possible to make comparisons of the specimens in other features such as height, rate of growth, flowering, fruiting and others because the potted plants available were planted at different times. What appears certain, however, is that these variations are not just environmental differences since the specimens have been raised as cuttings under the same greenhouse conditions for over twenty years, yet the differences have persisted (Geirnaert pers. comm.). This is suggestive of subdivisions within the species. More research is required to analyze more specimens and using proper methodologies to establish whether there is a need to subdivide the species into its lower taxonomic forms.

2.2.3 Plant description

Morphology of *E. tirucalli* has been extensively studied by Van Damme (1985, 1990, 2001). According to the author, *E. tirucalli* is an unarmed shrub or small tree that can grow 4-12 m high and about 15- 20 cm in trunk diameter. Its branches are evergreen, longitudinal, succulent, about 7 mm thick and usually produced in whorls, rarely single, giving it a broom- like structure. Branches usually end in smaller pencil-like twigs, dull green to red green in colour, with fine white striations and produced in whorls of 2 to 6. Its young stem is green, photosynthetic with grooves which in fact are small canal-like structures containing stomata protected from extreme conditions. The stem stomatal frequency is estimated at 12 per mm^2 in grooves on older stem parts, while it may reach 40 per mm^2 on a smooth younger part of the stem. Older stems become rough, brown and lose their photosynthetic ability with age.

Figure 2.1 Twig of *E. tirucalli* showing position of leaves

Courtesy: Van Damme (1989)

Plate 2.1 Red pencils of *E. tirucalli* specimens from US -New York State (*Photo by Mwine Julius*)

Plate 2.2

a) ***E. tirucalli* specimen from Morocco (*Photo by Julius Mwine*)**

closed-broom structure with a tendency for canopy formation

b) *E. tirucalli* specimen from Burundi (*Photo by Julius Mwine*)

open-broom structure with no observable tendencies for canopy formation

Plate 2.3 *E. tirucalli* efflorescence (*Photo by Julius Mwine)*

Plate 2.4 *E. tirucalli* potted plant as an ornamental (*Photo by Julius Mwine*)

Leaves are few, simple, scattered, entire, alternate, oblanceolate, about 1.3 to 2.5 cm long and 2 cm wide but broadest beyond the middle. They are present only at the tips of young branchlets. They have glandular, minute, dark brown stipules and are quickly deciduous (Van Damme, 1985 and 2001, also see fig 2.1).

According to the same author, inflorescences are stalkless and appear as yellowish heads in clusters of 2-6 cymes (Plate 2.3). The cymes produce a dense cluster of cynthia that develop only male flowers and occasionally a few female flowers but in some plants, cynthia are few and only female flowers develop. Cynthia have cup-shaped single sex involucres. Male involucres have bracteoles which are linear with plumose apices. Stamens are usually single per stalk and are about 4.5 mm long. Occasionally, an aborted female flower is present. In female involucres, the perianth is distinct and 3-lobed existing below a tomentose ovary which is lobular and about 0.5 mm long. The ovary is joined at the base with thickened bifid apices. Occasionally, a female flower exists within the involucres. Each involucre bears five independent nectaries that produce nectar and therefore flowers are insect-pollinated.

Fruits are glabrescent capsules on a tomentose pedicel, yellowish red when ripe and fall off easily. Seeds are ovoid, 3.5 x 2.8 mm in size, smooth, buff-speckled and with a dark brown ventral line. A caruncle exists which is about 1 mm across (Van Damme, 1985).

2.2.4 Ecology and distribution

E. tirucalli is probably the best known and most widespread of all tree Euphorbia species (Gildenhuys, 2006). According to the same author, the plant's origin is not known but Van Damme (1989) and Schmelzer and Gurib-Fakim (2008) believe it originated from tropical East Africa and it is endemic in countries such as Angola, Eritrea, Ethiopia, Kenya, Malawi, Mauritius, Rwanda, Senegal, Sudan, Tanzania, Uganda and Zanzibar. The same authors intimate that the tree is currently widely distributed in southern Europe, Asia and the Americas, having been steadily introduced due to its ornamental and medicinal features.

E. tirucalli can survive in a wide range of habitats. Van Damme (2001) states that the plant can grow under conditions in which most crops and other trees cannot grow. They include: tropical arid areas with low rainfall, on poor eroded soils, saline soils and high altitudes up to 2000 m but cannot survive frost. Its distribution is therefore limited by low temperatures. He goes on to say that like some other Euphorbias, *E. tirucalli* combines the C3 and CAM photosynthetic pathways which could probably be the reason for its survival in hardier conditions. To clarify this, the same author explains that CAM pathway entails a higher carboxylic acid accumulation (than C3 plants) at night, raising the osmotic potential of the plant which increases its salt tolerance. Also like other succulent plants, *E. tirucalli* stores extra water in the parenchyma and vacuoles which can be used to dilute salt ions entering the plant and as a reserve for survival in dry conditions.

As a crop, *E. tirucalli* can be grown in a variety of areas since it is tolerant to a variety of conditions. Propagation is by cuttings (from any part of the shoot) which root with ease and quickly form a bush. Experiments show that *E. tirucalli* can be grown at a density of 10,000-20,000 plants per hectare at a spacing of 1 m x 1 m whereas it coppices excellently at 20-30 cm height (Duke, 1983 cited at www.hort.purdue.edu). In many tropical areas, *E. tirucalli* grows wild often in abandoned sites of homesteads and kraals where they sometimes form thick woody vegetation tending towards a forest.

2.2.5 Chemical composition

E. tirucalli contains white milky latex in any part of the shoot. According to Kapaczewski (1947), the latex contains about 28% solid matter whose composition is: 21-27 % water soluble substances, 59-63 % resin-soluble substances and 12-14 % rubber-like substances. The chemical composition of the different parts of the plant has been extensively studied and a variety of chemical compounds have been isolated from them. Table 2.4 shows some examples. This great variety of chemical substances listed reveals the complexity of *E. tirucalli* latex and may explain most of its functions. For example, low herbivore pressure, poisonous nature, pesticidal features and medicinal characteristics may all be attributed to this chemical constitution (see sections a, c, d and f below).

Table 2.4 Chemical diversity of *E. tirucalli* parts

Chemical substance	**Source (plant part)**	**Reference**
4-deoxyphorbol di-ester	Latex	Kinghorn (2010)
Campesterol, Stigmasterol, Beta-sitosterol, Isofucosterol, Cycloartenol (sterols)	Stem callus	Uchida *et al*. (2010)
Cycloeuphordenol (triterpene)	Latex	Khan (2010)
Cyclotirucanenol (triterpene)	Latex	Khan and Ahmed (1988)
Diterpene ester	Latex	Khan and Malik (1990)
Euphol and beta-amyrin (triterpenoids)	Stem callus	Uchida *et al.* (2010)
Euphorbin A (polyphenol)	Stem	Yoshida and Yokoyama (1991)
Euphorcinol (pentacyclic triterpene)	Stem bark	Khan (1989)
Highly irritant euphorbia factors (not specific)	Latex	Furstenberger and Hecker (1977)
Serine proteases	Latex	Lynn and Clevett (1985)
Steroids	Latex	Nielson *et al.* (1979)
Taraxerane triterpene	Stem bark	Rasool (1989)
Tirucalicine (diterpene)	Latex	Khan (2010)
Tirucallin A (7) (tannin)	Stem	Yoshida and Yokoyama (1991)
Tirucallin B (11) and Euphorbin F (14) (dimers)	Stem	Yoshida and Yokoyama (1991)
Trimethylellagic acid	Latex	Chatterjee *et al.* (1977)

2.2.6 Pests and disease

There is a tendency to believe that *E. tirucalli* has no pests and diseases because of its poisonous latex. However, a few pests including *Meloidogyne incognita* (Trivedi *et al*., 1986) *Cuscuta* spp. (the witch weed) (Van Damme, 2001) and *Botrytis* spp. (Yanxia *et al.,* 2007) have been reported. The latter author notes that an infestation by *Botrytis* spp. causes the plant stem and roots to rot especially in warm and humid conditions. He reports that a combination of *Meloidogyne* spp. and *Botrytis* spp. infestations can wipe out a whole field in a short time.

2.2.7 Uses of *E. tirucalli*

a) Traditional medicine

Possibly due to a great variety of chemical substances found in *E. tirucalli* tissues (see table 2.4) medical folklore literature of different parts of the world (especially tropical and subtropical areas where it is endemic) is tainted with its curative ability. According to Schmelzer and Gurib-Fakim (2008) and Van Damme (1989), in East Africa, latex is used against sexual impotence, warts, epilepsy, toothache, hemorrhoids, snake bites, extraction of ecto-parasites and cough among others. In Peninsular Malaysia, a poultice of the roots or stems is applied to nose ulceration, hemorrhoids and swellings. Root scrapings mixed with coconut oil are taken for stomach-ache.

In India, Kumar (1999) notes that it is an unavoidable plant in most traditional homesteads and used as a remedy for ailments such as: spleen enlargement, asthma, dropsy, leprosy, biliousness, leucorrhoea, dyspepsia, jaundice, colic, tumors, and bladder stones. He further says that vesicant and rubifacient though it is, its latex is emetic in large doses but a purgative in small doses and applied against toothaches, earaches, rheumatism, warts, cough, neuralgia and scorpion bites. The same author points out that its branch and root decoctions are administered for colic and gastralgia while ashes are applied as caustic to open abscesses.

Duke (1983) and Van Damme (1989) mention that in Brazil, *E. tirucalli* is used against cancer, cancroids, epitheliomas, sarcomas, tumors, and warts although they argue that this has no scientific basis since the same tree is known to be co-carcinogenic. In Malabar (India) and the Moluccas, latex is used as an emetic and anti-syphilitic while in Indonesia, the root infusion is used for aching bones while a poultice of roots or leaves is used to treat nose ulcers, hemorrhoids and extraction of thorns. Wood decoctions are applied against leprosy and hands and feet paralysis following childbirth (Duke, 1983). The same author states that in Java, the plant latex is used to cure skin ailments and bone fractures.

b) Ornamental

E. tirucalli has increasingly become popular as an ornamental plant. Potted plants are placed in offices (see plate 2.4) and homes but can also be grown in lawns. It is preferred for its ease of maintenance and beautiful evergreen pencil-like branches which factors have increased its international trade resulting into a wide distribution in areas where it was not endemic. An extract of a report of the 17^{th} meeting of the Convention on International Trade in Endangered Species of wild fauna and flora (CITES) plants' committee, held in Geneva (Switzerland), 15-19 April 2006 (table 2.5) shows that *E. tirucalli* trade is booming. Possibly for a similar reason, the same report classifies it as an endangered species which should be protected. However, the latter conclusion was challenged by Van Damme and the Belgian CITES committee members (Van Damme, pers. comm.). Probably in recognition of this challenge, *E. tirucalli* is presently listed among the 'least concerned' category of the International Union for Conservation of Nature (IUCN) red list of threatened species 2010.3 (seen at www.iucnredlist.org).

Table 2.5 International trade figures of *E. tirucalli*

Plant part	Source/ country	2000	2001	2002	2003	2004	2005	2006
Extract (g)	Vietnam				36			
Leaves (g)	USA		4000					
Leaves (g)	Madagascar				3			
Plants	Kenya	32			12			
Plants	Belgium		23					
Plants	Brazil	4950	10000	2000	10000	10000		
Plants	Canada						204	
Plants	China			220				
Plants	USA	7481	10240	9163	20296	12935	14677	19396
Plants	Denmark							5000
Plants	Dominican Repub.	2500	1270			4200	3000	14239
Plants	Spain			165		331	80	
Plants	Haiti					108		
Plants	Netherlands	12500	6500	3000		100		
Plants	Madagascar	30			2			
Plants	Poland		1750	3040	3770	6450	17400	1100
Plants	Thailand				5			
Plants	Zambia	6		3	1		80	
Totals	All sources	27499	29788	17426	34251	34124	35441	39735

Source: CITES convention report on Euphorbias (2006) seen at www.cites.org/eng/com/PC/17/E-PC17-14.pdf.

Table 2.5 indicates that most of the exporting countries are not countries of *E. tirucalli* endemicity, which reveals how widespread and widely grown it has become.

c) Source of energy

Duke (1983) and Calvin (1978 and 1979) report that latex of *E. tirucalli* is composed of petroleum-like hydrocarbons largely C30 triterpenoids (also see table 2.4), which on cracking yield high-octane gasoline. They estimate a crude gasoline yield between 4 and 8 barrels per hectare from an *E. tirucalli*-planted field per year; and calculated at about three dollars per barrel, it is three times cheaper than normal crude oil. These postulations were validated by Calvin (1980) and although they were found to be true, extraction projects to this time have never materialized. However, *E. tirucalli* is still looked at as a potential source of biodiesel as it can produce a high biomass and grow in marginal areas unfit for production of other crops. Of late, there has been increasing attention on biodiesel production in order to reduce over-dependence on fossil fuel (Prusty, 2008). While agreeing with such a venture, Eshel *et al*. (2010) warn that emphasis should be put on non-food sources (such as *E. tirucalli*) to avoid hunger that can result from the use of food crops as a source of biodiesel.

Associated with biodiesel production is methane and biogas generation. Rajasekaran *et al.* (1989) and Van Damme (1990), considering its reported high biomass production and ease with which it ferments, note that it is a potential source of methane and biogas. Saw *et al*. (1989) experimentally demonstrated that *E. tirucalli* produces suitable biomass for biogas generation especially through chopped material under thermophilic conditions. Based on estimations from research carried out near Lake Baringo (Kenya), with 80,000 plants per hectare yielding 20 dry metric tons per year, they estimated *E. tirucalli*'s potential annual methane production (with a continuous digester), at around 3,000 m^3 per year, equivalent to approximately 3,000 1 of fueloil. The same authors estimated production of about 100 metric tons of compost per year as a byproduct.

Other forms of energy associated with high *E. tirucalli* biomass, are fuelwood and charcoal. Van Damme (1989 and 2001) names the provision of charcoal and fuelwood among its traditional uses. He further explains that the plant's ability to grow in semi-arid areas, devoid of forests, makes it one of the few alternatives for fuelwood production in such situations. For the same reason, it has been recommended for commercial fuelwood production projects for purposes of woodlot restocking in semi-arid parts of Kenya (Mahiri, 2002). Mahiri (1998) points out that *E. tirucalli* is preferred for this purpose due to its fast growth rate, high productivity, quick acclimatizion to an area and ease with which it dries.

d) Source of rubber

E. tirucalli is reported for possessing hydrocarbon polymers that are used for manufacturing rubber substitutes. Several researchers point out that its latex is an emulsion of terpenes and resins in water, which can easily be transformed into rubber at low cost (Calvin, 1979; Uzabakiliho *et al.*, 1987; Van Damme, 1990). The same authors further report that during the Second World War, its latex was used in South Africa to develop a rubber substitute which proved unprofitable and due to high resin content, could not yield high quality rubber. Also, due to the strong fixative power of the resin it has for long been used on the East African coast in local gum manufacture, for fastening knife-blades to wood handles and spear-heads to shafts (Van Damme, 1989). In the same view, Murali (1998) notes that the resin produces comparably good wood-based glue and adhesives whereas with a few modifications, it would compete favourably with other commercial resins.

e) Conservation and agroforestry

Due to its favourable agronomic features such as drought resistance, *E. tirucalli* is used in semi-arid areas to carry out afforestation and re-forestation for purposes of achieving soil conservation. Van Damme (2001) in the book '*Combating desertification with plants*' points out that such plants can be used as a soil cover in places where other plants (even grasses) cannot grow. Involvement of *E. tirucalli* has been mentioned in successful reforestation and conservation programs in: Tanzania (Smith *et al.*, 1996), Kenya (Macharia, 2004; Mahiri, 2002), and Sri Lanka (Melvani, 2009) among others. It has also featured in agroforestry programs (Jama *et al.*, 2003; Long and Nair, 1999; Mbwambo, 2004) as a hedge plant or as an intercrop.

Other related uses of *E. tirucalli* include: boundary demarcation (Kindt *et al.*, 2006; Van Damme, 1989), live fencing around compounds and kraals (Nascimento *et al.*, 2009; Simons *et al.*, 2004; Van Damme, 1989), cultural connotations e.g. as a sign of starting a new home in Luo culture of East Africa (Mahiri, 1998) and as a windbreak in semi-arid areas (Jama *et al.*, 2003). Simons (2004) points out that the plant plays these roles due to its latex toxicity and hence low herbivore pressure.

f) Pesticides

E. tirucalli latex has been reported to have pesticidal features against such pests as aphids (*Brevicoryne brassicae*) (Mwine and Van Damme, 2010), mosquitoes (*Aedes aegypti* and *Culex quinquefasciatus*) (Rahuman *et al.*, 2008), micro-organisms such as bacteria (*Staphylocococcus aureus*) (Lirio *et al.*, 1998) and molluscs (*Lymneae natalensis*) (Vassiliades, 1984) and *Biomphalaria gabrata* (Tiwari, 2006) among others. Siddiqui *et al*. (2003) report a dose-dependent latex toxicity to parasitic nematodes such as *Haplolaimus indicus*, *Helicotylenchus indicus* and *Tylenchus filiformis* in vitro, with increasing exposure period although some nematodes like *Meloidogyne* spp. are known to attack the plant.

The latex is also reported to be a hunters' tool applied in local fishing and arrow poisoning in tropical Africa (Neuwinger, 2004). Piscicidal feature has been validated by Kumat (1995) and Tiwari (2006). Although the plant is generally mentioned as a pesticidal plant, scanty experimental work has been performed to confirm this.

2.2.8 Disuses

A number of disuses have also been mentioned. Associated with its vesicant and rubifacient features, *E. tirucalli* latex is reported to cause conjunctivitis (Hsueh *et al.*, 2004; Joshi and Shingal, 2008; Scott and Karp, 1996; Shlamovitz *et al*., 2009) when it accidentally gets in contact with eyes. Eke *et al*. (2000) report that symptoms range from mild epithelial keratoconjunctivitis to severe keratitis with stromal oedema, epithelial sloughing, and anterior uveitis which usually heal in 2-7 days but can also result into permanent blindness. They advise that it should be handled with caution.

Research also shows that *E. tirucalli* is co-carcinogenic. Roe (1961) observed that papillomas and malignant tumors were elicited in mice treated with acetone extracts of *Euphorbia* lattices. Mizuno (1986) reports a high incidence of Burkitt's lymphoma - a latent Epstein-Barr virus (EBV) malignancy in East Africa where *E. tirucalli* is endemic. EBV causative factors were detected in soil and drinking water (where *E. tirucalli* grows) implying that people living in such areas run a high cancer risk. The findings have been further clinically validated in rats (Fürstenberger and Hecker, 1985; Imai *et al*., 1994; MacNeil *et al*., 2003; Silva *et a*l., 2007) some of which developed full blast lymphomas. However, folklore reports anti-cancer treatment by the latex (Cataluna and Rates, 1999), and there are scientific indications that it may modulate myelopoiesis and enhance resistance against tumor bearing (Valadares *et al*., 2006), both of which are suggestive of a cancer cure.

E. tirucalli is known to be an irritant to herbivores and due to its nasty and acrid features, most herbivores learn to avoid it. Howes (1946) and Simons *et al*. (2004) point out that this is one of the reasons why it is a good live fencing material.

Conclusively, *E. tirucalli* is a multi-purpose tree. This is expressed by the vast number of uses cited. Evidently, quite a lot has been done on exploration of its chemistry (Khan and Ahmed, 1988; Rasool and Khan, 1989; Uchida *et al.*, 2010) and evaluation of its potential as an energy plant (Calvin, 1980; Rajasekaran *et al.*, 1989). However, most of the medicinal uses mentioned have been left to folklore and need validation. For example, in spite of the vast number of ailments it is reported to cure e.g. (Cataluña and Rates, 1997; Kumar, 1999; Van Damme, 1989), to our knowledge, no substance of pharmaceutical importance has so far been obtained from it. Also scanty literature has been cited on validation of other functions like the reported insecticidal, nematicidal, piscicidal and molluscicidal features. This calls for more research/laboratory investigation, in order to establish scientific authenticity of these important functions and to ascertain with confidence, that *E. tirucalli* is a wonder plant for modern science.

Chapter Three

Use and Utility of Pesticidal Plants in Southern Uganda

Adapted from:

J. Mwine, P. Van Damme, J. Kamoga, C. Kudamba, M. Nasuuna and F. Jumba Ethnobotanical Study of Pesticidal Plants Used in Southern Uganda and Their Need for Conservation *Journal of medicinal plants research,* accepted December 9, 2010

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Abstract

Use of synthetic pesticides in developing countries is not only limited by their being expensive but also the small (uneconomic) fields whose limited production cannot offset costs for agricultural implements like agro-chemicals. Subsistence farmers, therefore, may have no choice but to use local methods of controlling pests, one of which is the use of traditional and, of late, introduced pesticidal plants' extracts.

In this study, whose main objective was to record all pesticidal plants used in southern Uganda, Masaka district, it was established that thirty four species belonging to eighteen families are currently used in traditional plant production. Most useful species are *Azadirachta indica* and *Tagetes minuta*. Most frequently cited families are Meliaceae and Euphorbiaceae. It was noted that of the plant species recorded, some species like *Azadirachta indica*, *Melia azedarach* and *Tagetes minuta* are already scientifically established pesticidal plants whereas others like *Euphorbia tirucalli, Bidens pilos* and *Vernonia amygdalina* may be known for other uses but not for this purpose, hence the need for their efficacy evaluation. Some important pesticidal plants like *Abrus precatorius*, *Euphorbia candelabrum* and *Phoenix reclinata* were reportedly becoming increasingly rare and would need conservation. The need to carry out such surveys in order to obtain inventories was observed and recording this knowledge before it disappears with the aging farmers was seen as urgent.

Key words: Indigenous knowledge, inventory, developing countries, subsistence farming, Pest management.

3.1 Introduction

In most developing countries, use of modern synthetic pesticides is limited and sometimes non-existent (Scialabba, 2000). This is so not only because they are expensive but also because of the small fields cultivated by subsistence farmers making the use of such pesticides uneconomic. Yet, most of these countries lie in (sub-) tropical areas where pests and diseases are abundant throughout the year. The implication of this is that in these areas, pests and diseases pose a major problem to agricultural production.

According to Oerke and Dehne (2004), pests account for 30-40 % crop loss (of total production) worldwide, while the loss in the tropics is reported to be even higher than 40 % (FAO, 2003). These figures show that the battle against pests is a difficult one even with the advanced technology that exists today.

In developing countries, where technological advancement is still low, and the use of modern methods is still in its infant stages, the most feasible means of production appears to be the use of traditional/cultural methodologies (Pei, 2001; Rates, 2001; Muhammad and Awaisu, 2008) - one of which is the use of traditionally-known pesticidal plants as a remedy for pest infestations.

The use of botanicals for pest control is as old as agriculture itself. Thacker (2002) reports the use of tobacco (*Nicotiana tabacum* L.) leaves for fumigation in stores as early as the late 1500s in North America, the use of *Sabadilla officinale* A. Grey ex Benth. by Indians in Venezuela for crop protection in the mid 1500s, and application of *Quassia* spp. extracts against aphids during the early 1600s in central America. Application of rotenone, ryanodine and veratridine, as well as other active ingredients of ryania (*Ryania specioza*), sabadilla (*S. officinale*), pyrethrum (*Chrysanthemum cinerariifolium*), marigolds (*Tagetes minuta*) and neem (*Azadirachta indica*) have been mentioned in early African and Asian agriculture (Casida and Quistad, 1998; Thacker, 2002).

While these botanicals were used as early as reported above, the discovery of synthetic pesticides in the early 1900s tended to overwhelm their use because of the many advantages synthetic pesticides appeared to have at that time. For example, DDT was reported to have a knockdown effect on most insects, high persistence in the environment, ease of application together with having a broad spectrum (DeLong, 1948; Walker, 2000) - advantages which the botanicals appeared not to possess.

It was only after environmentalists like Carson (1962) started realizing that these advantages were in effect potential disadvantages that scientists started to question the future prospects of synthetic pesticides. Therefore, botanicals are not new on the pest management scene but have recently been re-discovered as a route through which to bypass the many disadvantages of synthetic pesticides.

However, as Isman (2008) and Muhammad and Awaisu (2008) say, only a few are presently in commercial use. Therefore, there is need to explore, document and evaluate the use of more plants. Yet, Ankli *et al* (1999) and Gradé (2008) note that knowledge of such herbs is usually in the hands of a small group of people who selfishly guard it for their advantage. In many cases, it is passed on between generations by word of mouth or observing elders' activities and is often not documented. In many developing countries like Uganda, documentation and scientific evaluation of medicinal plants in general and botanicals for pest management in particular is still wanting. Available literature reveals that some ethnobotanical surveys have been made in Uganda on traditional plant use in human medicine (Freiburghaus *et al*., 1996; Kamatenesi-Mugisha *et al*., 2007a; Katuura *et al*., 2007; Ssegawa and Kasenene, 2007; Waako *et al*., 2007), veterinary medicine (Tabuti *et al*., 2003; Bukenya-Ziraba and Kamoga, 2007; Grade *et al*., 2007; Katuura *et al*., 2007), cultural values (Kakudidi, 2004a and b) but only limited amount of information concerning pesticidal/insecticidal plants used in this region is available (see Mugisha-Kamatenesi *et al*., 2008). This is in stark contrast with the ideal requirements of the ongoing campaigns to 'go organic' that are slowly but steadily catching up in this region. Organic farming discourages the use of synthetic agrochemicals and promotes ecological techniques like use of plant-based pest remedies.

Therefore, this study was set up to contribute to documentation of pesticidal plants used in Uganda especially in the agricultural district of Masaka (southern Uganda) where many farmers are converting to organic farming. The study objectives were: a) documentation of all known pesticidal plants in the area; b) establishment of pests against which these pesticidal plants are applied; and c) finding out their mode of application/formulation. Results will help to obtain a record of these plants from the farmers especially those who are elderly before they die/disappear with the knowledge and will stimulate further research on several aspects like conservation of the species and efficacy evaluation of plant substances against the pests they are claimed to control.

3.2 Materials and methods

3.2.1 Study area

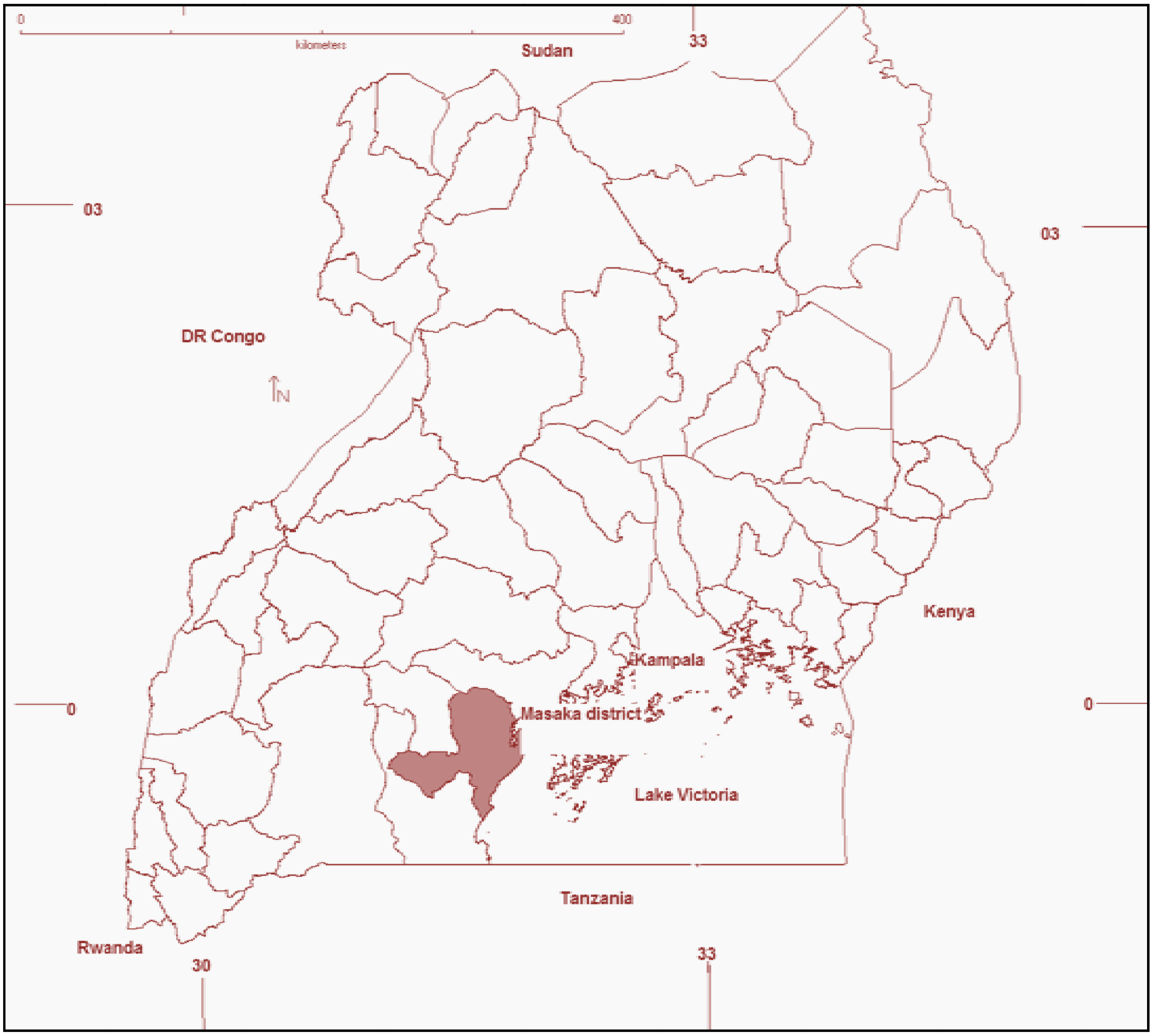
The study was carried out in Masaka district located between 31º 12´ and 32º 06´E and 0º 48´ and 1º 20´S in southern Uganda (fig. 3.1). Found on the shores of Lake Victoria, Masaka district is one of the most important agricultural areas in Uganda due to its favourable climatic conditions. The district has a bimodal type of rainfall with an annual average of 1200 mm and mild equatorial temperatures ranging between 22 and 26ºC. Following the bimodal type of rainfall, the district has two growing seasons i.e. March to June and October to December that support the growing of crops the whole year round. Main crops grown include bananas (*Musa* spp.), beans (*Phaseolus vulgaris* L.), cassava (*Manihot esculenta* Crantz.), maize (*Zea mays* L.), coffee (*Coffea* spp.) and a range of other (tropical) vegetables, fruits and cereals (Tenywa *et al*., 1999).

3.2.2 Data collection

The study was carried out with full knowledge and support of local councils (LCs) of the area that constitute the local administration at different levels of the district. Preparatory stages of the study involved holding meetings with local leaders who eventually introduced the research team to farmers. On the advice of district agricultural staff, it was found appropriate to include one agricultural field staff on the team to help with introduction and identification of the 'right' respondents. Respondents chosen were given simple on-the-spot interviews to ascertain their capability to carry out the whole exercise. Therefore, the survey team included an agricultural extension worker and a field officer recruited from the area, who also doubled as a translator where it was deemed necessary. The survey was conducted in the local language (Luganda) except for educated respondents who chose to use English during interview sessions.

During data collection, we opted for open-ended interviews in order to obtain quantitative data with a qualitative depth, devoid of researcher input. Mossholder *et al*. (1995) say that open-ended interviews offer a way of providing qualitative depth in a survey-based research and have an advantage of allowing respondents to answer in their own frames of reference, implicitly reducing the prime influence of leading questions following researcher suggestions. We also used direct observation to record observable data (especially on plant species morphology) as recommended by Etkins (1993).

Figure 3.1 Map of Uganda districts showing the study area (Masaka district)



Adapted from Google maps

The survey was carried out between October 2007 and March 2008 and five sub-counties including Bigasa (0º 04´59´´S; 31º38´00´´E), Bukoto (0º 23´09´´S; 31º37´38´´E), Kitanda (0º 44´59´´S; 31º35´58´´E), Lwengo (0° 24´ 58´´ S; 31° 24´ 29´´ E) and Buwunga (0° 22´ 22´´ S; 31° 47´ 35´´ E) were assessed. (GPS coordinates taken are for farms of lead farmers in the sub-county). Sub-counties were chosen on advice of Masaka agricultural staff and using the criterion that they were among the leading areas in organic farming adoption which implies extensive use of botanicals for pest management. In each sub-county, twenty-five farmers (about 1/3 of all farmers in a sub-county), and one extension staff were interviewed making a total number of 130 respondents. All respondents were adults (70 males and 60 females) between ages of 19 and 70.

During the survey, each respondent was requested to give information pertaining to the plants' local name, plant part used, method of formulation/use application mode of part used and pest(s) against which the latter were used. Finally, voucher specimens (JM 1-34) were prepared from each plant mentioned and deposited at the herbarium of Makerere University. Plants were identified by comparison with herbarium specimens with assistance from experts at Makerere University herbarium.

3.2.3 Data analysis

All data was recorded in previously designed data sheets according to the study objectives. For analysis, species and families recorded were assessed for User Value (UV) (Aburjai *et al*., 2007; Heinrich *et al*., 1998) - a quantitative method that demonstrates the relative importance of species locally,

$$UV = \frac{\sum U}{n} \qquad \text{Equation 3.1,}$$

where UV is the user value, U is the number of user citations and n is the number of respondents.

Informant consensus (ICF) (Aburjai *et al*., 2007; Ankli *et al*., 1999) was calculated to indicate information homogeneity. ICF = $(n_{ur} - n_t)/n_{ur} - 1)$ Equation 3.2,

where n_{ur} is the number of user reports in each category and n_t is the number of taxa used. According to the latter authors, ICF will be lower (closer to 0), if there is a large variation in plant use or when users do not exchange information about plant uses. High values (close to 1) reflect well-defined plant use or information exchange between respondents. All calculations and graphic presentations were carried out in Microsoft office Excel 2007. UV and ICF values are reflected in tables 3.1 and 3.3.

3.3 Results

3.3.1 Record of species

Table 3.1 shows the inventory of all species recorded from the survey. Thirty-four species belonging to 18 families were recorded. The most important pesticidal species were *Azadirachta indica*, *Tagetes minuta, Melia azedarach,* and *Jatropha carcus* with UVs of 0.96, 0.91, 0.85 and 0.79, respectively. Other species scored UVs ranging from 0.7 to 0.12 with *Phoenix reclinata* (0.12), *Abrus precatorius* (0.18) and *Chrysanthemum coccineum* (0.18) scoring the least.

The most used families were Meliaceae, Euphorbiaceae, Solanaceae and Verbenaceae with UVs of 0.90, 0.55, 0.53 and 0.53, respectively while Arecaceae (0.12) and Rutaceae (0.25) came out as the least used (table 3.3).

3.3.2 Plant parts/products used as source of pesticide

Leaves were named as plant parts most-used in formulation of pesticides. They were reported for 26 species out of the total 34 cited in the survey. They were followed by seeds/fruits and the bark with 11 and 10 species, respectively. Ash made by burning wood and flowers were named for only 2 species each (fig. 3.3). However, ash was generally mentioned as a constituent of many concoctions. Several intersections were observed where some plants were named for more than one part. *Azadirachta indica*, *Melia azedarach*, *Cannabis sativa* and *Jatropha curcas* were named for using almost all parts while others were named for one or two parts (table 3.1).

3.3.3 Formulation/mode of utilization of plant parts/products

Water extract was the most commonly named mode of formulation accounting for nearly half (49%) of all formulations. Others included use of the whole plant as an intercrop i.e. trap crop/repellant (10%), physical admixture of plant part with stored grain (10%), use of crushed seed cake (8%), application of plant oil extract (8%), latex spray (5%), ash admixture (5%) use of thorns as deterrents (2%), and use of smoke from burning plant parts (3%) (fig. 3.3). Most plants were reported to have several ways in which pesticides could be formulated from them. For example *J. curcas*, *E. tirucalli*, *Eucalyptus* spp., *N. tabacum* and *C. sativa* were named for three ways, while the majority was reported for two ways and a few others for only one (table 3.2).

Table 3.1 Record of species, their families, plant parts/products used and Use Values (UVs)

Species name and Voucher no.	Local name	Family	Part used	Times mentioned	UV
Abrus precatorius L. JM-001	Lusiiti	Fabaceae	L, S	23	0.176
Allium sativum L. JM-002	Katungulucumu	Alliaceae	L	56	0.430
Annona senegalensis L. JM-003	Kisitaferi	Annonaceae	L, B	55	0.423
Artemisia annua L. LM-004	Artemisia	Asteraceae	L, Fl	67	0.515
Asparagus africanus Lam. JM-005	Kadaali	Asparagaceae	L(spines)	45	0.346
Azadirachta indica A.Juss JM-006	Neem	Meliaceae	L, B, R, F	125	0.961
Bidens pilosa L. JM-007	Ssere	Asteraceae	L	36	0.276
Cannabis sativa L. JM-008	Njagga	Cannabaceae	L, S, F	52	0.400
Capsicum frutescens L. JM-009	Kamulari	Solanaceae	F	87	0.669
Carica papaya L. JM-010	Mupaapali	Caricaceae	R, B	32	0.246
Chrysanthemum coccineum Willd. JM-011	Pyrethrum	Asteraceae	L, Fl	23	0.176
Citrus aurantifolia Swingle JM-012	Nimawa	Rutaceae	F, L	32	0.246
Cupressus lusitanica L. JM-013	Kapripusi	Cupressaceae	L, B	54	0.415
Cupressus sempervirens L. JM-014	Ssedero	Cupressaceae	S, L	60	0.461
Cymbopogon nardus L.(Rendle) JM-015	Mutete	Poaceae	L	45	0.346
Datura stramonium L. JM-016	Ruziringa	Solanaceae	L	68	0.523
Eucalyptus globulus Labill. JM-017	Kalitunsi	Myrtaceae	L, B	60	0.461
Eucalyptus grandis W.Hill.ex Maid JM-018	Kalitunsi	Myrtaceae	L, B	56	0.430

Table 3.1 cont'd

Species name and Voucher no.	Local name	Family	Part used	Times mentioned	UV
Euphorbia candelabrum Tremaut. ex Kotschy JM-019	Nkukuulu	Euphorbiaceae	Latex, B, R	49	0.376
Euphorbia tirucalli L. JM-020	Nkoni	Euphorbiaceae	Latex, B, ash	46	0.353
Jatropha curcas L. JM-021	Kiryowa	Euphorbiaceae	Latex, F, S, B	103	0.792
Lantana camara L. JM-022	Kayukiyuki	Verbenaceae	L	69	0.530
Melia azedarach L. JM-023	Lira	Meliaceae	L, R, B	110	0.846
Mucuna pruriens Bak. JM-024	Mucuna	Fabaceae	L, R	32	0.246
Nicotiana tabacum L. JM-025	Taaba	Solanaceae	L	98	0.753
Phoenix reclinata Jacq. JM-026	Mukindo	Arecaceae	Sap, ash	15	0.115
Phytolacca dodecandra L'Herit JM-027	Luwoko	Phytolacceae	L, F	43	0.331
Plant ash N/A	Evuu	N/A	Ash	101	0.777
Ricinus communis L. JM-028	Nsogasoga	Euphorbiaceae	S	87	0.669
Schinus molle L. JM-029	Kishenda	Anacardiaceae	L, F	65	0.500
Solanum lycopersicum L. JM-030	Enyaanya	Solanaceae	F	24	0.184
Tagetes minuta L. JM-031	Kawunyira	Asteraceae	L	118	0.907
Tephrosia vogelii Hook.f. JM-032	Muluku	Fabaceae	L	88	0.676
Tithonia diversifolia (Hehsl.) A. Gray JM-033	Ekimyula	Asteraceae	F, L	75	0.577
Vernonia amygdalina Del. JM-034	Omululuza	Asteraceae	L	35	0.269

Key

L - Leaf, B - Bark, F - Fruit, Fl - Flower, S - Seed, R – Root

Table 3.2 Record of pesticide mode of formulation and pests they control

Species name	Mode of formulation	[1]Pest/ disease/deficiency treated
Abrus precatorius	Water extract	Liver flukes
Allium sativum	Trap crop, water extract	Field pests, storage pests
Annona senegalensis	Water extract	Fungicidal properties, insects
Artemisia annua	Water extract	Mosquitoes, flies
Asparagus africanus	Physical trap/thorns	Birds, moths, bats
Azadirachta indica	Water extract/crashed seeds	Most insects, mosquitoes
Bidens pilosa	Water extract	Aphids
Cannabis sativa	Water extract, smoke, trap crop	Storage pests, insect pests, coccidiosis, anti-biotic
Capsicum frutescens	Water extract, crashed seeds	Cut worms, ants, snails, storage pests
Carica papaya	Leaf water extract, crashed seeds	Blight, liver flukes
Chrysanthemum coccineum	Oil extract , water extract	Most pests
Citrus aurantifolia	Water extract, trap crop	Insect pests
Cupressus lusitanica, Sempervirens	Physical admixture, water extract	Storage pests, houseflies
Cymbopogon nardus	Trap crop, oil extract	Lepidoptera pests, beetles, aphids
Datura stramonium	Water extract	Insects
Eucalyptus globulus	Water extract, physical admixture, oil extract	Storage pests, repellent
Eucalyptus grandis	Water extract, physical admixture, oil extract	Storage pests, repellent
Euphorbia candelabrum	Latex spray, water extract	Termites, cutworms
Euphorbia tirucalli	Latex spray, ash dusting, physical admixture	Aphids, safari ants, cutworms
Jatropha carcas	Latex spray, water extract(seeds)	Insect pests, liver flukes

Table 3.2 cont'd

Species name	Mode of formulation	[1]Pest/ disease/deficiency treated
Lantana camara	Water extract, physical mixture	Insect pests, storage pests
Melia azedarach	Water extract	Most insects, liver flukes
Mucuna pruriens	Water extract, intercrop	Nitrogen deficiency
Nicotiana tabacum	Water extract, smoke, physical admixture	Storage pests, soil pests, domestic pests, repels snakes
Phoenix reclinata	Ash/dusting	Storage pests
Phytolacca dodecandra	Water extract	Snails, insect pests ,fungi
Plant ash	Dusting, water mixture	Cut worms, banana weevil, storage pests, fungi
Ricinus communis	Water extract, crushed seed /oil	Storage pests, domestic pests, liver flukes
Schinus molle	Water extract, crushed seeds	Liver flukes, storage pests
Solanum lycopersicum	Water extract, repellent crop	Aphids, thrips, weevils
Tagetes minuta	Water extract, repellent crop	Most insects, nematodes
Tephrosia vogelii	Water extract	Insect pests, ticks, fungi, mites, moles
Tithonia rotundifolia	Trap crop	Nematodes
Vernonia amygdalina	Water extract	Insect pests, malaria parasites, liver flukes

[1]Pests/diseases controlled were grouped into four broad uses: field pests, storage pests, domestic pests, veterinary pests and others/unclassified for calculation of ICF whose values were above 0.9 in all categories.

3.3.4 Pests the plants are used against

Numerous pests were mentioned during the survey but it was apparent that farmers were neither familiar with formal classification nor the names of pests and diseases. Most farmers gave broad answers such as weevils, storage pests, caterpillars, insects, moths or field pests. It was therefore difficult to obtain meaningful data for comparison. During interviews, respondents also pointed out the difficulty of naming particular pests controlled by certain plants because extracts are used whenever there is infestation without establishing particular pests being controlled. After all, many plants are used in combination or intercropped with others. Therefore, many plants were reported to be used against a range of pests. For analysis, pest data collected were organized in broad groups, i.e. field pests, storage pests, veterinary pests, domestic pests and others which could not be classified. All groups include the corresponding diseases. Table 3.3 shows that the most-cited pests were field pests named for 29 plant species in 1,270 user reports, while veterinary pests scored least with 5 species in 326 reports. However, all groups returned a high ICF i.e. above 0.9, indicative of the fact that there is a high user consensus among the farmers and a likelihood of sharing ideas about the use of botanicals for pest management.

3.4 Discussion

Ethnobotanical studies are made for different reasons. Some of these include assessment of functions of plants, for example identification of medicinal species (Jouad *et al*., 2001; Kamatenesi-Mugisha *et al*., 2007b; Katuura *et al*., 2007) and analysis of species diversity in a given area (Oryemoriga *et al*., 1995). Other reasons include determination of species conservation status (Schemske *et al*., 1994; van Jaarsveld *et al.,* 1998) or when trying to identify new plant species in an area that is not yet extensively studied (Oryemoriga *et al.,* 1995).

This study was meant to establish a record of pesticidal plant species used in Masaka district, southern Uganda and how they are utilized. It was found out that 34 species distributed in 18 families are being used in Masaka district as pesticidal plants (table 3.1). Some of these species are already confirmed pesticidal plants such as *A. indica*, *M. azedarach*, *J. curcas*, *T. minuta*, *T. rotundifolia, Chrysanthemum* spp. (Isman, 2006) but there are some species like *E. tirucalli, B. pilosa* and *V. amygdalina* that would require efficacy evaluation and publication/popularization for use as pesticidal plants. Therefore, there is a need to substantiate findings of this study by carrying out efficacy studies and other related work that may lead to confirmation and recommendation of such plants for more extensive use.

Meliaceae and Euphorbiaceae were reported to be the most useful families in this area for having a good number of species with pesticidal features. This means that they are good families to start from when looking for species of pesticidal importance.

Figure 3.2 Mode of formulation of pesticides among species recorded during the study

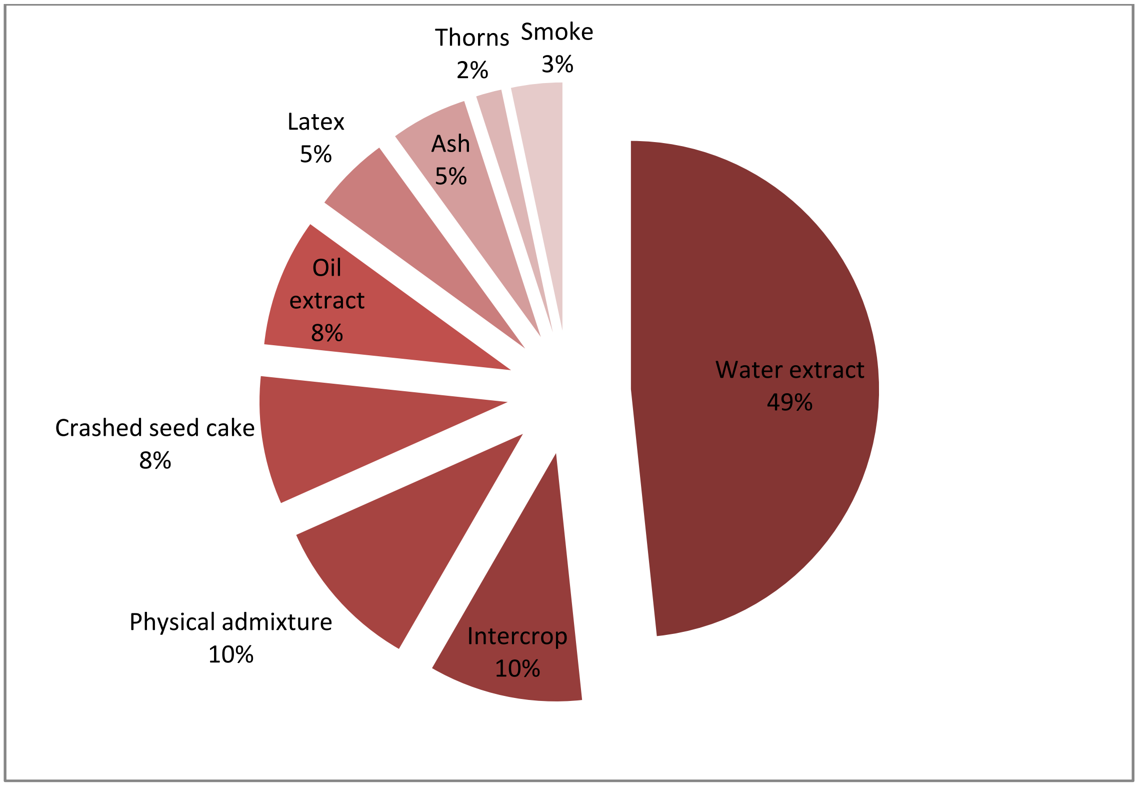


Figure 3.3 Plant parts used as source of pesticide

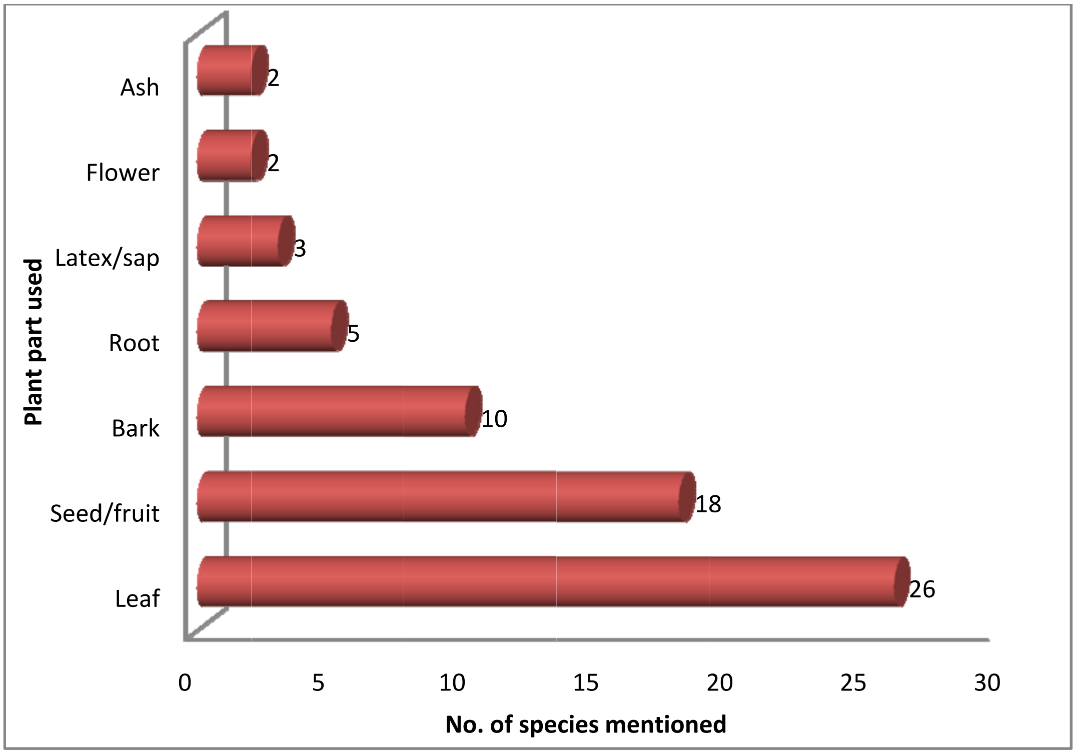


Table 3.3 Family use values showing the importance of each family in pest management in the study area

Family	No. of species	% of all species	Use citations	% use citations	UV
Asteraceae	6	17	354	17.41	0.453
Solanaceae	4	12	277	13.62	0.532
Euphorbiaceae	4	12	285	14.01	0.548
Fabaceae	3	9	143	7.03	0.366
Cupressaceae	2	6	114	5.60	0.438
Meliaceae	2	6	235	11.55	0.903
Myrtaceae	2	6	116	5.70	0.446
Alliaceae	1	2.9	56	2.75	0.430
Anacardiaceae	1	2.9	65	3.19	0.500
Annonaceae	1	2.9	55	2.75	0.423
Arecaceae	1	2.9	15	0.73	0.115
Asparagaceae	1	2.9	45	2.21	0.346
Cannabaceae	1	2.9	52	2.55	0.400
Caricaceae	1	2.9	32	1.57	0.246
Phytolacceae	1	2.9	43	2.11	0.330
Poaceae	1	2.9	45	2.21	0.346
Rutaceae	1	2.9	32	1.57	0.246
Verbenaceae	1	2.9	69	3.39	0.530

Plants produce a variety of secondary metabolites to protect themselves against pathogens and herbivores and/or to influence the growth of neighbouring plants (Fraenkel, 1969; Swain, 1977; Edwards, 1992; Sirikantaramas *et al*., 2008). The same authors go on to say that some of these metabolites are toxic even to the plant cells that produce them (when their target sites are present in the producing organisms) and must therefore be demobilized. Such substances include alkaloids (Dethier, 1980), tannins (Bernays, 1981), phenols (Palo, 1984), terpins (Schutte, 1984), and according to Gatehouse (2002), these are the substances man can exploit to make pesticides of botanical origin for pest control.

Our results are indicative of the fact that leaves constitute a large portion of the said secondary substances. This conclusion was reached at by considering our results (fig. 3.3) which show that most-exploited tissues of pesticidal plants are leaves (76%) followed by fruits and seeds (53 %) and then bark with 29%. These findings could be attributed to the positioning of plant parts on a plant. Leaves are exposed and conspicuous, which makes them easy targets for herbivore attack. It is not surprising, therefore, that plants tend to deposit and localize secondary substances in exposed parts such as leaves and fruit/flowers to act as deterrents to herbivores. Plants with inconspicuous leaves like *Euphorbia* spp. utilize their green stems/latex for such a purpose.

Similar findings have been reported by Kamatenesi-Mugisha *et al*. (2007a); Maregesi *et al*. (2007) and Ssegawa and Kasenene (2007) giving a clue to researchers on the cardinal parts to assess when in search of medicines/pesticides from plants.

Crops in Masaka district are heavily attacked by a variety of pests and diseases which are active throughout the year, probably due to tropical conditions. Farmers, therefore have to fight these pests in order to obtain tangible outputs from fields. However, during the present study, farmers could not point out exactly the particular pests managed by the mentioned pesticidal plants which could be a limitation for pest management. They reported pests generally as storage pests, insects, weevils, field pests and others (table 3.2). Such type of identification is incoherent and groups pests in categories that cannot help to draw scientific conclusions. While this could be attributed to the general low literacy rates of the farmers in this region which is rated at only 58% (UNHS 2002/3), it could also be due to the fact that a remedial concoction is often composed of a number of plant species and targets several pest species in the field or in storage. In such a situation, it might be difficult to tag particular pest species to individual plant species' extracts. Therefore, there is a need for research to elucidate chemical composition of the medicinal species and to evaluate specific pests managed by particular plant species through efficacy studies.

During our analysis, it was found appropriate to categorize the mentioned pests in broad groups namely field pests, domestic pests, veterinary pests and storage pests in order to give a broad view of pests managed by these plants. According to this categorization, field pests were the most-cited with 1270 user reports on 29 species, followed by storage pests (661 user reports on 16 species) indicative of the dominant type of occupation in the area studied. Masaka district is dominated by arable farming with little emphasis on pastoralism (Tenywa *et al*., 1999). It is likely that different results would have been obtained in a predominantly pastoral region (see Gradé, 2008).

During interviews with extension staff, it was revealed that certain medicinal plants are no longer available in the area studied and farmers have to travel long distances to harvest them. Species named include: *A. precatorius, P. reclinata,* and *E. candelabrum* which are mainly woodland species. Without substantiation, farmers also reported that some plants are completely unavailable and cannot be found anywhere, which could have implications of extinction. Most of southern Uganda and Masaka district in particular was originally a woodland area where these trees were once abundant (Hamilton, 1974). However, most of them have long been cut down for timber and while opening up agricultural land as the population increased. This is not surprising because over the years, an increasing number of plants have been red-listed as threatened species by IUCN in Uganda. For example, in 1997, IUCN had a red-list of 15 plants in Uganda (Walter and Gillet, 1999), which increased to 33 in 2002 (Earthtrends, 2003) and to 38 in 2008 (IUCN, 2009). This confirms our and other people's fears e.g. Hedberg (1993) and Cox (2000), that unless these plants and the traditional knowledge theirof are documented now, they will soon face extinction. Therefore, due considerations to conserve these species (for example by domestication) should be made as quickly as possible before they disappear completely.

3.5 Conclusion

This study has attempted to show that numerous plant species are used in this region for purposes of pest management. Notable ones such as *A. indica*, *M. azedarach*, and *T. minuta* dominate the application scene but a few 'new ones' like *B. pilosa* and *E. tirucalli* were also documented (for this purpose) for the first time in this region. Therefore, there is need to establish their efficacy and identify the pests against which their extract are most active. Also, the need for conservation of such species of pesticidal importance was noted. The earlier it is done, the better for pest management and biodiversity.

Chapter four

Evaluation of *E. tirucalli* Fresh Latex against Selected Agricultural Field Pests

Adapted from:

J. Mwine, P. Van Damme, C. Ssekyewa and K. Kalanzi Evaluation of Selected Pesticidal Plant Extracts Against Major Cabbage Insect Pests in the Field *Journal of medicinal plants research* submitted September 2010.

Presented at the Tropentag 2010 scientific meeting held at ETH Zurich, Switzerland on 14-16 September 2010.

Abstract

An evaluation of fresh extracts from three locally available pesticidal plants in southern Uganda was carried out against two important cabbage insect pests i.e. *Brevicoryne brassicae* and *Plutella xylostella* in order to assess their potential for use as pesticides. The research protocol followed the farmers' views that these plants could have pesticidal features as they had been observed to protect a number of leafy crops against pests. The assessment was carried out in the field using naturally infested cabbage plants (*Brassica oleracea*). Results suggest that *Euphorbia tirucalli* latex could reduce infestation of *B. brassicae* below economic threshold levels. Extracts from *Jatropha curcas* and *Phytolacca dodecandra* likewise reduced *B. brassicae* levels but could not do so to required threshold levels. The same extracts were evaluated on the diamondback moth *P. xylostella* but none was able to cause reduction of the moth's larvae to economic threshold level. It was concluded that *E. tirucalli* latex could be used as a management measure against *B. brassicae* and a factor in integrated pest management of *P. xylostella* infestations.

Key words: Integrated pest management, *Euphorbia tirucalli*, *Plutella xylostella, Brevicoryne brassicae*, natural pesticides.

4.1 Introduction

Due to high costs of synthetic pesticides, concerns over environmental pollution associated with continuous use of these chemicals and campaigns to 'live organic', there is a renewed interest in the use of botanicals for crop protection. Over the last few decades, there has been an increasing focus on plant-derived products to fight and reduce losses caused by agricultural pests and diseases (Devi and Gupta, 2000; Facknath, 2006; Ssekyewa *et al.*, 2008; Tewary *et al.*, 2005).

Most plant-derived products are easily biodegradable and therefore not to persist in the environment as opposed to synthetic products which often end up being pollutants. Plant products are also cheap especially if they are locally available (Isman, 2006) and can be locally processed.

During the search for plant-derived biocides, one approach used involves screening of plant extracts for deleterious effects against different organisms (Akhtar and Isman, 2003 and 2004; El Atta and Ahmed, 2002; Erler *et al.*, 2007; Guerra *et al.*, 2007; Hostettmann and Wolfender, 1997; Kartal *et al.*, 2006; Sadek, 2003; Ssekyewa *et al.*, 2008; Tewary *et al.*, 2005).

In most cases, research has focused on chemical composition of sap, latex or juices occurring in different plant parts. For example, the Euphorbiaceae family is known for its potent latex available from the leaves and barks of most of its species. The latex is particularly rich in diterpene and triterpene esters (Fürstenberger and Hecker, 1977; Khan *et al.*, 1989; Rasool *et al.*, 1989) which are known to be pesticidal (Rahuman *et al.*, 2008). Phytolaccaceae sap on the other hand is rich in triterpenoids (Spengel, 1996) and saponins (Dorsaz and Hostettmann, 1986; Ndamba *et al.*, 1994; Slacanin *et al.*, 1988) which are both reported to be molluscicidal (Ndamba *et al.*, 1994).

The term latex refers to the milky exudate of plants that coagulates upon air exposure. On the other hand, sap extracts or teas are obtained from plant parts, mostly leaves, by squeezing or pounding plant parts or mixing them with (hot) water. Succulent plants easily yield copious quantities of sap when squeezed while non-succulent leaves have to be pounded.

The present study focused on extracts from *Phytolacca dodecandra* L. (Phytolaccaceae), also known as endod; *Jatropha curcas* L. (Euphorbiaceae), commonly known as physic nut; and *Euphorbia tirucalli* L. (Euphorbiaceae), usually referred to as the pencil-tree.

Extracts from these three plant species have been mentioned in literature to display pesticidal properties (Adebowale and Adedire, 2006; Devi and Gupta, 2000; Emeasor *et al.*, 2005; Gebre-Amlak and

Azerefegne, 1999; Rahuman *et al*., 2008). In addition, they have been identified by local farmers as being able to improve health of several crops when applied. For example, farmers at Nkozi organic demonstration field in central Uganda intimated that *P. dodecandra* is ‘good for the growth of cabbage’ (sic) while farmers in western Uganda mentioned that latex of *E. tirucalli* promotes general health of leafy plants (K. Kalanzi, Masaka, Uganda, personal communication).

Although extracts of these 3 species are generally claimed to improve crop health and reduce leaf damage, it is not entirely clear what pests they are specifically used against. Available literature shows that *E. tirucalli* has (mosquito) larvicidal (Rahuman *et al*., 2008; 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 properties (Lirio *et al*., 1998). *J. curcas* and *P. dodecandra* are known for their molluscicidal and larvicidal features (Abebe *et al*., 2005; Isharaza, 1997; Lemmich and Thiilborg, 1995; Rahuman *et al*., 2008) but also for being insecticidal in grain storage (Adebowale and Adedire, 2006; Emeasor *et al*., 2005). However, these attributes are not directly related to controlling insect pests in the field.

It is in this context that the present study was set up by screening three plant extracts for their insecticidal features against major field cabbage pests in Uganda, including the diamondback moth *Plutella xylostella* L. (Plutellidae) and the cabbage aphid *Brevicoryne brassicae* L. (Aphididae).

It should be noted that these pests have for some time now, been a serious problem to production of cruciferous crops not only in East Africa (Badenes-Perez and Shelton, 2006; Macharia *et al*., 2005) but also in the rest of the world (Furlong *et al*., 2008; Guilloux *et al*., 2003; Isayama *et al*., 2004). A research of this kind is therefore highly necessary in order to develop possible new bio-controls for such pests for integrated pest management.

4.2 Materials and Methods

4.2.1 Experimental field

The experiment was carried out at the organic demonstration plot of Uganda Martyrs University (GPS: 0º 00´ 49´´S, 32º 00´ 32´´E) on the shores of Lake Victoria in central Uganda, between December 2008 and June 2009.

The experimental block was divided into 21 plots each 6 m² in size to accommodate six treatments and controls, which were all replicated three times. Treatments were arranged in a randomized complete block design.

4.2.2 The cabbage experimental plants

Cabbage (*Brassica oleracea* L. Brassicaceae) plants used were a Copenhagen hybrid of the capitata type that makes heads and matures in 8-10 weeks. Seeds were bought from a local farmers' shop and germinated in a nursery bed near the experimental block. Transplanting took place three weeks after sowing. Fifteen cabbages were planted in each plot at a spacing of 0.5 m by 1 m as recommended by the seed producers (RK seeds Ltd, India).

4.2.3 Plant extracts

Three plant extracts were used: latex from *E. tirucalli* and *J. curcas,* and sap from *P. dodecandra. E. tirucalli* latex was obtained locally by making incisions on mature branches of hedge trees near the experimental site. Latex was let to ooze into a small bottle that was subsequently closed with a cover and wrapped with aluminum foil (brand KMD 0.01 mm usually used for food packaging) to prevent photo-deterioration (Oliveira-Filho and Paumgartten, 1997). The bottle was put in a cooler icebox (Model GEO-006-12, made in China) and kept at about 4°C to prevent coagulation. Likewise, *J. curcas* extract was obtained by making incisions on upper stem parts of trees (originally used to support *Vanilla* orchids) growing near the experimental site. The extract was collected in sample bottles and taken directly for use.

On the other hand, extract from *P. dodecandra* was obtained by pounding leaves and squeezing them. A clean white piece of linen was used to squeeze and sieve the extract out of the pounded mass of leaves, which was taken directly for use in the experiment.

Following our laboratory trials on the efficacy of these extracts (Mwine, unpublished), two dilutions (v/v) of each extract were applied: 25% and 12.5% *E. tirucalli* latex (labeled P2 and P1); 50% and 25% latex of

J. curcas (J2 and J1) and 50% and 25% sap of *P. dodecandra* (E2 and E1), respectively. Control plots were sprayed with previously collected rain water. Extract dilutions were made by mixing pure extracts of the plants with appropriate quantities of rain water (v/v) to obtain required percentages. It should be noted that like most pesticides, these extracts should be handled with care since for example *E. tirucalli* latex is known to be toxic and irritating to eyes (Shlamovitz *et al*. 2009). Hands should be washed thoroughly with soap after manipulation.

4.2.4 Spraying

Spraying was carried out with a 1 L Knapsack sprayer (Farmate NS-1N, made in China) twice every week for the whole experimental period (Karagounis *et al*., 2006). Each experimental plant in plots was sprayed to cover both the inner and outer surfaces of leaves. Field infestation was already visible by the time of first spraying i.e. three weeks after transplanting. This experimental set-up was used to be able to observe the potential 'curative' effect of extracts i.e. the extracts' abilities to manage already existing (natural) infestation (Karagounis *et al*., 2006). As the latter author explains, the set up is in line with IPM principles where an intervention is applied only when the pest incidence has reached economic threshold levels. This is made possible by constant monitoring of pest levels in the field. With this set up, 'calendar spraying' is avoided and minimum quantities/interventions are applied with the benefits of economizing the remedial resources as well as protecting the environment.

4.2.5 Sampling

Sampling of pests (resulting from natural infestation) was done by field counts on a weekly basis just before spraying. The first sample acted as a base-line for the latter levels of infestation. Assessment of *B. brassicae* was done on ten randomly selected plants by direct counting. All aphid colonies on both sides of the first three leaves starting from the core were counted. This implies that every week, a new set of leaves was considered so as to assess potential preventive capacity of extracts. Infestation rated by colonies of aphids per plot, was ranked on a 0-3 scale, where 0 = absence; 1 = 1-5 colonies; 2 = 5 – 10 colonies; 3 = over 10 colonies (Karagounis *et al*., 2006).

Presence of *P. xylostella* moth was determined by opening up young folds of cabbage leaves where eggs are laid and hatch into caterpillars. Presence levels were determined by recording numbers of larvae observed (Phillips, 1983). Six plants randomly chosen in each plot were considered in all treatments. This was considered a reasonable sample since the direct counting method, which was used, provides accurate results (Strickland, 1961).

Leaf damage levels were obtained by estimating the damaged area of cabbage leaves/gaps by means of a squared paper grid. The third leaf starting from the cabbage core for six randomly chosen plants was considered per treatment. The third leaf was used since it was considered to have received reasonable exposure to pest encounters without however, showing signs of senescence.

At the end of the experiment, the number of harvestable cabbage heads was counted and each cabbage weighed, using a Salter® weighing scale (model SKU 235-6S, made in England) with a maximum capacity of 25 kg and $\pm$ 0.1 g precision level. The number of marketable heads i.e. with leaf damage level less than 10%, was recorded to establish levels of protection (efficacy) of different extracts (Liu *et al*., 2003)

4.2.6 Data analysis

Data obtained from counts of *B. brassicae* and *P. xylostella* infestations were subjected to Kruskal-Wallis one way analysis of variance by ranks (H test) to establish trends of infestation between treatments. Means were differentiated by Mann-Whitney U test where the H test was found significant. Results with $P \leq 0.05$ % were considered statistically significant. Data for leaf damage and number of marketable cabbages were presented as percentages. All calculations and graphic presentations were carried out using Microsoft office Excel 2007.

4.3 Results

4.3.1 Levels of *B. brassicae* infestation in the field

Mean *B. brassicae* scores fluctuated between the first and fourth week of spraying for all treatments but experienced a fall for most treatments from the fourth week onwards tending to level off towards the end of the growing season (fig. 4.1). This trend was observed also for the control treatments. However, analyzed by Kruskal-Wallis one way analysis of variance, on average, there was a significant difference in *B. brassicae* scores between different extracts (H=28.27, df=6, P=0.000084). P1, P2 and J2 appeared to show relatively good results while J1 and E2 returned nearly as poor results as the control. A comparison of treatment means (overall average infestation) against the control with Mann-Whitney U test shows that there is a significant variation in infestation between extracts of *E. tirucalli* (P1 and P2), *J. curcas* (J2) and control (P1: U=9.86, P=0.0017; P2: U=11.83, P=0.0056; J2: U=9.10, P=0.0026). However, closer inspection reveals that only *E. tirucalli* formulations (P1 and P2) reduced the *B. brassicae* levels to below one colony per 5 plants, i.e. the economic threshold as proposed by PIP/PSM (2007).

4.3.2 Levels of *P. xylostella* infestation in treatments

Mean number of *P. xylostella* larvae decreased in all treatments after the third week of spraying (week 5) but experienced a rise again starting from week 7 until the end of the growing season. Analyzed using Kruskal-Wallis one way analysis of variance (H test) there was a highly significant difference in larvae average infestation between treatments (H=32.45, df=6, P=0.000013). Mann-Whitney's post-hoc test revealed that both *E. tirucalli* dilutions (P1 and P2) were more potent than other treatments but showed no difference between the two, implying that the latex is potent even at lower dilutions (P1: U=10.646, P=0.0011; P2: U=10.694, P=0.0011). Percentage reduction of larvae counts against the control was evident to the tune of: P2; 45%, P1; 40.2%, E2; 12.9%, E1; 7.24%, J2; 11.1% and J1; 9% (also see fig. 4.2). However, in all treatments, mean numbers of larvae counted remained significantly higher than one larva per plant, which is the recommended economic threshold for *P. xylostella* larvae in cabbages as proposed by PIP/PSM (2007).

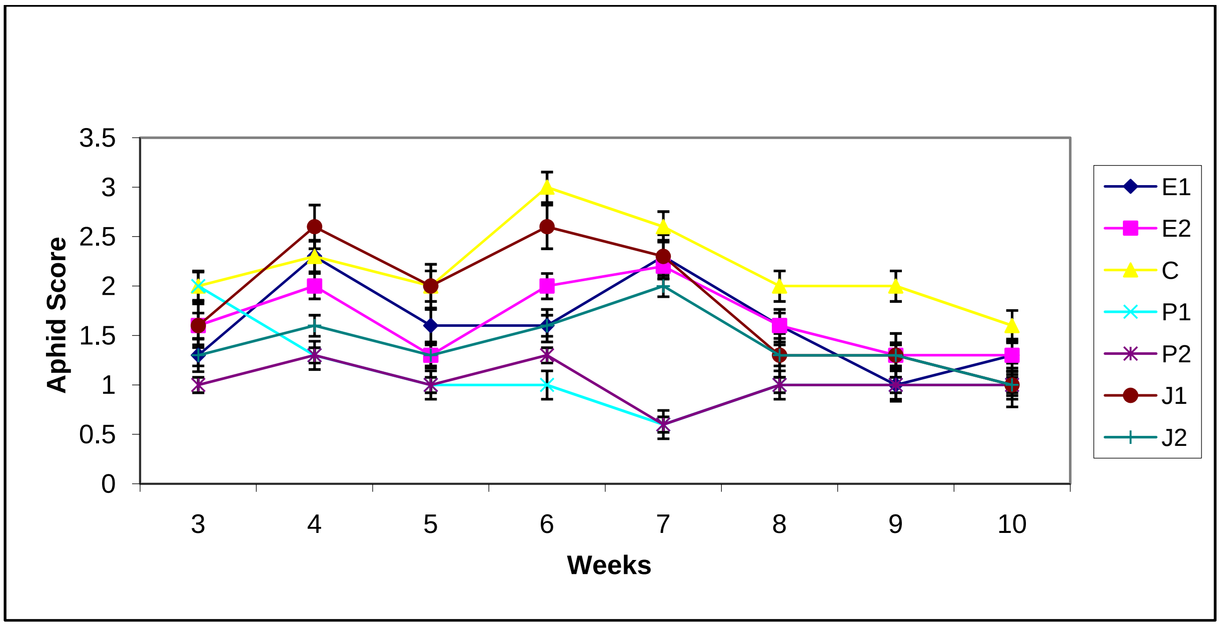
4.3.3 Cumulative leaf damage in the field

Leaf damage levels continuously increased in all treatments throughout the season. In some cases, damage levels in plants under treatment were as high as in plants from the control (fig. 4.3). Only extracts from *E. tirucalli* and *P. dodecandra* brought about reduction in leaf damage as compared to control. Percentage reduction in leaf damage as calculated against the control were E1 - 17%; E2 - 15%; P1- 22.5%; P2 - 25.6%; J1 - 11.8% and J2 - 11.8%. Mean leaf damage levels over the season ranged from 12.5% for P2 which was lowest to 20% for E1 which was highest.

4.3.4 Percentage of marketable cabbages per treatment

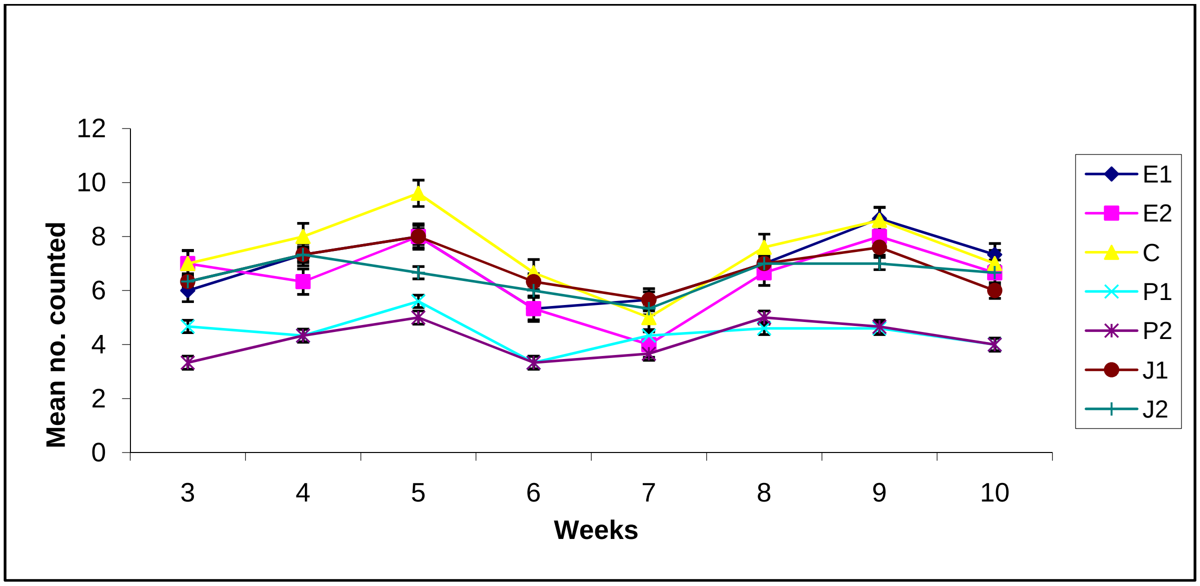
Marketable percentage of cabbages per plot was generally very low ranging from 6.7% to 33.3%. Only one formulation of *E. tirucalli* (P1) showed a slightly higher marketable harvest percentage at 33.3% as compared to control at 20%. Some formulations of *P. dodecandra* (E1) and *J. curcas* (J1) even showed lower percentages than control (fig. 4.4). All figures were below the usual range of 75 – 85% commonly observed in Uganda's organic farms.

Figure 4.1 Mean weekly *Brevicoryne brassicae* scores*per plot among treatments (E1 and E2 represent 25% and 50% *J. curcas* dilution, C represents rain water, P1 and P2 represent 12.5% and 25% *E. tirucalli* dilution while J1 and J2 represent 25% and 50% *P. dodecandra* dilution respectively); Error bars show mean + SD



*Scores per plot: 0=total absence, 1=1-5 colonies, 2=5-10 colonies, 3=0ver 10 colonies.

Figure 4.2 Mean number of *Plutella xylostella* larvae counted per treatment (E1 and E2 represent 25% and 50% *J. curcas* dilution, C represents rain water, P1 and P2 represent 12.5% and 25% *E. tirucalli* dilution while J1 and J2 represent 25% and 50% *P. dodecandra* dilution respectively); Error bars show mean + SD



4.4 Discussion

A good pesticidal substance should protect a crop against target pests to levels below economic threshold. Our results indicate that all extracts displayed considerable effectiveness to reduce *B. brassicae* infestation levels in the field but only *E. tirucalli* extracts (P1 and P2) were potent enough to reduce aphid levels below the economic threshold level i.e. one aphid colony per five cabbage plants in the field, as proposed by PIP/PSM (2007). This result might not be surprising in view of the chemical composition of *E. tirucalli* latex. The latex is known to be composed of a range of toxic substances including phenolic compounds, ellagic acid and tannins (Lin *et al*., 2001), diterpene esters (Khan and Malik, 1990), triterpenes (Furstenberger and Hecker, 1986; Khan *et al*., 1989; Khan *et al*., 1987; Rasool *et al*., 1989). All of these compounds may individually, additively or synergistically contribute to explain the pesticidal action of the latex against *B. brassicae*.

Berger (1994) and Duke (1983) had earlier observed pesticidal properties of *E. tirucalli* against aphids but to the present time, it has never been experimentally demonstrated. Therefore, to the best of our knowledge, our study is the first to report on efficacy of E. *tirucalli* latex against a number of field pests and particularly some agricultural insect pests.

On the other hand, several researchers have reported pesticidal properties of *Jatropha curcas* (Adebowale and Adedire, 2006; Alyelaagbe *et al*., 2007; Emeasor *et al*., 2005; Rahuman *et al*., 2008) and *Phytollaca dodecandra* (Abebe *et al*., 2005; Gebre-Amlak and Azerefegne, 1999; Lemmich and Thiilborg, 1995). Also during the present study, extracts from both species caused a reduction in *B. brassicae* infestation levels albeit not to acceptable threshold levels.

The diamondback moth (*P. xylostella)* is known to be a very difficult pest to control (Sarfraz *et al*., 2005; Wang *et al*., 2007). It easily develops resistance against synthetic pesticides (Bautista *et al*., 2007; Sarfraz and Keddie, 2005; Wang *et al*., 2007; Zhao and Grafius, 1993; Zhao *et al*., 2006) or else it conceals itself in a cabbage head (Macharia *et al*., 2005) thus making it difficult to eliminate. In many cases, this pest is controlled using a combination of pest management techniques which may or may not include the use of plant extracts (Attique *et al*., 2006; Facknath, 2006; Farrar and Shapir, 2005; Li *et al*., 2006; Raymond *et al*., 2006; Talekar and Shelton, 1993).

Our results show a reduction of *P. xylostella* larvae in the field ranging from 7 % for J1 to 45% for P2. However, these percentage reductions may not mean much especially if the level of infestation at the moment of application of the treatment was already high.

According to our observation, larvae counts ranged from four (P2) to ten (E2) per plant. These counts are so high that even if a pesticide would bring about a reduction of 40% it would still not provide reasonable protection to the crop. However, combined with another technique such as biological control e.g. through the use of *Bacillus thuringiensis* (Schroer and Ehlers, 2005) or trap crops (Badenes-Perez and Shelton, 2006), pest load could be brought down to economic threshold levels.

Therefore, extracts from *E. tirucalli* whose potency resulted into an infestation reduction amounting to 45% and 40.2% (P2 and P1) respectively, could be used as contributory and/or supplementary therapy against the *P. xylostella* moth. However, more research is needed in order to substantiate this hypothesis.

As an additional indicator to the failure or success of these extracts to manage pests in cabbage fields, we used leaf damage levels in the field and percentage of marketable cabbages among treatments. Results suggest that *E. tirucalli* extracts protected cabbage plants better than other extracts to the extent of causing a 22.5% and 25.6% reduction in leaf damage for P1 and P2, respectively. Although this was a fair result, it does not necessarily mean that the protection was satisfactory because marketable cabbages harvested fell short of at least 75% of the harvest which would be reasonable for an organic farmer. This shortfall is, however, more attributed to *P. xylostella* than *B. brassicae* infestation.

These results are in agreement with what was observed in our earlier laboratory trials where *E. tirucalli* latex was observed to provide protection to sweet pepper *Capsicum annuum* L. (Solanaceae) against green peach aphid *Myzus persicae* Sulzer Aphididae; Homoptera (Mwine, unpubished).

In sum, the present experiment has attempted to demonstrate the possibility of using extracts from *E. tirucalli* latex to manage *B. brassicae* in cabbages. Where the extracts are used in presence of *P. xylostella* (and they usually infest together!), another contributory/complementary therapy should be opted for to bring down infestation levels below economic thresholds. An integrated pest management approach is recommended.

As already reported, *E. tirucalli* latex is toxic. However, this is equally so for many other pesticidal substances especially synthetic ones. While the latex has been evaluated on cabbages to represent leafy crops (as recommended by farmers) and to take advantage of a crop that is infested by both *B. brassicae* and *P. xylostella* at the same time, there is a possibility of compromising safety issues as cabbage leaves are eaten directly (as leaves) and sometimes raw. Although plant-based pesticides are known to have a short half-life and easily get biodegraded, there is need to establish the residual and toxicity levels of the latex on cabbage before it is recommended for extensive use. If the latex toxicity levels were shown to be beyond acceptable margins, then the latex could be recommended for use with other non-leafy crops that are affected by aphids.

Results of this experiment have also helped to validate local farmers' observations that the extracts are beneficial for protection of leafy crops. Although it does not give total protection (and really no pesticide does), in our opinion, *E. tirucalli* is a welcome pest management measure especially for African (organic) farmers who do not use synthetic pesticides and may not have many alternatives. After all, *E. tirucalli* grows wildly and abundantly in many arid and semi-arid areas in Africa and is readily available.

Figure 4.3 Cumulative percentage of leaf damage in treatments (E1 and E2 represent 25% and 50% *J. curcas* dilution, C represents rain water, P1 and P2 represent 12.5% and 25% *E. tirucalli* dilution while J1 and J2 represent 25% and 50% *P. dodecandra* dilution respectively); Error bars show mean ± SD

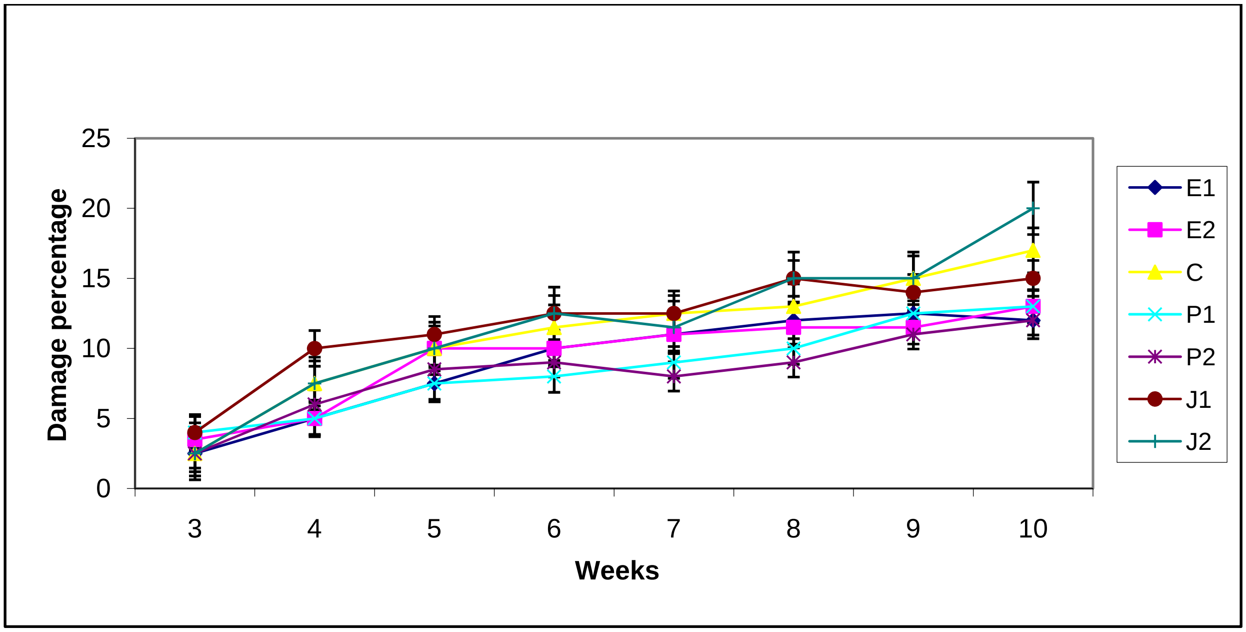
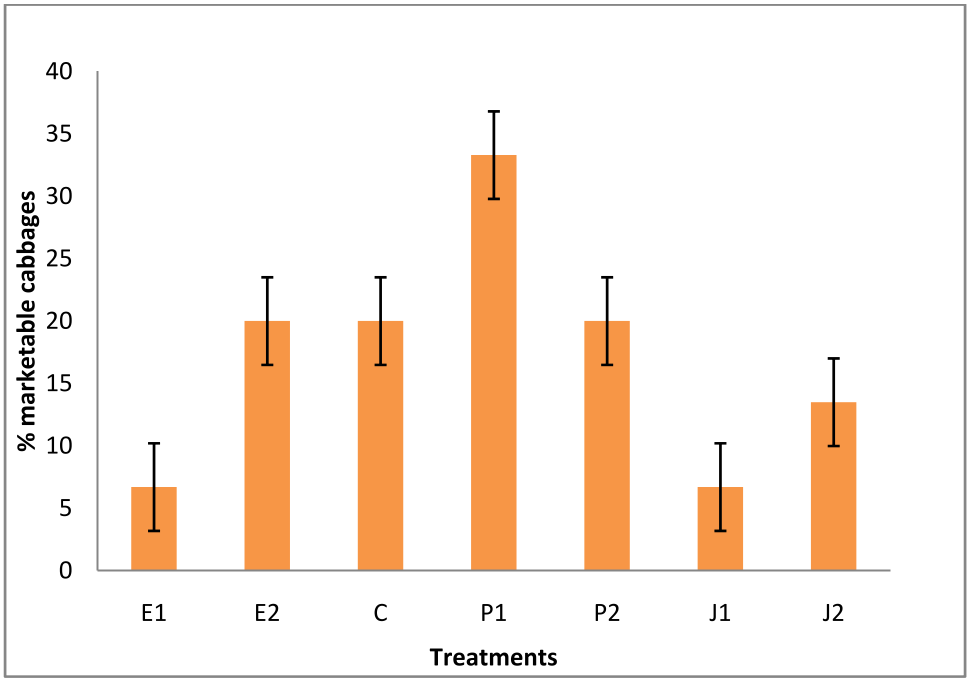


Figure 4.4 Percentage of marketable cabbages per plot in the treatments (E1 and E2 represent 25% and 50% *J. curcas* dilution, C represents rain water, P1 and P2 represent 12.5% and 25% *E. tirucalli* dilution while J1 and J2 represent 25% and 50% *P. dodecandra* dilution respectively); Error bars show maen ± SD



Chapter five

Evaluation of Larvicidal Properties of *E. tirucalli* Latex against Larvae of *Anopheles* Mosquitoes

Adapted from:

J. Mwine, P. Van Damme and F. Jumba Evaluation of Larvicidal Properties of the Latex of *Euphorbia tirucalli* L. (Euphorbiaceae) against Larvae of *Anopheles* Mosquitoes *Journal of medicinal plants research* 4(19): 1954-1959

Presented at the National Council of Higher Education (NCHE) exhibition held at Kampala, Uganda (poster) 23-27 January 2010.

Abstract

Malaria is the most important vector-borne disease in (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 from affected areas has proved impossible. Therefore, any measure that attempts to fight the parasite or its vector (*Anopheles* spp.) would be of great help.

In this experiment, we assessed the efficacy of *E. tirucalli* latex both as a herbal mosquito remedy and larvicide against *Anopheles fenestus* Giles and *A. gambae* Giles (which are important malaria vectors in Uganda) 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 (1: 250) in 5 days. LT 50 and LT 90 for the same dilution were attained at 12 and 36 hours, 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: Integrated Pest Management, larvicide, *Anopheles* spp., ethnobotany

5.1 Introduction

Malaria is the most important vector-borne disease in the (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 where every one in 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 – 40% of all visits to 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. 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 sanitation, 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 current control measures, indoor spraying with DDT (which was banned in many countries) has been revived 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, in countries such as Brazil, Egypt and Zambia (Killeen *et al*., 2002b). According to the latter 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 biodegradability and lower toxicity to most organisms (Frédérich *et al*., 2002).

Several researchers have investigated application of plant extracts to fight malaria vectors. Plant species cited in literature for this purpose include *Achyranthes aspera* L. Amaranthaceae; (Bagavan *et al*., 2008), *Azadirachta indica* A. Juss Meliaceae; (Aliero, 2003), *Jatropha curcas* L., *Euphorbia tirucalli* L., *E. hirta* L., *Phyllanthus amarus* Schumach. & Thonn. and *Pedilanthus tithymaloides* L.(Poit) Euphorbiaceae; (Rahuman *et al*., 2008a), *Piper nigrum* L. Piperaceae; (Rasheed *et al*., 2005), *Chenopodium album* L. Chenopodiaceae; (Sharma *et al*., 2006), *Solanum xanthocarpum* Schrad et Wendl. Solanaceae; (Mohan *et al*., 2005), *Ajuga remota* Benth. Lamiaceae; (Sharma *et al.*, 2004), *Thymus capitatus* (L.) Hoffsgg. & Link Lamiaceae; (Mansour *et al*., 2000), *Tagetes erecta* L. Asteraceae; *Cleome icosandra* L. Capparaceae; *Ageratum conyzoides* L. Asteraceae; and *Eichhornia crassipes* (Mart.) Solms Pontederiaceae; (Saxena *et al.,* 1992) among others. These authors have gone further to report chemical substances such as flavonoids, diterpenoids, triterpenoids, esters and alkaloids among others, found in the respective plant tissues and define their degree of anti-malarial efficacy.

In this paper, we focused on assessing the larvicidal properties of *E. tirucalli* against *Anopheles* mosquitoes. Common features and medicinal properties of *E. tirucalli* against a number of pests and disease causing organisms are discussed in sections 2.2.7 and 4.1. In particular, petroleum extracts of *E. tirucalli* have been tested against *Aedes aegypti* and *Culex quinquefasciatus* and were found potent enough to cause larval mortality for *A. aegypti* and *C. quinquefasciatus* (LC 50 = 4.25 ppm and 5.52 ppm, respectively; Rahuman *et al*., 2008a). Having shown a high larvicidal efficacy against *Aedes* and *Culex* 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 to or kill *Anopheles* mosquito larvae in their semi-natural environment so as to be adopted for use as a mosquito-control measure.

5.2 Materials and methods

5.2.1 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 as a test site. The pond was 28 m by 19 m in size and 1.2 m deep when it was active. At experiment time, it contained shallow rainwater only about 0.2 m deep.

5.2.2 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 the 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 (fig. 5.1).

All tubs were provided with approximately 6 kg of top soil drawn from the pond to provide adequate abiotic and biotic breeding and living 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). Data presented in this paper are pooled results of both experiments.

5.2.3 *Euphorbia tirucalli* extract

E. tirucalli latex used was locally obtained from hedge trees in the vicinity of the experimental site in the same manner as in section 4.2.3.

Figure 5.1 Schematic layout of the experimental plot (tubs were buried 2m from each other)

T2	T3	T1	C
T3	T2	C	T1
T1	T3	T1	T2
T2	C	C	T3

Key

C – Control sprayed with tap water

T1 – 1/250 latex/water dilution (v/v)

T2 – 1/200 latex/water dilution (v/v)

T3 – 1/150 latex/water dilution (v/v)

5.2.4 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 entomologists at Vector Control Division (VCD), Ministry of Health, Kampala. Mosquitoes assessed were those found naturally breeding in the area in order to approximate a semi-natural environment.

5.2.5 Experimental essay

Following our preliminary experiments carried out at VCD on lethal doses, (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 consisted of tap water. These initial dilutions were obtained by mixing appropriate quantities of latex with 1 L of water drawn from the respective experimental tubs. A randomized complete block design was used as experimental set-up 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 subsequent sampling (Fillinger *et a*l., 2003). Following the methodology of the same authors, 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 (equidistant to each other). Four dips per tub were taken for this initial test.

5.2.6 Sampling and assessment

Sampling of larvae was carried out after an interval of 0 h, 12 h, 24 h, 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 dilutions were taken consecutively with the same dipper. One dip was taken from each tub at a time, coming back to it later for the next dip 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 pair of forceps. Both dead and living larvae were recorded and returned to their respective tubs in order to maintain the semi-natural conditions of the experiment.

5.2.7 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≤ 0.05 % were considered statistically significant.

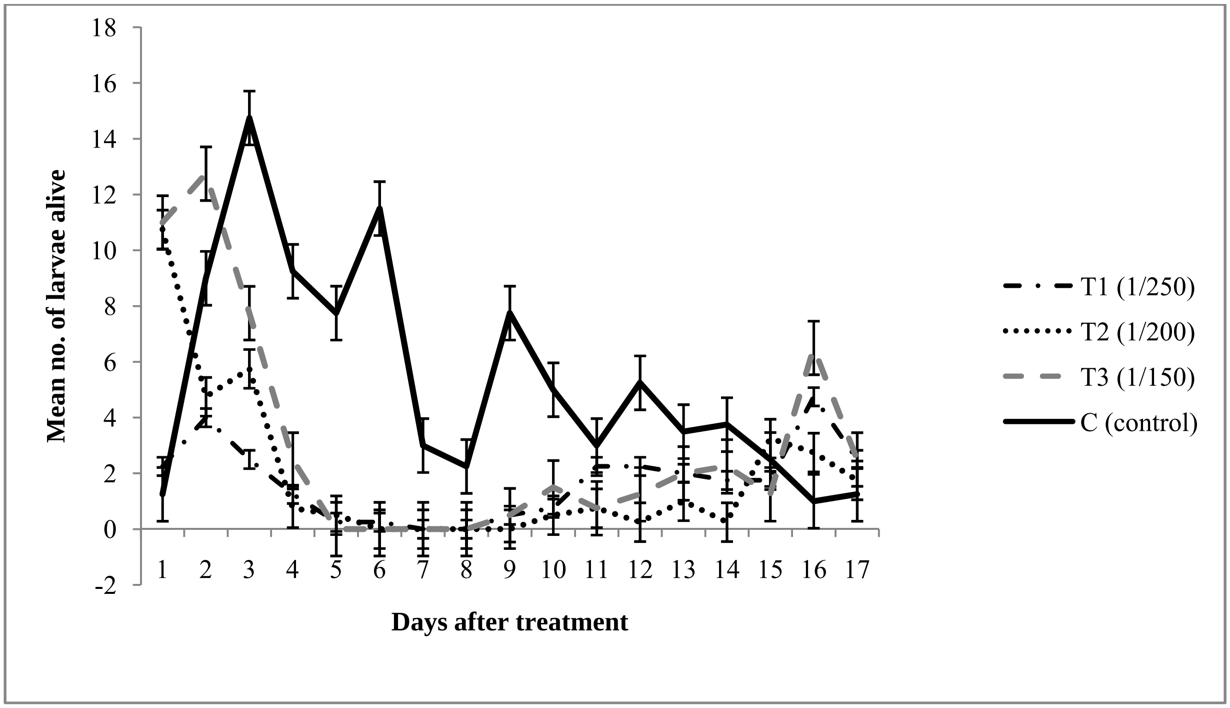
Percentage mortality was calculated using the formula of Mulla *et al.* (1971) which takes into account natural factors of population change in an ecosystem. Mortality = 100 – (C1/T1 xT2/C2) x100, where C1 and C2 represent average numbers of larvae in control tubs before and after treatment respectively, while T1 and T2 represent larvae in treated tubs. Microsoft Excel for Windows 2007 was used for performing calculations.

5.3 Results

A high efficacy against *Anopheles* mosquito larvae was returned by all *E. tirucalli* treatments unlike the control as shown in fig. 5.2. The three *E. tirucalli* dilutions appear to follow a similar efficacy trend from the beginning of the experiment to the end. 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 control (T1: U=11.27, P=0.0008; T2: 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 attained within 12 and 36 hours of application, respectively, for the highest dilution (T1=1:250) reaching total mortality in 5 days (see fig. 5.3). 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. It is also evident from the figure that *E. tirucalli* latex may not have a knockdown effect since larvae remain active for more than 24 hours.

Figure 5.2 Means of *Anopheles* spp. larvae alive after treatment with *E. tirucalli* latex; Error bars show mean + SD



5.4 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. Fig. 5.3 shows that at a dilution as low as 1: 200, fresh latex was able to cause 80 % larval mortality in only 12 hours rising to 100% in 4-5 days. Of the dilutions tested (see fig. 5.3), all three attained LT 50 within 12 hours and LT 90 in 36 hours. 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. Fig. 5.2 and 5.3 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 use of heavy doses which may cause problems. After all, *E .tirucalli* is known to be toxic (Fürstenberger and Hecker, 1977; Shlamovitz *et al*., 2009) and has been pointed out to be co-carcinogenic (Fürstenberger 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 when they are ready.

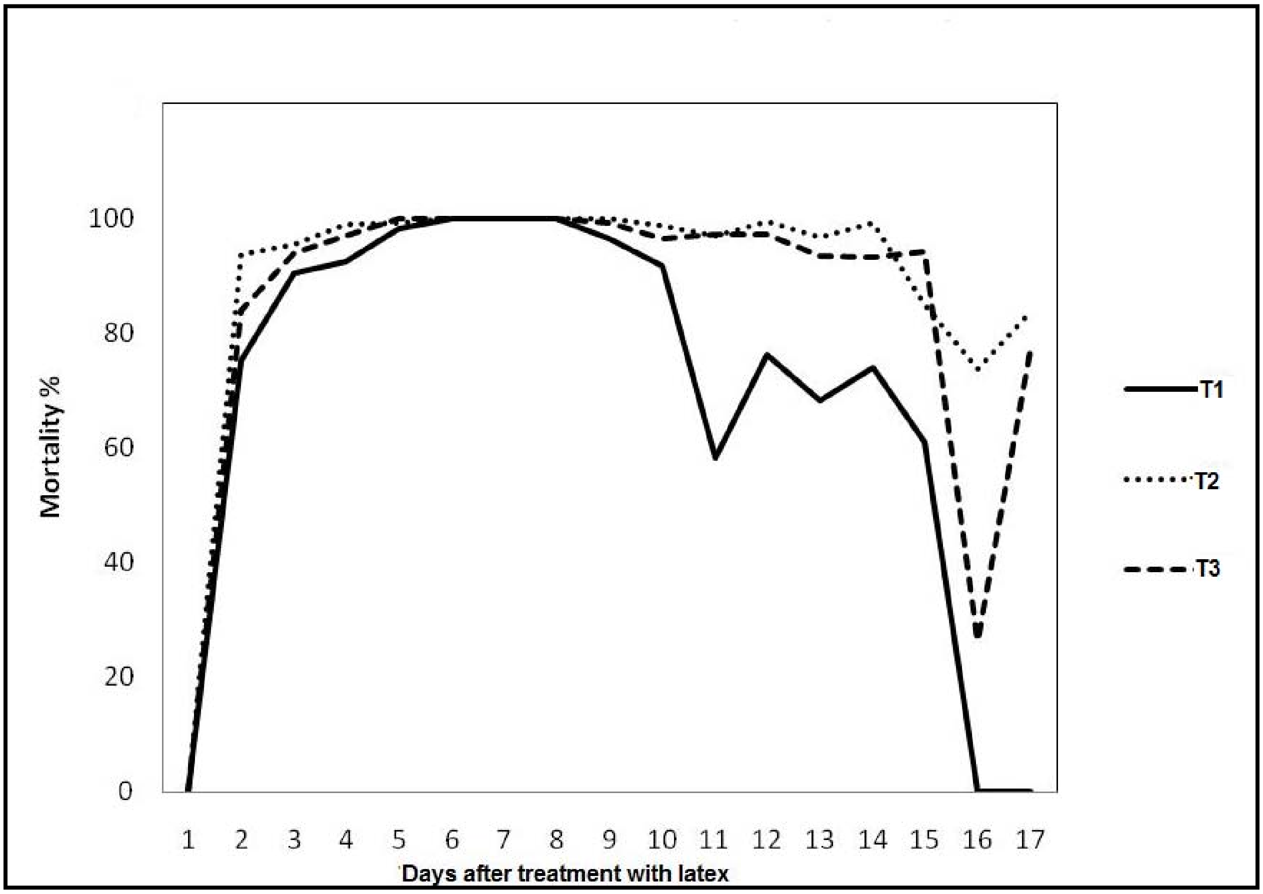
5.5 Conclusions

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 hours, 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.

Figure 5.3 Larval mean mortality in the treatments during the experimental period



Chapter six

Determination of Nematode-*E. tirucalli* Host Status

Adapted from:

J. Mwine, G. Nabulya, P. Van Damme, J. Masereka and G. Bwogi Is *Euphorbia tirucalli* L. (Euphorbiaceae) Nematicidal or a Nematode Victim? Preliminary Research: Establishment of *E. tirucalli*-Nematode Host Status *scientific research and essays* submitted, September 2010

Abstract

Phytoparasitic nematodes are important crop pests in (sub-) tropical areas. They cause severe damage to a variety of crops leading to serious yield losses. Synthetic nematicides are the most used management methods against them but are reported to be toxic, environmental pollutants and ozone layer depleters. Therefore, application of ecological and environmentally friendly alternatives/options like trap crops, resistant cultivars, and plant-based nematicides is generally recommended. In the present study, we screened *Euphorbia tirucalli* roots and surrounding soil (in natural conditions) to establish its nematode host status as an indicator to its nematicidal position. A number of important phytoparasitic nematode species in East Africa and coincidentally the most abundant in our samples obtained from Uganda were ranked. Results revealed that *E. tirucalli* may either be a poor host to or even nematicidal against *Radopholus similis*, *Pratylenchus goodeyi* and *Helicotylenchus multicinctus* whereas it was shown to be parasitized by *Meloidogyne* spp. It was concluded that while *E. tirucalli* is a victim to *Meloidogyne* spp., to the first three species, it should be considered as a nematicidal plant.

Key words: plant-based remedies, integrated pest management, natural nematicide, ethnomedicine.

6.1 Introduction

Phytoparasitic nematodes are among the most difficult crop pests to control. They cause severe damage to a variety of crops leading to serious yield losses especially in (sub-) tropical areas, where environmental factors favour their multiplication, survival and dispersal. Yet, most peasant farmers in these areas are usually unaware of them hence no steps are taken to properly control them (Kagoda *et al.*, 2010). Where control is attempted, particularly in intensive agricultural systems, measures rely heavily on synthetic nematicides. The latter, however, are usually very expensive. With their continued use, their efficacy continues to decline as nematodes develop resistance to them (Chitwood, 2002). In addition, many are persistent and accumulate in the environment, whereas they have also been reported to be ozone layer depleters (Fuller *et al.*, 2008). Therefore, many of them are being withdrawn in favour of new, safe, effective and natural options which include among others: trap crops, use of nematode-resistant species/cultivars and plant-based nematicidal compounds (Chitwood, 2002).

A number of plant-based compounds named in this direction include: alkaloids from pyrrolizidine alkaloid-producing plants e.g. *Clotolaria* spp. (Thoden and Boppre, 2010), polythienyls from *Tagetes* spp. (Marotti *et al.*, 2010; Vasudevan *et al.*, 1997), tannins such as those obtained from *Lotus corniculatus*, *Dorycnium rectum* and *Rumex obtusifolius* (Molan and Faraj, 2010), saponins from *Medicago* spp. (Argentieri *et al.*, 2008), triterpenes from *Euphorbia* spp. (Siddiqui *et al.*, 2003), polyphenols from *Quillaja saponaria* (San Martin and Magunacelaya, 2005), esters from various sources (Davis *et al.*, 1997), among others. According to Chitwood (2002), these compounds can be developed for use as nematicides themselves, or else they can serve as model compounds for the development of chemically-synthesized derivatives with enhanced activity or environmental friendliness in form of repellents, attractants, hatching stimulants or inhibitors and nematotoxicants.

Apart from the classic application of these plant-based nematicidals, plants themselves have been documented for suppressing nematodes 'using' these substances (Roberts, 1992; Williamson and Kumar, 2006). In this respect, numerous mechanisms of operation have been suggested including: plants' release of nematicidal compounds from roots (Li *et al.*, 2008; McPartland and Glass, 2001), stimulation of nematode-antagonistic organisms (Kaya and Koppenhofer, 1996) or action of endophytic bacteria (Insunza *et al.*, 2002; Mekete *et al.*, 2009). Plants can also be trap crops whereby nematodes are hosted by the plants' roots but remain harmless (LaMondia, 1996; Scholte, 2000). Such mechanisms can help to explain specific species' susceptibility, tolerance or even resistance to nematodes. According to Trudgill (1991), plant resistance to parasitic nematodes involves both physical and chemical barriers, which are either constitutively present or induced by infection; for example: production of phenolic compounds, synthesis of phytoalexins, accumulation of toxins, host cell death and necrosis, and modification of cell walls to a variable extent depending on plant susceptibility.

One of the approaches that can be applied to establish species' susceptibility to a pest or disease is to ascertain the pest's presence and survival within its tissues, i.e. establishing pest host status (Buena *et al*., 2008). According to Williamson and Kumar (2006), resistance to nematodes in plants is generally characterized by nematode failure to produce functional sites in a host after invasion and to subsequently develop into reproducing females. The same authors further explain that as a sign of plant resistance, colonizing nematodes mostly end up producing a dominance of males with very few females resulting in a severe reduction in reproducing individuals and hence population reduction. However, some invasive nematodes do not reach this far. Hawes *et al*. (2000) report that epidermal root layers act as biological 'goalies' by allowing or refusing organisms to penetrate the root, implying that nematodes can be accepted or immobilized at the root border depending upon the plant-nematode interaction success. In the same line of thinking, Chitwood (2002) mentions a case of nematode repulsion where root exudates of asparagus (*Asparagus officinalis* L., Liliaceae) conveyed resistance to the stubby-root nematode (*Paratrichodorus christiei*) at a distance from the plant itself. The implication of all this is that there is a whole continuum of plant-nematode colonization levels depending on nematode-plant interaction.

To measure nematode-plant host status or colonization success, Bridge and Page (1980) formulated an index that expresses different ranges of nematode establishment into a host. They explored a range of host status variations by utilizing visually determined numbers of galled roots as an index of nematode establishment (for galling nematodes). On a scale of 1 to 10, 1 is equivalent to 10% infected roots. In related cases, Davis and Webster (2005) applied percentage increase in egg counts to determine host susceptibility of different plants while Anwar *et al*. (1994) utilized numbers of nematodes in roots to establish nematode-plant host status. Such methods could be used to assess the host status of plants that are said to be anti-nematodal in terms of nematode colonization success. Screening such plants for evidence of variables like levels of necrosis/galling, nematode presence or absence in the plant's tissue and in the surrounding soil can help to ascertain the plant's host status with a good level of precision.

In this research, we focused on *Euphorbia tirucalli* L. (Euphorbiaceae) which has often been reported to possess nematicidal features (Siddiqui *et al*., 2003; Van Damme, 1989a and b). Common features and the wide variety of medicinal properties of *E. tirucalli* against a number of pests and disease causing organisms are presented in section 2.2.7 and 4.1. In this respect, Siddiqui (1984) reports that *E. tirucalli* latex is anti-nematodal against *Haplolaimus indicus*, *Helicotylenchus indicus* and *Tylenchus filiformis*.

Of late, organic farmers in Uganda have been trying different plant species either as a source of pesticidal extracts, trap crops or repellents for management of different pests. *E. tirucalli* is one of the plants thus identified as pesticidal. Apart from having been successfully tried on aphids (Mwine *et al*., 2010), there is a growing tendency to believe that intercropped with different crops or grown as a hedge plant, it can help to rid fields of nematodes (Kalanzi, pers. comm.). Whether it is used as a trap crop or as a repellent and if so, against which species of nematodes, is not clear. However, Muhammad *et al*. (1999) found no activity of its methanoic extracts against *Bursaphelenchus xylophilus* and *Meloidogyne* spp. which have been reported as its main nematode pests (Van Damme, 1989a,b; Yanxia *et al*., 2007) All these are indicative that *E. tirucalli* nematicidal status is not clear and needs ascertaining.

The objective of this study was therefore to establish whether *E. tirucalli* possesses nematicidal features, by acertaining its nematode host status. Results will give a broader scientific basis to support decisions on agronomic management of nematodes using *E. tirucalli* latex/plant material and should stimulate further research on its extract's nematicidal efficacy.

6.2 Materials and Methods

6.2.1 Soil and root samples

Three soil samples of approximately 0.5 kg each were scooped from different sides of each of the 75 selected *E. tirucalli* hedge plants at about 50 cm diameter and 10 cm depth. Soil was loosened with a hand hoe before sampling. Also, several pieces of roots were taken from each plant from where soil samples were taken. Both sets of (soil and root) samples were placed in polythene bags, sealed and transported in a plastic iced cooler box (brand GEO-006-012, made in China) to the laboratory where they were kept at about 10°C (Hooper, 1993) until screening. Screening was done as soon as possible but in any case not more than seven days after sampling. Prior to screening, the three samples from each tree were thoroughly mixed to constitute a representative sample for each particular tree. Twenty-five *E. tirucalli* trees were sampled from a representative location in each of three assessed regions of Uganda, namely Western, Central and Eastern. These regions were chosen because our preliminary studies revealed that the species is used for pesticidal purposes by organic farmers in these areas (Mwine unpublished). More specifically, samples were taken from Kikise (00° 17´ 59´´ S; 31° 47´ 56´´ E) in Masaka district; Kashaka (0° 50´ 00´´ S; 30° 11´ 00´´ E) in Mbarara district and Budumbuli (0° 34´ 66´´ N; 33° 20´ 31´´ E) in Jinja district.

To rule out possibilities of complete absence of nematodes in areas sampled, two other common plants known to be hosts and to be parasitized by a variety of nematodes, i.e. *Musa paradisiaca* L. (Musaceae) and *Solanum nigrum* L. (Solanaceae) growing in close proximity (same crop field) to the experimental hedge plants were likewise screened (control experiment). Guided by organic farmers in the region, care was taken to obtain samples from areas that use pesticides minimally.

6.2.2 Nematode extraction

a) Soil extraction

Nematode soil extraction was carried out using conventional methods as described by Rodriguez-Kabana and Pope (1981) and Viglierchio and Schmitt (1983). In this particular experiment, 100 cm^3 of soil sample were spread on a two-ply tissue paper (manufactured by Tesco industries L.L.C P.O Box 6720, Sharjah, U.A.E., tesco@emirates.net.ea) mounted on a sieve consisting of a 2 mm mesh fiber glass screen, glued between two 15 cm diameter PVC pipe sections. The sieve with the soil was placed in a plastic mixing bowl and water was added slowly between the sieve and bowl walls so as just to cover the soil. This set-up was then incubated at room temperature (25-27°C) for 72 hours after which the sieve was removed and the bowl contents passed through a 38 mm mesh stainless steel sieve. Twenty five ml of the solution were then drawn off, concentrated, thoroughly mixed and five ml aliquots transferred to a counting dish for examination and nematode enumeration under a compound microscope (model Laborlux S brand 020-505.030, made in Portugal). Taxonomic identification of nematodes was carried out by expert nematologists at National Agricultural Research Laboratories, Kawanda, P.O. Box 7065, Kampala, Uganda.

b) Root extraction

For roots, extraction technique used was modified from existing Baermann funnel methodology i.e. maceration – filtration technique (Bridge and Page, 1980; Gowen and Edmunds, 1973; Hooper, 1993) and as used by Kashaija *et al*. (1994). Root samples were washed clean, chopped into small pieces, mixed thoroughly, and 5 g weighed off for nematode extraction. Maceration was carried out using a blender under tap water which was run for 5 seconds to attain full speed, 5 seconds at full speed and allowed to come to a halt in another 5 seconds, in order to obtain a uniform suspension (Hooper, 1986). Blender contents were then poured on a double ply tissue paper (as used in soil extraction above) supported by plastic sieves with a 2 mm mesh, plastered on plastic supports. After a 72 hour incubation period at room temperature, the extract was collected in sample bottles and let to stand for 5 hours to allow nematodes settle. The supernatant was then decanted and the final suspension reduced to 25 ml, mixed thoroughly and 5 ml aliquots drawn off at a time for examination under a compound microscope (see section 6.2.2a) for nematode enumeration and identification.

6.2.3 Data analysis

Extrapolated from methodologies used by other authors (Anwar *et al.*, 1994; Bridge and Page, 1980; Davis and Webster, 2005), data obtained from both soil and root extractions were ranked to reflect *E. tirucalli* host status for different nematodes. The four most important plant parasitic nematodes in East Africa (Bridge, 1988) and those most occurring in our samples, namely *Radopholus similis*, *Pratylenchus goodeyi*, *Helicotylenchus multicinctus* and *Meloidogyne* spp., were ranked (ordinal ranking) according to variables that best reflect colonization success (nematode-plant host status) (table 6.1).

Table 6.1 Variables used to rank nematodes

Variable	Ordinal rank
Presence in tissue with necrosis or galls and juveniles	++++
Presence in tissue with necrosis or galls	+++
Presence without necrosis or galls	++
Absence in tissues but presence in soil	+
Absence in soil and tissue	0

We also calculated Percentage Relative Abundance (PRA) from mathematical formulae and used it as an index of preference of a nematode to a host where:

$$\text{PRA} = \frac{No.\ of\ nematodes\ in\ a\ \ host}{Total\ no.\ of\ nematodes\ in\ all\ hosts} \text{ x } 100 \qquad \text{Equation 6.1.}$$

The formula assumes that all nematodes have equal chances of being attracted to any host (plant species) growing in an area. Preference will therefore be determined by the nematode-host relationship. A higher PRA (close to 100 % indicates colonization of the plant species while a low RPA (close to 0 %) shows inhibition.

6.3 Results and discussion

6.3.1 General presence of nematodes

Results indicate that nematodes assessed were generally present in the three areas considered for the three plants screened (table 6.2). *Meloidogyne* spp. were the most abundant in both roots and soil followed by *R. similis*, then *P. goodeyi* and *H. multicinctus*. Presence of nematode species assessed in both soil and roots samples of alternative host plants (*M. paradisiaca* and *S. nigrum*) is an indication of equal chances of occurrence in *E. tirucalli*, if it were indeed a host plant. The implication is that nematode absence within the tissues/soil of *E. tirucalli* is attributed to inherent features of the plant itself (e.g. nematicidal) and not to other factors such as application of nematicides in the area.

6.3.2 *E. tirucalli* host status

According to our rankings (table 6.3), *E. tirucalli* displays various levels of host status for different nematodes. *Meloidogyne* spp. was ranked as the most successful in colonizing *E. tirucalli* since large numbers of the species were found in the surrounding soil and it was the only one found in its roots. However, close observation showed a slightly low PRA (7.14%), indicating that a small number of nematodes was either attracted or managed to survive in its tissues. Also, the fact that no galls were observed in this experiment may indicate that *E. tirucalli* is a poor host. Based on these results, *Meloidogyne* spp. is considered to be a resident and since neither galls nor immature stages were observed, it is difficult to ascertain that *E. tirucalli* is actually parasitized by the species (Buena *et al*., 2008). What is certain, however, is that the plant is not nematicidal to this species lest they would not survive in its tissues. But *Meloidogyne* spp. are reported to be among the few parasites of *E. tirucalli* (Van Damme, 1989a and b; Yanxia *et al*., 2007) and it was earlier reported to successfully colonize and form galls (in *E. tirucalli* roots) in Senegal (Van Damme, 1989b). All these imply that the observed presence of *Meloidogyne* spp. in *E. tirucalli* roots is indicative of parasitization and establishment.

Helicotylenchus multicinctus and *Radopholus similis* were only found in soil samples but shown to be absent from the roots (table 6.2). This could be attributed to either coincidental migration from surrounding roots (of other plant species) or failure to penetrate *E. tirucalli* tissues. Failure to penetrate certain plant tissues may be due to ability of the plant to suppress nematode attack (Hawes *et al*., 2000) using any of the mechanisms already mentioned (also see Williamson and Kumar, 2006). However, a high PRA (48.78%) shown for *H. multicinctus* suggests the opposite i.e. a tendency towards colonization. Yet, this could be a false signal since most of the nematodes that brought about the high PRA were recorded in the soil not in the tissues. Siddiqui and Alam (1984) and Van Damme (1989b) earlier reported antinematodal features of *E. tirucalli* latex against *H. indicus* which belongs to the same genus as *H. multicinctus*. This indicates that there are high chances of *E. tirucalli* being nematicidal to it just as *H. indicus.*

R. similis is described as a migratory nematode with a wide habitat range including citrus (*Citrus* spp.) and *Musa paradisiaca* (O'Bannon, 1977) with which our results agree (see table 6.2). The species was found abundantly in *M. paradisiaca* (both soil and roots) but absent in *E. tirucalli* roots and surrounding soil. Similarly, it had a very low PRA (0.03 %) implying that it was unable to colonize it. This is suggestive of nematicidal properties of *E. tirucalli* against *R. similis* just like *H. multicinctus.* These two species were equally ranked (table 6.3) and belong to the same order (Tylenchida).

On the other extreme, *P. goodeyi* was completely absent in soil and roots of *E. tirucalli* although abundant in roots of *M. paradisiaca*. *P. goodeyi* is described as a root-lesion nematode and was reported to be the most abundant and widespread nematode on *M. paradisiaca* in Uganda (Kashaija *et al.*, 1994; Sarah, 1989). But Pestana *et al*. (2009) report *S. nigrum* as a poor host or being resistant to *P. goodeyi*, recommending its application as a green manure or nematicidal fumigant in *P. goodeyi*-infected banana plantations. Our results show a high abundance of *P. goodeyi* in *M. paradiasica* and absence in *S. nigrum* (table 6.2) in agreement with their findings. Absence in both soil and root samples of *E. tirucalli* in all regions combined with a PRA of 0.00 %, gives a strong indication of the potential nematicidal behaviour of the plant. Given that *Solanum* spp. have been recommended for its management and yet a few adults were observed in its roots unlike in *E. tirucalli* roots (table 6.2), is indicative of the fact that the latter plant may either be a poorer host or possess stronger anti-nematodal features than the former.

6.4 Conclusion

This work has attempted to show a variation in *E. tirucalli* host status regarding nematode species assessed. Although it cannot be concluded with ultimate accuracy, the following can probably be added to the prevailing facts about *E. tirucalli*-nematode behaviour: *H. multicinctus* may fall in the same category as *H. indicus* (same genus) and its observed failure to penetrate *E. tirucalli* roots implies possible anti-nematodal features against it. *E. tirucalli* may also be considered anti-nematodal to *R. similis* and *P. goodeyi* for their failure to penetrate its roots and colonize it.

Pending further confirmation, these results will help to make agronomic decisions on management of nematodes with *E. tirucalli*. While it might be considered for management of *H. multicinctus, R. similis* *and P. goodeyi*, it is documented and has been shown here that *Meloidogyne* spp. parasitize it and should therefore, not be used in its management unless it is used as a trap crop.

Table 6.2 Abundance of nematode numbers per 10 ml aliquot of sample

Plant	Area	HM		RS		PG		ML	
		Soil	Roots	Soil	Roots	Soil	Roots	Soil	Roots
E. tirucalli	Kashaka	0	0	0	0	0	0	42	0
	Kikise	48	0	1	0	0	0	13	2
	Budumbuli	12	0	0	0	0	0	34	0
M. paradisca	Kashaka	8	3	0	0	0	85	0	0
	Kikise	12	16	33	7	7	0	47	0
	Budumbuli	16	4	200	86	23	48	8	23
S. nigrum	Kashaka	2	0	0	0	1	0	106	46
	Kikise	2	0	0	0	0	0	64	230
	Budumbuli	0	0	0	0	0	0	59	658
Total abundance		**100**	**23**	**234**	**93**	**31**	**133**	**273**	**959**
% Relative abundance		**6.59**		**17.54**		**8.79**		**66.09**	

Key to species

HM - *H. multicinctus* RS - *R. similis*

PG - *P. goodeyi* ML - *Meloidogyne* spp.

Table 6.3 Ranking* *E. tirucalli* susceptibility against different nematodes

Nematode	Mean abundance in sample		Rank	RPA (%)
	Soil	Root		
HM	30	0	+	48.78
RS	0.3	0	+	0.03
PG	0	0	0	0.00
ML	30	0.6	+ +	7.14

* Ordinal ranking was used to reflect increasing susceptibility of nematodes to *E. tirucalli.*

Key to ranks

Presence in tissue with galls/necrosis and juveniles	+ + + +
Presence in tissue with galls/necrosis	+ + +
Presence in tissue and soil	+ +
Presence in soil only	+
Complete absence	0

Chapter Seven

Final Discussions, Conclusions and Recommendations

7.1 General discussion

At the beginning of this work, it was clear that numerous extracts of pesticidal plant species were being used in pest management in southern Uganda. However, neither a record of these species nor their extract efficacy studies had been documented. Only limited information was available concerning these issues. As a result, the initial work of this study was devoted to establishing the importance of pesticidal plant species in this region (chapter three). A number of species that previously had not been documented to be of pesticidal importance (including *E. tirucalli*) were recorded for the first time. Whereas some farmers recognized them as being pesticidal species, they also expressed a strong desire to ascertain their efficacy against particular pests. Responding to this desire, numerous efficacy evaluation trials were carried out in the laboratory. *E. tirucalli* was chosen for this work due to its multi-functional properties. We employed appropriate methodologies for each pest species to evaluate (in the laboratory) *E. tirucalli* extracts against a number of pests. Like all pesticidal remedies, there was a marked variation in efficacies recorded. Using efficacy/mortality levels adapted from Throne *et al*. (1995), their performance was ranked from poor to very good for different pests (table 7.1). Within the limits of the available resources for this work, only pests against which our tests showed good to very good laboratory results were evaluated in the field and reported in detail (see section 1.4) in this thesis. Some of the chapters have been published in journals, presented at symposia and conferences, whereas others have been submitted for publication.

Table 7.1 *E. tirucalli* extract performance rating* against assessed pest species

Pest category	Species evaluated	Treatment	Extract performance	Reference
Field/agronomic pests	*Brevicoryne brassicae*	Latex spray/colony	Very good	Chapter 4
	Myzus persicae	Latex spray/colony	Very good	Mwine (unpub.)
	Plutella xylostella	Latex spray/larvae	Good	Chapter 4
	Spodoptera littoralis	Latex spray/larvae	Poor	Mwine (unpub)
	Radopholus similis	Soil/root screening	Good	Chapter 6
	Helicotylenchus multicinctus	Soil/root screening	Good	Chapter 6
	Pratylenchus goodeyi	Soil/root screening	Very good	Chapter 6
	Meloidogyne spp.	Soil/root screening	Poor	Chapter 6
Health/human vectors	*Anopheles funestus*	Latex spray/larvae	Very good	Chapter 5
	Anopheles gambae	Latex spray/larvae	Very good	Chapter 5

*Rating: Very good – over 90 % mortality; Good – 50-90 %; Poor - below 50 % (adapted from Throne *et al*., 1995).

In general, *E. tirucalli* extracts assessed in this work have shown remarkable performance against a number of organisms (see chapters 4, 5 and 6). However, a number of issues need to be pointed out in relation to the practical applicability of the extracts. In chapters 4 and 5, we pointed out that when applying the extracts, low doses should be adhered to in order to minimize their poisonous effects. Even when minimum doses were to be used, there could still be a problem of eco-toxicity. For example, it is known that *E. tirucalli* latex is piscicidal at low doses (Neuwinger, 2004) which points to its toxicity to even larger organisms than arthropods. Combined with a high application frequency (once or twice a week recommended in chapter 4), there is also a possibility of cumulative environmental toxicity. Such a scenario has been observed for waste residues of tobacco (*Nicotiana tabacum)* which produces nicotine that pollutes ground water (Mumba and Phiri, 2008). The extracts therefore, need to be used with caution and with as low a frequency of application as possible. However, plant extracts are generally known to get biodegraded easily since they have relatively shorter half-lives than synthetic pesticides (Ansari and Kafeel, 2010; Stalin *et al.* 2008) which may point to less danger of long term eco-toxication.

Crude extracts may have an advantage of being broad spectrum in their action because they are likely to contain a mixture of active ingredients (Abate *et al*., 2000). This is even more likely especially when it is a concoction made from several plant species. However, this attribute could come with a disadvantage of being harmful to non-target organisms since no particular pest is targeted. Although we did not observe the 'silent spring scenario' in our experiments, we presume that some other organisms (other than the target pests) could have been affected by the extracts since they were crude. There is a belief that plant-based pesticides generally have a more mild effect on non-target organisms (Isman, 2005; Stalin, *et al.* 2008). However, there is a need to evaluate *E. tirucalli* extracts against beneficial insects/organisms to see whether and to what extent there is an effect and in order to avoid affecting the ecosystem. The latter problem could be overcome by extraction and purification of active ingredients from the crude extracts. The pure ingredients would then be used against targeted organisms.

In most developing countries, there is a general tendency to apply crude plant extracts in agriculture and health without policy restrictions nor the application of control mechanisms or standardization of formulations. According to Mosihuzzaman and Choudhary (2008), this is probably because they are considered safer and more acceptable, based on their long-standing use in various cultures compared to modern synthetic products. For example, their preparation/standardization, dosage, storage and other factors which are important (and monitored) in synthetic products are usually not paid attention to in the case of botanical extracts. While they may pose fewer eco-toxicological problems probably due to the ease with which they are biodegraded, a few such extracts e.g. from *N. tabacum* are said to be even more toxic than synthetic pesticides (Avery, 2006). This could be a pointer that many other botanicals are not as safe as they are presumed to be. There is therefore a need to evaluate such properties like eco-toxicology for *E. tirucalli* and other botanical extracts whenever they are used. Essential features like pre-harvest interval as well as food and soil residual levels should be paid attention to even for botanical extracts.

7.2 General conclusions

Chapter one set the background for the research by reviewing the dangers caused by some modern technologies used in agriculture such as application of synthetic pesticides in agronomy. Dangers resulting from this application which include pollution, demise of non-target organisms, reduction in biodiversity, acid rain, depletion of the ozone layer and climate change were reviewed and safer alternatives such as use of plant-based remedies, biological control and integrated pest management were suggested. Advantages of applying ecological/environment-friendly methods were emphasized drawing the conclusion that there is a need to reduce or completely abandon synthetic pesticides in order to protect the environment for the present and next generations.

Chapter two provided available knowledge on the subject of study. Euphorbiaceae family in general and *Euphorbia tirucalli* in particular were reviewed exploring their rich chemical diversity and the accruing medicinal properties. Origin and reasons for possession of large chemical assemblage from which important pharmaceutical substances (both folklore and modern) are/can be prepared were discussed. The conclusion that Euphorbiaceae family is a good starting point for the search of medicinal/pesticidal plants was well-deserved since Euphorbiaceae is a 'parent family' of genus *Euphorbia* in which *E. tirucalli* species is found. In the same chapter, the multi-functional properties of *E. tirucalli* were reviewed. It was noted that most of the species' functions depend upon its possession of chemical-rich latex that confers upon it the characteristics of having both low herbivore pressure and toxicity to a number of organisms. This helped to draw the conclusion that *E. tirucalli* could have pesticidal properties.

In chapter three, an ethnobotanical survey was carried out to establish the utility of pesticidal plants in southern Uganda in general and *E. tirucalli* in particular. The present study established that 34 species are used as pesticidal plants in this region. Most of the species were the usual, scientifically-confirmed pesticidal plants like *Tagetes* spp., *Azadirachta indica* and others. However, a few 'new' species like *E. tirucalli* and *Bidens pilosa* were associated with pest management (in crops) for the first time in this region. The need to evaluate their efficacy on particular crop pests was seen as urgent. It was concluded that for such pesticidal plants to be put to extensive and commercial use, efficacy studies should be carried out. Also, owing to observations that some of the pesticidal plants are disappearing (may be threatened by extinction), it was concluded that their conservation probably by domestication should be effected as soon as possible.

In chapter four, *E. tirucalli* together with *Jatropha curcas* and *Phytolacca dodecandra* fresh extracts were evaluated against cabbage pests i.e. *Brevicoryne brassicae* and *Plutella xylostella* in the field (natural infestation). Results revealed that there is a large variation between efficacies of the three extracts on the pests assessed. While *E. tirucalli* extracts showed the capacity to manage *B. brassicae* infestation to economic threshold levels, the same extracts could not do likewise for *P. xylostella.* On the other hand, *J. curcas* and *P. dodecandra* extracts controlled neither of the two pest infestations assessed to the required threshold levels. This led to the conclusion that *E. tirucalli* extracts should be recommended for management of *B. brassicae* infestations and a factor in integrated pest management of *P. xylostella.* The same experiment evaluated the extract dilutions that can be applied against the assessed pests. Both extract dilutions i.e. 25 % and 12.5 % showed no significant difference in *B. brassicae* control. This finding led to the conclusion that lower extract dilutions are to be preferred to avoid over- dosing that could lead to poisoning of non-target organisms. As it is, *E. tirucalli* latex is known to be toxic and co-carcinogenic, therefore lower doses to minimize these drawbacks should be applied. This work also helped to validate farmers' views that *E. tirucalli* latex can protect leafy crops against a variety of pests.

Evaluation of larvicidal properties of *E. tirucalli* latex against *Anopheles* spp. larvae in the field was carried out in chapter five. The experiment was carried out in a neglected pond where *Anopheles* species were found to breed naturally. Results revealed a high efficacy for the latex extracts tested. LT 50 and LT 90 for the highest dilution (1:250) were achieved at 12 and 36 hours, respectively. Total mortality was obtained on the fifth day after treatment with the extracts. It was also observed that the latex extracts were active for only one week after which the larvae population started increasing again. This led to the conclusion that the extracts should be applied twice every week when the larvae populations are high but only once a week when infestation is low. On comparison of the dilutions applied (1:250, 1:200, and 1:150), no significant difference was observed between their performance. It was therefore concluded that the highest dilution (1:250) be preferred for application to avoid overuse of the latex which could lead to safety problems. In addition, since *E. tirucalli* extracts are known to have piscicidal properties (Neuwinger, 2004), it was recommended that latex application should be limited to water bodies which are not utilized for fish rearing.

Chapter six revealed a variation of *E. tirucalli* nematicidal properties against different nematodes. Having established that some farmers believe that *E. tirucalli* has nematicidal properties, its roots and the surrounding soil were screened for evidence of nematode colonization. This experiment was based on the hypothesis that *E. tirucalli* root exudates would repel nematode species to which it is nematicidal but would be a victim to those it is not. Consequently, *Meloidogyne* spp. were found to occur in its roots whereas other nematode species screened showed variable plant-host relationships. *Radopholus similis* and *Helicotylenchus multicinctus* were absent in *E. tirucalli* roots but present in the surrounding soil as opposed to *Pratylenchus goodeyi*, which was found in neither of the two areas screened. It was therefore concluded (according to these preliminary findings) that *E. tirucalli* is likely to be nematicidal against the latter three species while it is a victim to the first. As for agronomic management of nematodes using *E. tirucalli*, these results are only indicative and not conclusive enough to recommend its application. Further research is necessary to validate these findings.

In summary, like all other pest remedies, *E. tirucalli* extracts efficacy varies with different pests through a whole continuum and spanning different pest taxa. High efficacy was observed against *Anopheles* spp. larvae, *B. brassicae*, *R. similis, H. multicinctus,* and *P. goodeyi*. For the organisms against which the extracts did not show a high efficacy (see table 7.1), this study believes that it is possible that other parts of the plant (not assessed in this work) could be efficacious against them or the organisms are able to protect/detoxify themselves against the chemical substances found in the extracts. Even when some pests use it as a host (e.g. *Meloidogyne* spp.), nevertheless, *E. tirucalli* pesticidal properties cannot be underestimated.

7.3 Recommendations for further research

This work is just the first step towards elucidation of pesticidal properties of *E. tirucalli* in particular but also numerous other pesticidal plant species whose extracts are used in southern Uganda. Extracts used in this study were crude/not extracted to march the famers' set-up and to establish their initial efficacy before incurring costs of extraction. Extracted substances are expensive, especially in field experiments. There is a need to process and purify these extracts for purposes of preservation, ease of application and standardization which would lead to commercialization of the final product. Further research is therefore, recommended in this direction.

For most of the results reported in this thesis, only a limited number of extract dilutions were applied within the scope of this work. Trials with a larger number of extract dilutions would help to better determine the actual lethal dose of the extracts to avoid over-dosing. In the same spirit, such experiments would also help to improve on pests' lethal time as well as the frequency of application. We recommend further research in this area.

Co-carcinogenic properties of *E. tirucalli* have been widely reported. In spite of this feature, its extracts have continued to be used in alternative medicine even as a remedy against cancer. Laboratory reports have also pointed to this anti-cancer property. This study recommends that further research should be carried out to establish the truth about this controversy to help make informed decisions on the application of the species for medicinal purposes.

Further research is also needed in the area of the working mechanism of *E. tirucalli* latex. Although available literature points to systemic pathway of action for Euphorbiaceae lattices in animals (de Silva *et al*. 2008), there appears to be no information regarding the mechanism of action in plants. There is a need to know whether the latex penetrates the plants such that the target pests can take it up in the plant sap or if it protects the plants ectopically. There is also a need to find out if the latex penetrates the pests from the chitin, spiracles or by ingestion. Such information will help to determine the right methodology of application and possibly reduce overdosing by aiming at the right target.

As observed earlier (section 7.1) policies related to toxicology and hazards of pesticides are often relaxed/silent or non-existent for botanical extracts in many countries. Yet some plant substances are said to be very toxic and could pose hazardous residual effects. The advantages of *E. tirucalli* as a plant-based pest remedy notwithstanding, this work recommends that research should be carried out to establish the pre-harvest interval as well as residual levels of the product whenever it is applied .

Material reported here is an endeavour to satisfy the present thesis objectives but also, an attempt to answer the farmers' call for researched data on some medicinal/pesticidal plant species. While the thesis objectives may have been achieved, there is still quite a lot of inconclusive work concerning the latter aspect. For example, only preliminary findings are reported on nematicidal properties of *E. tirucalli* and no evaluation has been carried out against micro-organisms that are reported to be controlled by the species' extracts. All such inconclusive work need to be (further) investigated in order to obtain more conclusive results. Such results will help to make better informed recommendations concerning the application of *E. tirucalli* extracts for ecological pest management.

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Publications

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Research interests:

- Evaluation of crude botanical extracts of medicinal/pesticidal importance;
- Research on biodiesel plants in Uganda.