REPRODUCTIVE INHIBITION EFFECTS OF *Azadirachta indica* A. JUSS AND *Ricinus communis* LINN. SEED OILS IN ALBINO FEMALE MICE

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DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been submitted or presented for examination in any institution.

Signature..... Date..2/6/2020.....

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Recommendation

This thesis has been submitted with our approval as supervisors for examination according to Egerton University regulations.

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DEDICATION

To all my family members especially my children Justin and Fabian for their support and encouragement during my study.

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ABSTRACT

Rodents are the greatest vertebrate pest problem in developing countries causing substantial damage to structures, food, crops, industrial and domestic property. They also act as reservoirs and vectors of zoonotic diseases. The current method for controlling rodents is the use of rodenticides which are toxic to non-target organisms, pollutants to environment and trigger resistance. In an effort to find eco-friendly alternatives to synthetic rodenticides, reproductive inhibition of *Ricinus communis* and *Azadirachta indica* seed oils were evaluated in albino female mice. Seed oils of *A. indica* and *R. communis* were extracted from mature dried seeds by cold pressing and boiling respectively and chemical compositions were determined using Gas Chromatography (GC)-Mass Spectrometry (MS). The constituents of both seed oils were dominated by saturated and unsaturated fatty acids, cyclic esters and methyl esters. The predominant constituents of *R. communis* were (Z)-6-Octadecenoic acid (37.33%), Ricinoleic acid (30.22%) and 13-Hexyloxacyclotridec-10-en-2-one (26.67%) while those of *A. indica* were 2-hexyl-1-decanol (30.97%), Octadecanoic acid (29.69%) and Oxalic acid, 6-ethyloct-3-yl ethyl ester (15.55%). Mature fertile female mice received *A. indica* and *R. communis* seed oils at doses (0.0-0.8 ul/kg body weight) for 14 days. The experimental design was a randomized block design (RBD) with six replicates of mice per treatment. Data on mating success was analyzed using *Chi*-Square Test while oestrous cycle, fertility index, gestation period, litter size and weekly body weight were analyzed using one-way analysis of variance (ANOVA). Data on percentage fertility and anti-implantation were corrected for homogeneity of variances using arcsine-transformation before being subjected to one-way ANOVA. *Azadirachta indica* and *R. communis* seed oils caused disruption of the oestrous cycle by increasing the frequency of diestrus and metestrus phases. At higher doses *A. indica* and *R. communis* seed oils reduced mating success to 67% and 83% respectively with a similar significant reduction in fertility index at 17% compared to the negative control. At a dose of 0.4ul, *A. indica* and *R. communis* seed oils caused a significantly prolonged gestation period of 24.33±0.33 and 23.33±0.33 days with reduced litter size of 3.33±0.33 and 2.67±0.88 young ones respectively. Subsequently, at higher dose none of the mice littered compared to the negative control. At concentrations of 0.6ul and 0.8ul, *A. indica* and *R.communis* showed anti-implantation activity of 100% compared to the negative control. Therefore *A. indica* and *R. communis* seed oils reduce rodent population and can be used as potential rodenticides.

TABL.E OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND ACRONYMS

CDC	Centers for Disease Control
FSH	Follicle-Stimulating Hormone
GC-MS	Gas Chromatography-Mass Spectroscopy
GnRH	Gonadotrophin Releasing Hormone
HPG	Hypothalamic-Pituitary-Gonadal
HPO	Hypothalamic-Pituitary-Ovarian
ICIPE	International Centre of Insect Ecology and Physiology
LH	Luteinizing Hormone
mRNA	messenger Ribonucleic Acid
MUFA	Monounsaturated Fatty Acid
NPMA	National Pest Management Association
PGCs	Primordial Germ Cells
RBD	Randomized Block Design
TXA_2	Thromboxane A_2
VO	Vaginal Opening

CHAPTER ONE

INTRODUCTION

1.1 Background information

Rodents are one of the major postharvest pests that affect food security by impacting on both food availability and safety. They are problematic to agriculture and public health since they can inflict considerable economic damage associated with their diversity, generalist feeding habits and high reproductive rates (Malebane, 2017). Rodents have been estimated to damage more than 1% of the world's cereal crops on annual basis. In developing countries including Kenya, estimates of 3-5% have commonly been reported (Belmain *et al*., 2015). The three most important rodent species that damage crops in Kenya are the black or house rat (*Rattus rattus)*, the house mouse (*Mus domesticus*) and the Norway or common rat (*Rattus norvegicus*). In Kenya, rodents contributed 30% of the total postharvest losses on maize stored in stores according to a survey conducted in 2014 (Ognakossan *et al*., 2018).

The grain damage by rodents in stores is often associated with removal of the germ, which causes germination failure when the seeds are used for planting. The contamination of the grain with feaces, hair and urine results in poor quality and lower market value. It is extremely difficult to remove contamination as infested batches are often declared unfit for human consumption (Ognakossan, 2017). In addition, due to their gnawing and burrowing habits, rodents destroy many articles including packaging materials, clothes, building structures and furniture. For instance, by gnawing through electrical cables they cause fires with consequent losses (Belmain *et al*., 2015).

Rats and mice easily proliferate in areas with poor hygiene, domestic waste, poor housing structures and improper handling of leftover food. Apart from being pests they are also known to be reservoirs and carriers of many diseases to humans and livestock. They act as reservoirs by harbouring disease-causing organisms and serve as potential sources of disease outbreaks but always via vectors such as fleas, ticks, mites and sandfly. These diseases include toxoplasmosis, lymphocytic choriomeningitis, trichinosis, plague, giardiasis and leishmaniasis. Rodents are also carriers harbouring the disease-causing agent and capable of passing it directly onto humans as the case in *Salmonella*, Hantavirus, toxascariasis, *Escherichia coli*, tularemia and leptospirosis (Murphy *et al*., 2008; Meerburg *et al*., 2009; Witmer and Aaron, 2017). The diseases are spread directly through rodent bites, handling of live or dead rodents and contact with rodent feaces, urine and saliva. Mice urine triggers allergies, particularly in children because their immune systems are still developing. Mice

and rats are known to be vectors of *Salmonella typhi,* a bacterium found in their digestive tract. The rodents spread the disease by contaminating food and water in homes. The disease can also spread to household pets and often results in death. The Centers for Disease Control and Prevention (CDC) reports that every year over 42,000 people become ill due to *Salmonella* infection transmitted by rodents (NPMA, 2014).

Rats and mice are vectors of a larger number of domestic livestock diseases like swine fever and foot and mouth disease since they are infested with fleas, mites, ticks and lice. However, rodents have ecological, scientific, social, and economic values. Rodents are important in seed and spore dispersal, seed predation, energy and nutrient cycling, the modification of plant succession and species composition as well as food source for many predators. Additionally, various rodent species are economically important as sources of food or fur in many parts of the world, and others are used extensively in biomedical research (Bradford, 2015). Laboratory rats and mice serve as models to study a number of human diseases such as obesity, diabetes, cancer, cardiovascular diseases (high blood pressure and heart failure), neurological diseases (Parkinson's disease), inflammatory and immune mediated diseases (kidney disease and multiple sclerosis) (Harvey *et al*., 2011). By using rodents as models, researchers have built up an incredible wealth of knowledge about basic biology and complex physiological interactions about humans. This has continued to provide a very valuable contribution to research. Hence, the indiscriminate removal of native rodents from ecosystems, including agro-ecosystems, is not the best management option in many cases (Witmer and Aaron, 2017).

Rodents require control because of economic losses and ecological damages they cause. In the last decade, there has been increased awareness in the general community of the environmental pollution and animal welfare issues associated with various pest control methods. At the small scale farmer level, synthetic pesticides are costly and inaccessible in rural areas. Synthetics are often adulterated by dilution, mixed incorrectly and used beyond their expiry date (Stevenson *et al*., 2012). It is therefore prudent to develop alternative control techniques that are cost effective, species specific, environmentally friendly and more humane. In this regard, fertility control is considered to be one of the potential options because many approaches have been reported to suppress reproduction, for example, the use of steroid agonists and antagonists of gonadotropin releasing hormone (GnRH), immunocontraceptive vaccines, natural plant extracts and chemicals.

Evaluation of herbs has been in progress worldwide for several decades to identify effective and safe substances for fertility regulation (Tran and Hinds, 2013). Anti-fertility activity of *Azadirachta indica* (Neem) and *Melia azedarach* (Dharek) have been reported earlier that it disrupts ovarian function in rodents (Roop *et al*., 2005). Plant extracts inhibit fertility by adversely affecting, directly or indirectly, reproductive processes ranging from gonadal development to gestation.

Other researchers have reported effects on the phases of the oestrous cycle and histological changes in the ovary and uterus (Rao and Alice, 2001; Nandwa, 2016). Few studies have directly focused on fertility using breeding/mating success for more than one cycle and even fewer have monitored changes in the hormones of the hypothalamic-pituitary-gonadal (HPG) axis (Tran and Hinds, 2013; Kaingu, 2016). The treatment duration and route of treatment as well as doses of plant extracts are also inconsistently applied. The current study investigated the effect of both *A. indica* (Neem) and *R. communis* (Castor bean) seed oils on reproduction in female mice.

1.2 Statement of the problem

Small-scale farmers are the backbone of global food security but they face several challenges in the process of food production including pest menace, social, economic and environmental among others. Rodents are the most destructive vertebrate pests of agricultural produce. The persistence of rodent problem is due to their high rate of reproduction, complex behaviour and ability to adapt to diverse ecological conditions. Management of rodents is often limited because of the high cost of rodenticides and inaccessibility in rural farming areas. Most pest control measures in Kenya focus on invertebrate pests, such as stem borers, armyworm and locusts. Very little attention has been paid to vertebrate pests like rodents and research on rodent pest control tends to be neglected.

Managing rodent pests on a broad scale using lethal methods is not an appropriate long-term strategy given their extraordinary breeding capacity and high mobility. Moreover, environmental, animal welfare and ethical concerns regarding the use of poisons and trapping has decreased the acceptance of mortality methods in recent times. Another reason for avoiding lethality is that it may promote a strong selective pressure for resistance to the lethal agent. In addition, rodenticide baits are a health risk because they may come in contact with children and pets. Traps can be used to control rodent populations but they require monitoring and follow-up. Therefore there is need to introduce nonlethal, non-toxic alternatives that focus on reducing reproduction, rather than increasing the mortality of the

pest species. Fertility control aims to reduce a specific population size by reducing the number of young produced and recruited into the population. The use of plant extracts as a control of rodent population could be a better option because they are environmentally friendly and locally available. Therefore, the current study evaluated the reproductive inhibition effects of *A. indica* and *R. communis* seed oils as a control method of rodent population.

1.3 Objectives

1.3.1 General objective

To evaluate the reproductive inhibition effects of plant extracts in the management of mice for improved food security and protection of household goods.

1.3.2 Specific objectives

i) To determine the chemical constituents of *A. indica* and *R. communis* seed oils.

ii) To determine the effects of *A. indica* and *R. communis* seed oils on oestrous cycle in mice.

iii) To determine the effects of *A. indica* and *R. communis* seed oils on mating success, fertility index, gestation length, litter size and body weight in mice.

iv) To evaluate the effects of *A. indica* and *R. communis* seed oils on embryo implantation in mice.

1.4 Hypotheses (Ho)

i) *Azadirachta indica* and *R. communis* seed oils have similar chemical constituents

ii) *Azadirachta indica* and *R. communis* seed oils have no effect on oestrous cycle in mice.

iii) *Azadirachta indica* and *R. communis* seed oils have no effect on mating success, fertility index, gestation length, litter size and body weight in mice.

iv) *Azadirachta indica* and *R. communis* seed oils have no effect on embryo implantation in mice.

1.5 Justification

Rodents cause more economic loss and human suffering than any other vertebrate pest. Controlling rodents using plant extracts which are readily available to small scale farmers can reduce field and stored crop losses and also damage to household items. Pesticidal plants degrade rapidly with negligible persistent ecological impacts and can thus provide

environmentally-benign pest control. Impacts of plant extracts on beneficial organisms and other non-target species are negligible compared to synthetic pesticides. Encouraging farmers to plant pesticidal plants could provide sustainable and environmentally-benign pest management control and boost their income. Most African countries rely on imported synthetic pesticides and generally are only involved in their re-packaging, marketing and distribution. This over–reliance on importation of pest management products could be corrected by developing cottage industries to process and package botanical rodenticides in rural areas. Considering that many African governments actually subsidize the use of imported synthetic pesticides, these subsidies could be more effectively redirected towards the development of local enterprise that improves pest management through use of pesticidal plants. Using botanical rodenticides could therefore, improve quality and quantity of food crops, nutritional value, higher market value, create jobs, increase business opportunities and enhance achievement of the BIG four agenda specifically food security and health.

CHAPTER TWO

LITERATURE REVIEW

2.1 Rodent species

Rodents are the largest group of mammals, constituting almost half of the class Mammalia's approximately 4,660 species. They are indigenous to every land area except Antarctica, New Zealand, and a few Arctic and oceanic islands. However, some species have been introduced even to those places through their association with humans (Keirn, 2011). The huge order Rodentia encompasses 27 separate families, including not only the "true" rats and mice (family Muridae), but also such diverse groups as porcupines, beavers, squirrels, marmots, pocket gophers, and chinchillas. Rodents in this study refer to the rats and mice since they are by far the most prevalent and important pests in homes and businesses worldwide due to their ability to adapt to the human environment. The rats are the brown rat *Rattus norvegicus*, also called the common or Norway rat, ship rat *Rattus rattus*, also called the black or roof rat and house mouse *Mus domesticus* (Bradford, 2015).

Despite their great diversity, all rodents share common features. They are characterized by upper and lower pairs of ever-growing rootless incisor teeth. The incisors have thick enamel layers on the front but not on the back; this causes them to retain their chisel shape as they are worn down (Lupo, 2017). House mice are active all year round and they invade homes or businesses at any time. They are small, slender rodents with a pelage that is grayish-brown on the dorsal surface and gray to buff on the ventral area. The adult house mouse size is 70 – 95 mm in length, with a tail around the same length and weighs between 12 – 30 g. It has relatively small feet and head with large eyes and ears which distinguish them from the "true" rats, *R. norvegicus* and *R. rattus* (Witmer and Aaron, 2017).

2.2 Rodent oogenesis and ovulation

Like in all mammals, the development of a normal ovary during foetal life is essential for the production and ovulation of oocytes in rodents (Sarraj and Drummond, 2012). Oogenesis is the differentiation of the ovum (egg cell) into a cell competent to further development when fertilized. The process of oogenesis begins with migratory primordial germ cells (PGCs) and results in an ovulated egg containing genetic material, protein, mRNA transcripts and organelles that are essential to the early embryo. Several studies reveal that some plant secondary compounds such as flavonoids, sterols, saponins and alkaloids disrupt meiosis

which compromise oogenesis and result in infertility (Koul *et al*., 1990; Sharma *et al*., 2013; Kaingu, 2016).

Ovulation is characterized by the rapture of the follicle wall and the release of cumulus-oocyte complex. Most mammals including rodents, however, have a periodic ovulation pattern, in which the female ovulates only at specific times of the year. This ovulatory time is called estrus. In these cases, environmental cues, most notably the amount and type of light during the day, stimulate the hypothalamus to release gonadotropin-releasing factor. This factor stimulates the pituitary to release its gonadotropins namely, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) which causes the follicle cells to proliferate and secrete oestrogen. The oestrogen enters neurons and evokes the pattern of mating behavior characteristic of the species. The gonadotropins also stimulate follicular growth and initiate ovulation. Thus, estrus and ovulation occur close together (Hill, 2017).

2.3 Life cycle of mice

Mice reach sexual maturity earlier than rats and produce more litters at a more frequent rate than rats. Maturity occurs between 5-8 weeks in mice and 7-10 weeks in rats (Lupo, 2017).The mean age of puberty in female rats and mice is based on the occurrence of vaginal opening (VO). The first oestrous cycle begins within approximately one week after vaginal opening and reoccurs regularly every 4 or 5 days for a variable proportion of the animal's lifespan (Falkner and Tanner, 2013). The female mice and rats, like most placental mammals, demonstrate intrinsic reproductive cyclicity characterized by regular occurrence of an oestrous cycle. During this cycle numerous well defined and sequential alterations in reproductive tract physiology, histology and cytology occur, initiated and regulated by the hypothalamic-pituitary-ovarian (HPO) axis (Hill, 2017).

The oestrous cycle consists of four stages, namely, proestrus, estrus, metestrus and diestrus. Diestrus is immediately followed by the proestrus phase of the next cycle (Russel, 2008). The oestrous cycle is polyestrous (cycle constantly throughout the year) with an estrus period of approximately 12 hours. Anoestrus is a period of reproductive quiescence between oestrous cycles that is not usually observed in healthy, cycling female mice. The oestrous stages can be identified using vaginal visual method and vaginal slide-smear technique. The proestrus phase is characterized by the development of antral follicles on the ovary and increase in the concentrations of oestrogen which leads to gaping vagina with reddish-pink moist tissues and nucleated epithelial cells. At the estrus phase, the female is receptive to the male which is a behavioural consequence of elevated concentration of oestrogen. The vagina

tissues are lighter pink, less moist with more pronounced striations and anucleated epithelial cells. The metestrus phase has low concentration of oestrogen following the LH surge and ovulation. It is characterized by pale dry vagina, leukocytes, cornified and nucleated epithelial cells in equal proportions. The diestrus phase is reffered to as quiescent phase which is characterized by nearly static state of the reproductive tract. It has small opening vagina with very moist bluish purple tissues and leukocytes within the vagina. These phases last an average of 4-5 days (Pritchett and Taft, 2007; Hamid and Zakaria, 2013; Paccola *et al*., 2013; Kaingu, 2016). Oestrous cyclicity only ceases during pseudopregnancy, pregnancy, and lactation, although a fertile postpartum estrus does occur within 24 hours after birth (Figure 1) (Hill, 2017). As a female approaches menopause at about 18 months of age, her cycle will become more irregular until it stops completely, and if she is bred during this time, the size of her litters will decrease as her fertility wanes (Falkner and Tanner, 2013).

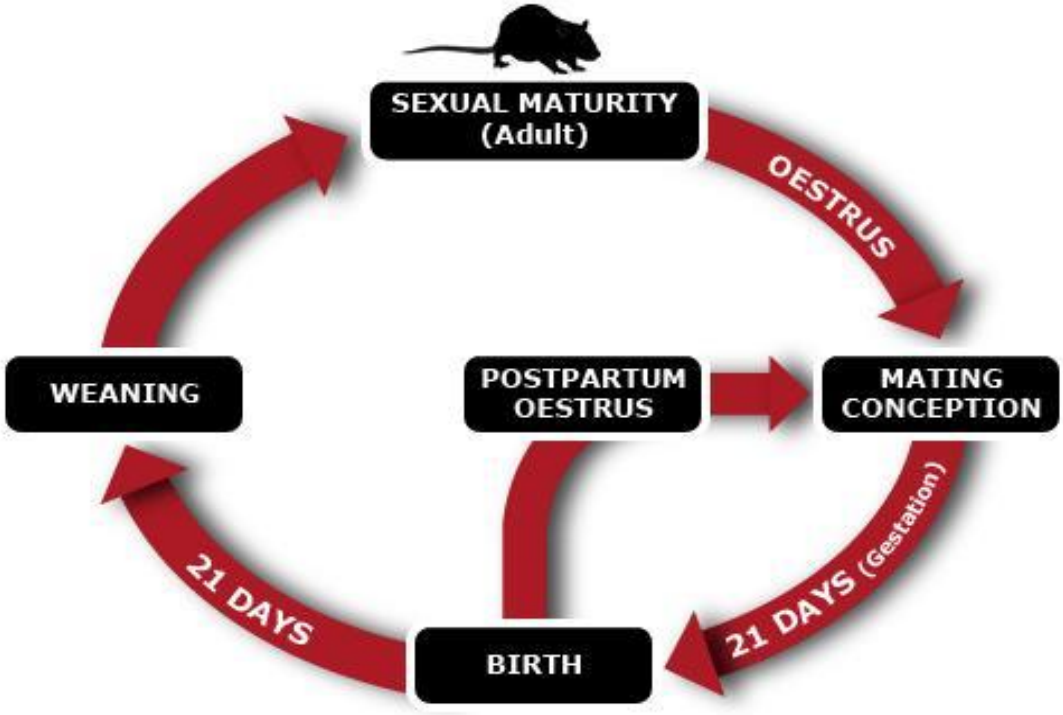


Figure 1: Life cycle of mice (*http://*007pestcontrol.co.uk)

2.4 Rodent mating, gestation period and litter size

Female mice produce a pheromone to attract the male. After sensing the pheromone, the male emits an ultrasonic mating call. Successful mating in the mouse shows presence of sperm in the vaginal smear or presence of a copulation plug. Copulation plug is a firm, white to off-white object, often completely filling the vagina of the female which provides a physical barrier to the re-mating of that female by another male, physical stimulation

necessary to sustain luteal formation and helps to retain the ejaculate in the uterus (Mangels *et al*., 2016). After mating, successful fertilization requires complex spermatozoa-ova interactions. Fertilization involves many sequential steps, beginning with the binding of spermatozoa to the zona pellucida, followed by the acrosome reaction and penetration of spermatozoa through the zona pellucida, and ending with the fusion of the sperm and egg pronuclei (Kaingu *et al*., 2017). Pregnancy begins with fertilization of the ovulated oocyte by the sperm. After fertilization, the egg undergoes time-dependent mitotic division while trying to reach blastocyct stage and the uterus for implantation. Uterine preparation for implantation is regulated by coordinated secretions and functions of ovarian sex steroids (Deb *et al*., 2006). Preparation for implantation begins with the preovulatory increase in oestrogen that initiates proliferation of uterine epithelial cells. Although many of these undergo apoptosis during metestrus, uterine stromal cells begin to proliferate by 3 days postmating as progesterone concentration increases. On day 4, as oestrogen concentrations rise again, uterine stromal cells are stimulated to proliferate and differentiate under the influence of high concentrations of oestrogen and progesterone setting the stage for implantation to occur (Pritchett and Taft, 2007).

Rattus norvegicus breed throughout the year, producing six litters of twelve young per year. Very hot or cold temperatures reduce breeding, but a female mouse breeds up to ten litters of five to six young. The *R. rattus* has smaller litters of up to eight young and have eight litters per year (Lupo, 2017). Their gestation period is normally 22 days, but varies from 21 to 23 and rarely to 26. A postpartum pregnancy lasts 28 days. Two weeks into the pregnancy, the mother's abdomen expands and the mammary glands enlarge. Mice usually live for nine to 12 months while rats live for 12 to 18 months. Controlling rodents becomes necessary due to their capability to breed at a very fast rate (Bradford, 2015).

2.5 Rodent habits and behaviour

Rattus norvegicus, *R. rattus* and *M. domesticus* are nocturnal; they have very poor eyesight, but have very strong sense of smell, taste, and hearing. They locate their food and mates through smell (Harding *et al*., 2004). They are omnivores but the *R. norvegicus* and *M. domesticus* prefer cereals, while *R. rattus* prefer fruits and foods with high moisture content. *Mus domesticus* seek food in the same places and when eating it ‘kibbles’ the grain by removing the outer husk to eat the white endosperm. Rats consume about 25g of food and drink more than 30ml of water per day while *M. domesticus* eat approximately 3-4g of food and drink 3ml of water per day (Belmain *et al*., 2015).

Rattus norvegicus use social learning in a wide range of situations especially in acquiring food preferences (Jozefowiez *et al*., 2009*)*. *Mus domesticus* builds its nest using any soft material or finely shredded paper in a hidden area near a food source. *Rattus norvegicus* lives mostly in burrows while the *R. rattus* nests in walls, attics, and trees. *Rattus norvegicus, R. rattus* and *M. domesticus* are highly intelligent rodents capable of remembering each path they take and work as a team (Foote and Crystal, 2007). One of the most important differences in behaviour between them is that *M. domesticus* are very curious and investigate anything new while *R. norvegicus* and *R. rattus* are very cautious and choose to avoid new things in their path until they have had time to get used to them. Signs of rodent presence include droppings, gnawing marks, tracks, runways and burrows. In buildings, a *M. domesticus* infestation is a nuisance because of the noise, odours and droppings. Its droppings are approximately 3-8mm in length, granular in shape, black in colour and are often found scattered randomly during an infestation (Lupo, 2017).

2.6 Control of rodent populations

The social, economic and environmental impact of pest rodent is high in developing countries. Chemical control is widely applied as the primary method for managing rodent pests, weeds and insect pests in agricultural production. Chemicals such as rodenticides provide effective short-term control of rodents, but are neither target specific nor cost-effective in the long term (Hinds *et al*., 2003).

Furthermore, synthetic rodenticides are often adulterated by dilution, mixed incorrectly and used beyond their expiry date (Stevenson *et al*., 2012). Many synthetic chemical compounds, especially zinc phosphide is a very dangerous chemical that have been used to control rodents. Due to their reckless use, these compounds are risky and dangerous and cause food, soil, air, surface and underground-water contamination. Moreover, residues of these pesticides may have harmful effects on beneficial organisms and also bring about an increase in the resistance of the pests to these pesticides, which ultimately result in their outbreak. Since there is a great concern about the pollution and toxic effects of synthetic pesticides to the environment all over the world, there is an effort to replace them with safer and less toxic rodenticides that are environmentally friendly such as biorodenticide (Morovati *et al.*, 2008). Biorodenticides can potentially reduce the risks resulting from the use of synthetic rodenticides (Mkenda *et al.*, 2015). Plant extracts used to protect crops against pests and diseases should be integrated in pest control management programmes of rodents.

Numerous herbs have been used historically to reduce fertility and modern scientific research has confirmed anti-fertility effects in at least some of the herbs tested. There are many different ways in which herbs impair fertility. Some herbs such as *Jatropha gossypifolia* leaf extract, *Acacia leucophloea*, *Aspilia africana*, *Croton menyharthii*, *Uvariodendron kirkii* and *Anethum graveolens* disrupt the oestrous cycle (Jain *et al*., 2012b; Sharma *et al*., 2013; Kaingu, 2016). *Piper bitle*, *Ocimum gratissium* and *Butea monosperma* show anti-oestrogenic activity (Umadevi *et al.*, 2013). *Ficus religiosa*, *Calotropis procera* and *Leonotis ocymifolia* have anti-implantation activity (Devi *et al.*, 2015) while *Melia azedarach*, *Coriandrum sativum* and *Cannabis sativa* have abortifacient effect (Sharma *et al*., 2013; Umadevi, *et al.*, 2013). The effects of most plant extracts are short term and reversible once treatment ceases and the specific chemicals responsible for the effects have not been isolated (Tran and Hinds, 2013).

The use of plant extracts in fertility control of mice populations could provide a number of advantages such as potentially improved ecological safety and biologically active substances that are easily metabolized. In addition, since some of the promising plant species are widely available, the production is cost effective. The fertility control approach can be applied in the field as part of an integrated rodent management strategy. When incorporated into integrated pest management programmes, pesticidal plants including *A. indica* and *R. communis* may reduce the need for synthetic rodenticides.

2.6.1 *Azadirachta indica* plant

Azadirachta indica A.Juss (Neem) tree is also called "Wonder Tree" in most parts of the world because of its numerous useful characteristics such as medicinal and pesticidal properties (Morovati *et al*., 2008). Neem tree is an evergreen or deciduous, fast growing plant that may reach a height of 25 meters (Figure 2) and thrives primarily in tropical climates that have annual rainfall of 400-800mm (Schmutterer, 1990). *Azadirachta indica*, a native of India, grows in nutrient-poor soils in arid habitats and has tremendous potential for human use. In recent years, neem has been grown in tropical and sub-tropical areas of Africa, America and Australia. In Kenya, it is found along the coast and other parts including Baringo regions (Anjarwalla *et al*., 2016). During the last 20 years, neem tree has been introduced in many countries mainly for afforestation and fuelwood production in dry areas, but also used as an avenue or shade tree and as producer of natural pesticides (Morovati *et al*., 2008).

Neem tree has been found to contain a vast array of biologically active compounds, which are chemically diverse with therapeutic potential. Its chemical constituents include steroids, triterpinoids, reducing sugars, alkaloids, phenolic compounds, flavonoids and tannins (Sharma *et al*., 2013). The active compounds available in the plant materials may vary considerably across geographical locations and depending on soil and growth limitations. Furthermore, different extracts from the same plant material using different solvents for extraction may give different responses (Brooker and Kleinig, 2006; Bett, 2015). Various derivatives of the tree have potential use in toiletries, pharmaceuticals, the manufacture of agricultural implements and furniture, cattle and poultry feeds, nitrification of soils for various agricultural crops, and pest control (Koul *et al*., 1990; Kumar *et al*., 2018).

The seed oil of *A.indica* is used in traditional medicine for its antidiabetic, spermicidal, antifertility, antibacterial, and wound healing properties (Roop *et al*., 2005; Quraishi *et al*., 2018; Shareef and Akhtar, 2018). *In vivo* studies showed that intravaginal application of neem oil prior to coitus can prevent pregnancy (Sinha *et al*., 1984) and also neem oil proved spermicidal against rhesus monkey and human spermatozoa *in vitro* (Asif, 2013). Antifertility effect of neem oil has also been studied and suggested to be a novel method of contraception (Upadhyay *et al*., 1990). The available evidence indicates that the ethnopharmacological properties come from its chemical constituents that possess low toxicity and rapid biodegradability (Koul *et al*., 1990).

Figure 2: *Azadirachta indica (*Neem) fruiting stage

2.6.2 *Ricinus communis* plant

Ricinus communis (Euphorbiaceae) commonly known as castor oil plant is a soft wood perennial shrub found throughout the tropics and warm temperate regions. It grows to 2-4 m, branched, completely glabrous, a glaucous green with yellow parts that are often reddish. The leaves are simple, alternate, downy and with a long petiole bearing shield-like epidermic

glands. The fruit is a 2-3 cm capsule composed of three prickly shells; each loculus contains a shiny seed about the size of a haricot bean, with a caruncle, covered with a very hard yellow/brown marbled integument (Figure 3) (Sharma *et al*., 2013).

Castor plant has been used to treat several ailments. Its leaves, roots, and seed oil are used in treatment of inflammation, digestion, joint pains, allergy, menstrual disorders, liver disorders, hypoglycemia, antioxidant, antimicrobial, cytotoxic activities and as a laxative (Lord *et al*., 2003; Jombo and Enenebeaku, 2008). In India and Africa the seeds are considered a contraceptive and a seed taken could prevent pregnancy for a year and also used to induce abortion. In China it is used to treat dystocia and retained placenta. In Malaysia the roots combined with other herbs in a concoction is given to women after delivery to help relieve postnatal pains and the heated leaves when applied over the breast promote lactation. It has been reported that the methanolic extract of the seed has significant anti-fertility property in adult rats (Raji *et al*., 2006). Methanolic extract of *R. communis* seeds given to albino mice at a dose level of 200 mg/kg body weight induced anti-implantation activities (Sani and Sule, 2007). It suppresses ovarian function and hence the number of ova released at estrus stage (McNeil *et al*., 2003).

Castor plant seeds contains about 30-35% oil which can be extracted by a variety of processes or combination of processes, such as hydrate presses, continuous cold presses and solvent extraction (Jimoh and Mohammed, 2006). Phytochemical screening indicated the presence of alkaloids, saponins, phenols, flavonoids and tannins (Sharma *et al*., 2013). The exact phytochemical causing the anti-implantation activity is yet to be established to make a suitable antifertility drug from *R. communis*.

Figure 3: *Ricinus communis* fruiting stage

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study site, experimental conditions and design

The study was carried out at Egerton University, Njoro Campus, Kenya at the Integrated Biotechnology Laboratory. Egerton University is situated at 0°22'11.0"S, 35°55'58.0"E with altitude of approximately 2,250 meters above sea level. The site receives annual rainfall of about 1000 mm and has a mean annual temperature of 17-22 ºC (Waithaka *et al*., 2017). Rearing of mice and bioassays was carried out in a ventilated room with a relative humidity of 45-55% and temperature of 27±2ºC under a photoperiod of 12 hours light and 12 hours darkness. The experimental design was a randomized block design (RBD) with six replicates of mice per treatment.

3.2 Laboratory mice rearing

Mature Swiss albino female mice weighing between 25-35 g were used in *in vivo* bioassays due to low cost of rearing, short generation time and availability (Harding *et al*., 2004). They were obtained from Kenya Medical Research Institute (KEMRI), Nairobi, Kenya. The mice were housed in cages made of wire mesh with husk beddings and fed on standard mice pellets from Unga Feed and Flour Mills Limited, Nakuru, Kenya. They were provided with water *ad libitum* and maintained under standard laboratory conditions as indicated above in Section 3.1. The animals were kept in this condition for two weeks to become acclimatized to the laboratory conditions.

The oestrous cycle of each mouse was monitored daily by Pipette smear technique to ascertain oestrous cyclicity prior to any experimental manipulation (Marcondes *et al.*, 2002). Only those with regular 4-5 day oestrous cycles were used for the experiment. The mice were assigned numbers with indelible ink for the purpose of identification. Their body weights were taken using electronic balance and recorded prior to the bioassays.

3.3 Collection of *A. indica* and *R. communis* seeds

The ripe drupes of *A. indica* and capsules of *R. communis* each weighing 5kg were collected from Marigat, Baringo County, Kenya. Marigat is situated at 0.47055°N, 35.9792°E and about 700 meters above the sea-level, most of which is rangelands. Temperatures in this zone are high (above 32°C), with low average rainfall (500-600 mm) (Mala *et al*., 2014). The samples of both plants were identified by a plant taxonomist and voucher specimens (number

Kip1 (*Azadirachta indica*) and Kip 2 (*Ricinus communis*) were deposited at the herbarium in the Department of Biological Sciences, Egerton University, Kenya.

3.4 Extraction of seed oils

3.4.1 *Azadirachta indica* oil

The ripe drupes of *A. indica* were washed using tap water and rinsed with distilled water to remove dirt and other impurities. They were dried in the open sun until the casing dried. Then they were further dried in an oven at 60°C for 7 hours to a constant weight in order to reduce moisture content. Oil from 500g of seeds was extracted using a cold pressing machine KickStart Ram press, Model VE 963 OP (Technology Exchange Lab, Inc. USA). The ram press uses a piston inside a cage to crush the seed and force out the oil (Bachmann, 2004). The seed oil obtained was stored in a sealed glass vial (Bijoux bottle) at 4 °C until use (Bett *et al.,* 2016).

3.4. 2 *Ricinus communis* oil

Extraction of *R. communis* seed oil was done using traditional method according to Oluwole *et al.* (2012). The process included shelling the pods, dehulling the seeds and winnowing to remove any unwanted materials, boiling the dehulled seeds in water at 90^{o}C for 10 minutes. The boiled seeds were spread in the sun to dry in order to reduce the moisture content. Dried seeds were ground using a mortar and pestle to form a paste. The paste was mixed with water in proportion of 500g of paste to 1 litre of water. The mixture was cooked and as the water evaporated, the oil started gushing out and settled on the surface. The oil was scooped using a spoon to another container and then dried by heating.

3.5 GC-MS analysis of chemical constituents of *A. indica* and *R. communis* seed oils

Analysis of the chemical constituents of *A. indica* and *R. communis* seed oils was carried out at the laboratories of the International Centre of Insect Ecology and Physiology (ICIPE), Nairobi. One milligram (1mg) of each of the sample was separately weighed and dissolved in 1 mL dichloromethane, dried using anhydrous Na_2SO_4 to make a stock solution (1 mg/mL) from which an experimental sample whose final concentration was 100 ng/µL was prepared. The samples were analyzed on an Agilent Gas Chromatograph HP-7890A (Agilent Technologies, Wilmington, USA) connected to an HP 5975 C (Agilent, Wilmington, USA) Mass Spectrometer in full scan mode. The GC equipment was fitted with a non-polar HP-5MS low bleed capillary column (30 m × 0.25 mm internal diameter; 0.25 µm film thickness) with 5%-phenyl methyl silicone as the stationary phase (J & W Scientific, Folsom, CA,

USA). One microliter of each sample was injected in the splitless mode, and helium was used as carrier gas at 1.0 ml min^{-1}. The oven temperature was maintained at 35°C for 5 min, increased to 280°C at 10°C min^{-1} and then held at this temperature for 5.5 min then to 285°C at 50°C min^{-1} for 14.9 minutes. Spectra were recorded at 70 eV in the electron impact (EI) ionization mode. Compound identities were determined using NIST'11, 08, 05, Adams and chemecol mass spectral databases (Adams, 2007; Bett *et al.*, 2016). The chemical constituents, retention time and retention index of *A. indica* and *R. communis* oils were recorded.

3.6 Effects of *A. indica* and *R. communis* on oestrous cycle

Mature female mice with regular oestrous cycle were randomly grouped into nine groups of six mice each. They received orally, seed oils of *A. indica* and *R. communis* at doses of 0.0, 0.2, 0.4, 0.6, 0.8ul/kg body weight for 14 days. Each dose was micropipetted using a micropipette into a syringe containing 0.1ml olive oil. Vaginal smears were collected using a glass pipette filled with 10 ml of normal saline (NaCl 0.9%) daily in the morning between 9-10 am for oestrous cycle cytological features. The vaginal fluid was placed on clean glass slides stained with Haematoxylin and counterstained with Eosin. The stained slides were viewed using a light microscope under low power (× 4) and high power (×10) objectives**.** The observed types of cells were used to determine the four stages of oestrous cycle (Diestrus, Proestrus, Estrus and Metestrus phases) (Marcondes *et al.*, 2002). The frequency of the oestrous phases of each mouse was recorded for 18 days and the mice were weighed on day 1, 7 and 14 using an electronic balance, Model N0091537 and also recorded.

3.7 Effects of *A. indica* and *R. communis* on mating success, fertility index, gestation length and litter size.

The female mice used in Section 3.6 above were also used for determination of mating success, fertility index, gestation length and litter size. A positive control group was added which received subcutaneous injection of 0.5ml of contraceptive (Depo provera). Male mice were obtained from Kenya Medical Research Institute (KEMRI), Nairobi, Kenya. They were tested for fertility prior to experiment by housing with female mice. Fertile male mice were introduced into female cages at a ratio of 1:2 male to female post- treatment. Mating success was determined by observing vaginal smear microscopically to check for presence of spermatozoa. The presence of spermatozoa was considered day one of gestation. The litter

size was counted at the end of gestation period. Fertility index was calculated using the formula (Kaingu, 2016) in Equation 1.

$$\text{Fertility Index } (\%) = \frac{TP}{TM} \times 100 \quad (1)$$

Where,

TP=Total number of females pregnant, and

TM= Total number of females mated

The mating success, fertility index, gestation period and litter size were recorded**.**

3.8 Effects of *A. indica* and *R. communis* on embryo implantation

Successfully copulated mice were selected randomly into nine groups of three mice each. They received doses as in Section 3.6 above for 6 days. On day 7 of pregnancy the mice were euthanized using 100% carbon dioxide at very-low–flow (10%) and low-flow (30%) volume displacement per minute (VD/min) rates (Hickman, 2019). Uteri of both sides were dissected out and assessment of implantation sites was carried out by counting the number of implantation sites in each uterine horn. Mean number of implantations was compared to the control. Mean number of implantations was calculated as total implantation sites divided by the number of pregnant mice in the group.

Anti-implantation activity (%) was calculated using the formula (Kaingu, 2016) in Equation 2.

$$\text{Anti-implantation activity } (\%) = \frac{Ic - It}{Ic} \times 100 \quad (2)$$

Where,

Ic = number of implantation sites in control group, and

It = number of implantation sites in test group.

The total number of embryos (implant) in both uterine horns gave an estimate of litter size. The number of implantations, mean implantation sites and anti-implantation activity were recorded.

3.9 Data analysis

Data on mating success was analyzed using *Chi*-Square Test while oestrous cycle, fertility index, gestation period, litter size and weekly body weight were analyzed using one-way analysis of variance (ANOVA) (SPSS, 2015). Data on percentage fertility and anti-

implantation were corrected for homogeneity of variances using arcsine-transformation before being subjected to one-way analysis of variance (ANOVA). The significance level adopted for all tests was 5% ($P<0.05$). Differences between treatment means was determined using Tukey's Studentized (HSD) test (SPSS, 2015). The data obtained was reported in tables and figures.

CHAPTER FOUR

RESULTS

4.1 Chemical constituents of *A. indica* and *R. communis* seed oils

Azadirachta indica and *R. communis* seeds each weighing 500g were extracted with percentage oil yield of 0.59% and 9.52% respectively. *A. indica* seed oil was golden yellow in colour while *R. communis* seed oil was colourless. The samples of *A. indica* and *R. communis* seed oils were subjected to Gas chromatography-mass spectrometry and the results obtained are indicated in Tables 1 & 2 and Figures 4 & 6.

From the results in Table 1, the most abundant compounds in the extract of *A. indica* when percent concentration was measured against retention index and retention time (min) were 2-hexyl-1-decanol (30.97%), Octadecanoic acid (29.69%), Oxalic acid, 6-ethyloct-3-yl ethyl ester (15.55%). Others were Octadec-9-enoic acid (9.92%), Oxalic acid, cyclobutyl tridecyl ester (5.46%), Methyl hexadecanoate (3.85%), n-Hexadecanoic acid (2.38%), Sulfurous acid, nonyl 2-propyl ester (1.41%) and 1- octadecene (0.8%).

Table 1: Retention time (min), retention index and mean (Mean ± SE, n=3) percent concentration of chemical constituents of seed oil obtained from *A. indica*

No.	Retention Time (min)	Compound name	Retention Index	% Concentration (Mean ± SE, n=3)
1	11.5227	Sulfurous acid, nonyl 2-propyl ester	998	1.41±1.00
2	16.7117	1- octadecene	1320	0.80±0.38
3	20.0696	Oxalic acid, cyclobutyl tridecyl ester	1579	5.46±0.38
4	20.3972	Oxalic acid, 6-ethyloct-3-yl ethyl ester	1606	15.55±7.26
5	20.7891	2-hexyl-1-decanol	1640	30.97±18.86
6	22.6436	Methyl hexadecanoate	1832	3.85±2.76
7	22.9946	n-Hexadecanoic acid	1865	2.38±1.52
8	24.6502	Octadec-9-enoic acid	2038	9.92±4.69
9	24.8491	Octadecanoic acid	2059	29.69±21.91

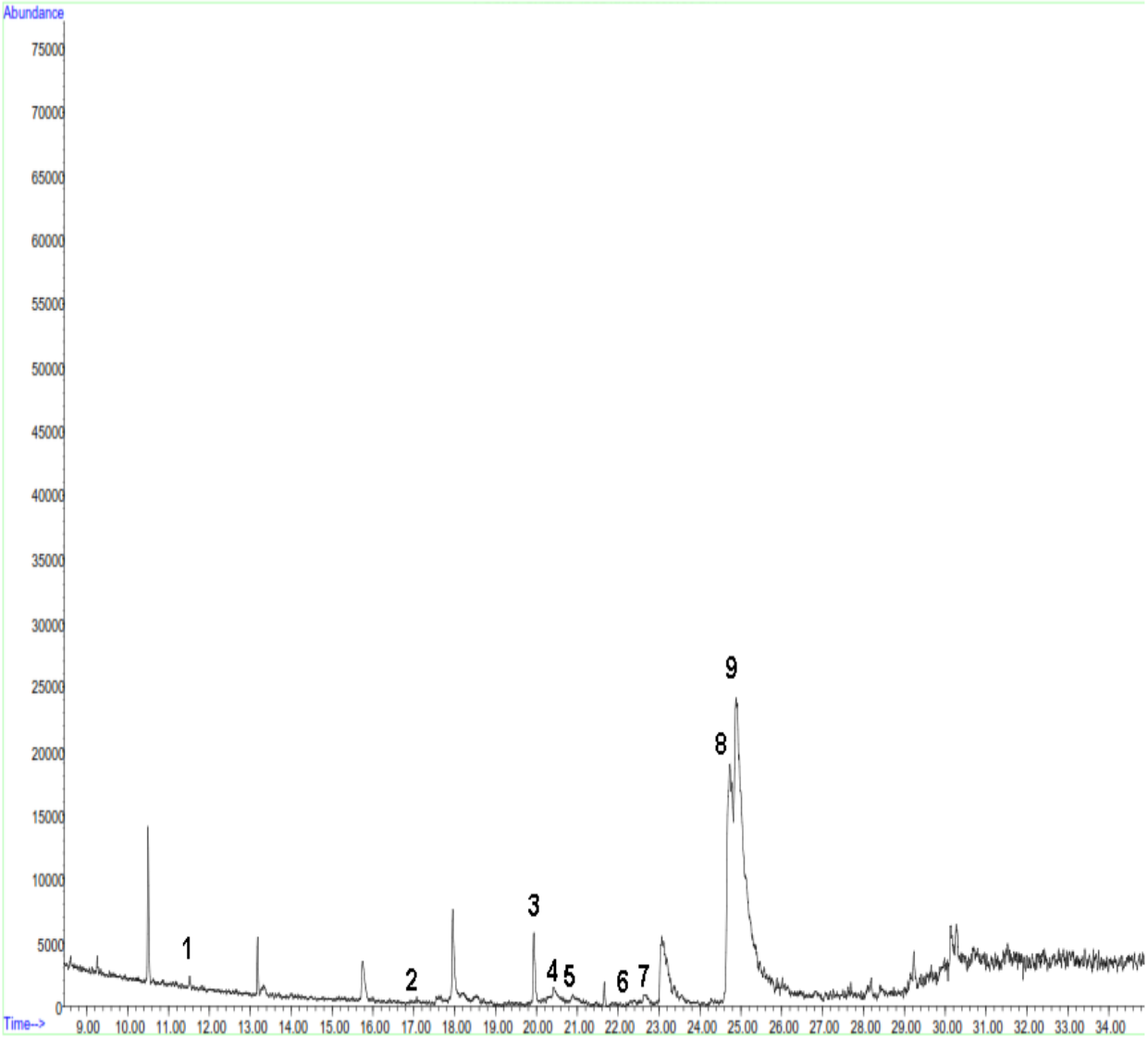


Figure 4: Chromatogram of the seed oil of *Azadirachta indica*. Peaks 1–9 show the components identified (Table 1)

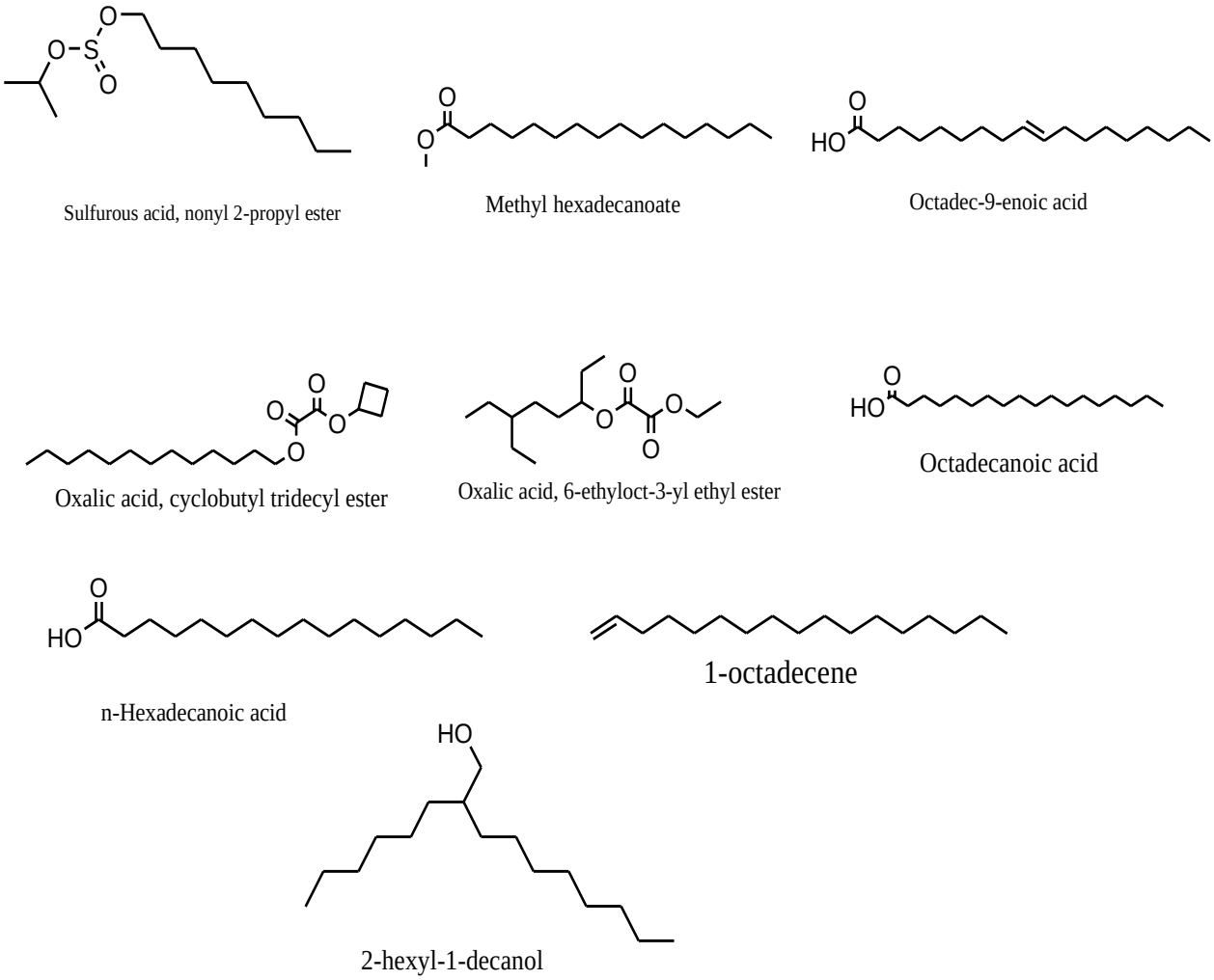


Figure 5: Structures of the nine compounds obtained in the seed oil of *A. indica*

From the results, *A. indica* seed oils were dominated by esters 44.4%, fatty acids (33.3%), alkanes and alkenes each at 11.1% (Figure 5).

In Table 2, the most abundant compounds in the extract of *R. communis* when percent concentration was measured against retention index and retention time (min) were (Z)- 6-Octadecenoic acid (37.33%), Ricinoleic acid (30.22%) and 13-Hexyloxacyclotridec-10-en-2-one (26.67%). Others were Tetradecane (3.73%) and 2-Ethylbutyric acid, 4-methylpent-2-yl ester (2.06%).

Table 2: Retention time (min), retention index and mean (Mean ± SE, n=3) percent concentration of chemical constituents of seed oil obtained from *R. communis*

No.	**Retention Time**	**Compound name**	**Retention Index**	**% Concentration (Mean ± SE, n=3)**
1	11.5169	Tetradecane	997	3.73±0.25
2	22.3043	2-Ethylbutyric acid, 4-methylpent-2-yl ester	1800	2.06±1.42
3	24.0301	13-Hexyloxacyclotridec-10-en-2-one	1971	26.67±1.40
4	24.7087	(Z)-6-Octadecenoic acid,	2044	37.33±12.64
5	26.4169	Ricinoleic acid	2231	30.22±12.37

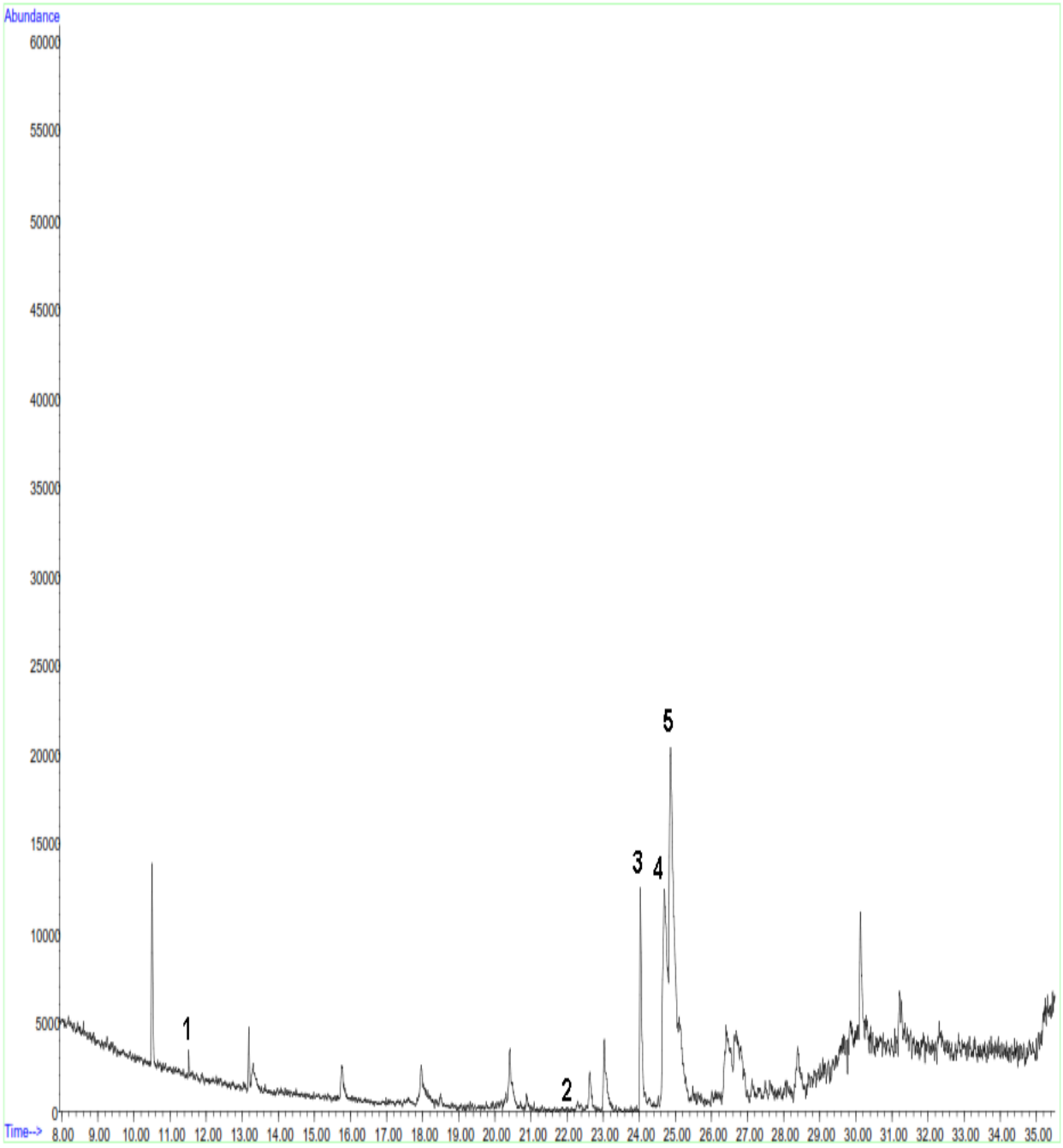


Figure 6: Chromatogram of the seed oil of *Ricinus communis*. Peaks 1–5 show the components identified (Table 2)

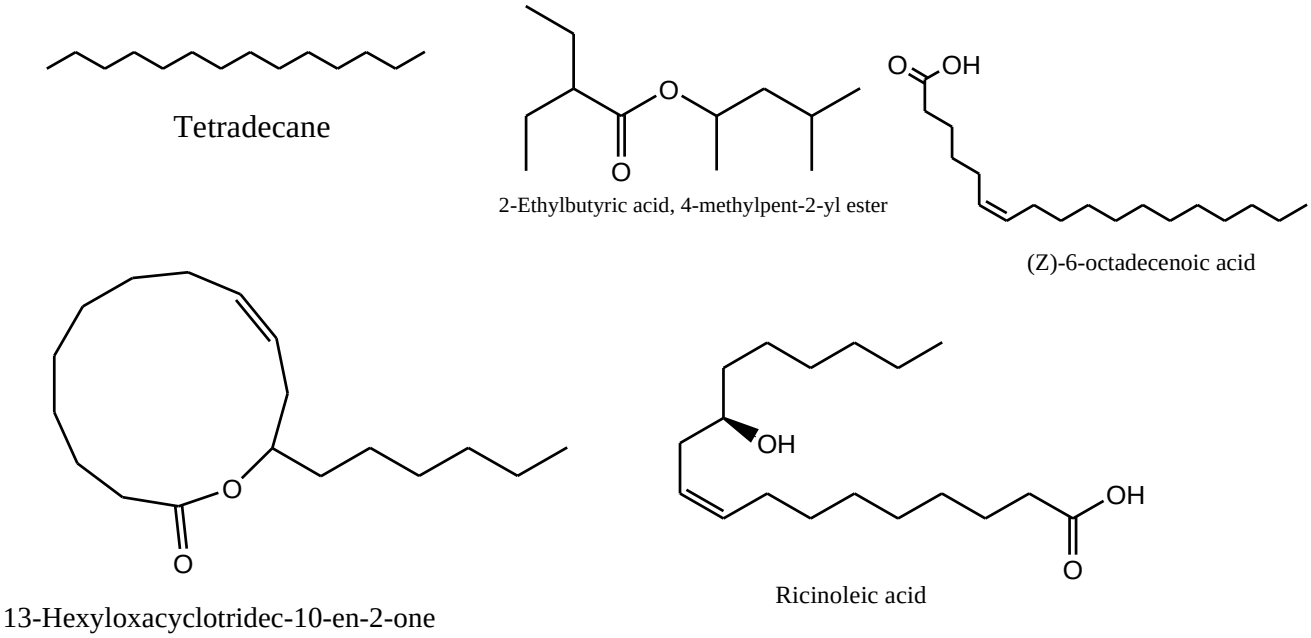


Figure 7: Structures of the five compounds found in the seed oil of *Ricinus communis*

Similarly, *R. communis* seed oils were dominated by esters and fatty acids each at 40 % and alkanes 20% like *A. indica* seed oils (Figure 7).

4.2 Frequency appearance of oestrous cycle phases in mice treated with *A. indica* and *R. communis* seed oils

A normal oestrous cycle in laboratory mouse lasts four to five days and consists of four phases namely; proestrus, estrus, metestrus and diestrus (Figures 8-11). The appearance of oestrous phases at control level and different doses of *A. indica* and *R. communis* were observed from the vaginal smear over a period of 18 days.

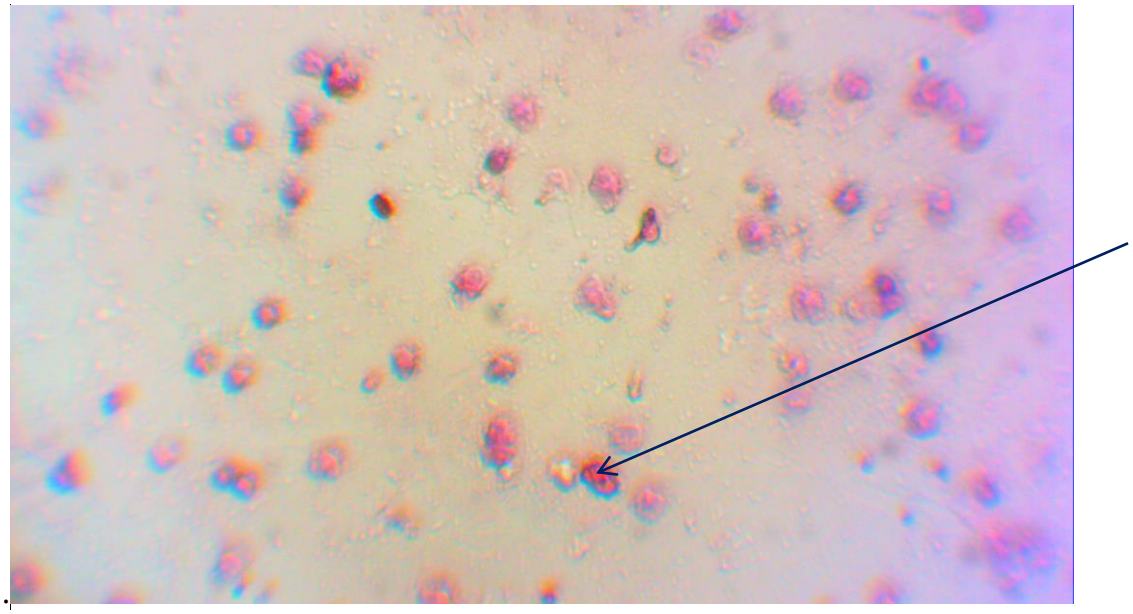


Figure 8: Proestrus phase with nucleated epithelial cells as indicated by the arrow (X400)

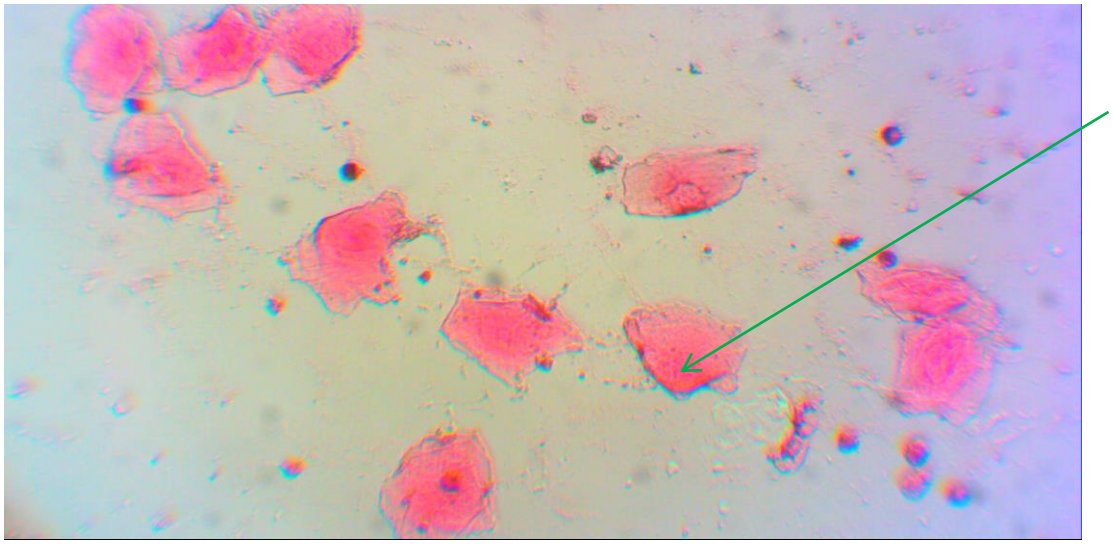


Figure 9: Estrus phase with anucleated epithelial cells as indicated by the arrow (X400)

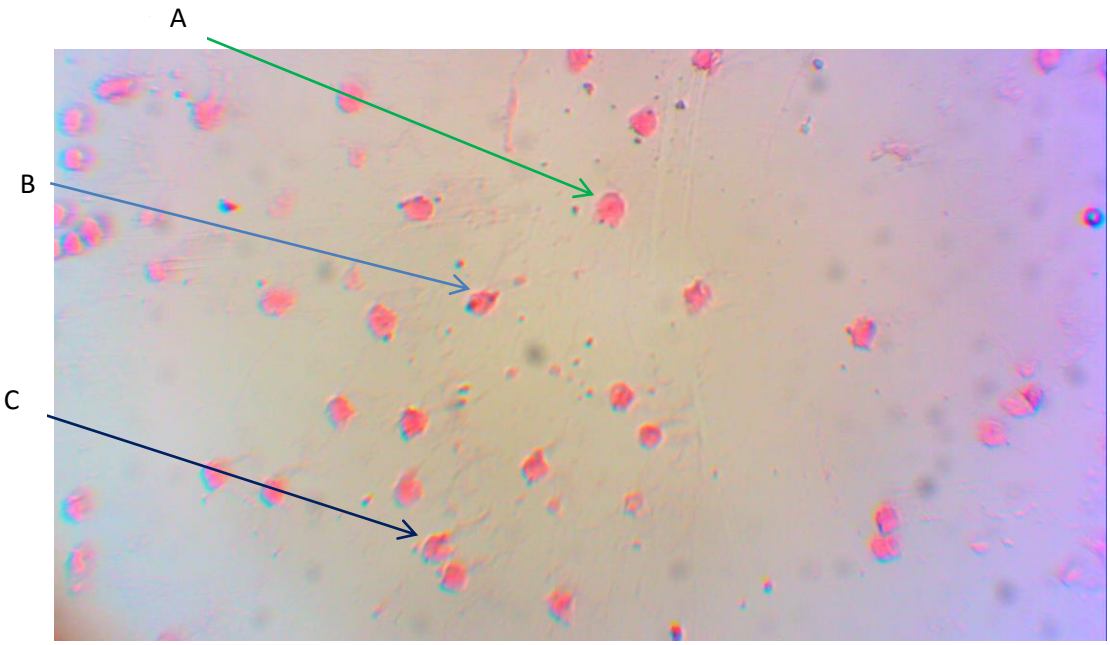


Figure 10: Metestrus phase with cornified epithelial cells (A), leukocytes (B) and nucleated epithelial cells (C) (X400)

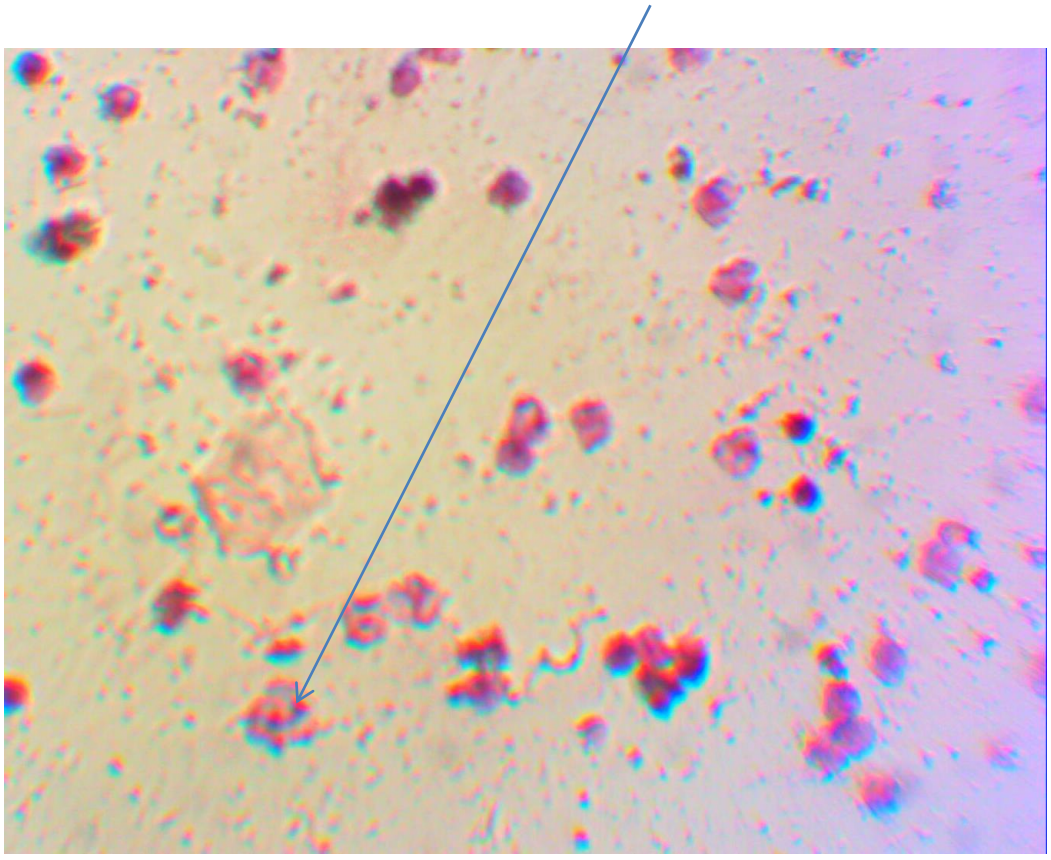


Figure 11: Diestrus phase with leukocytes as indicated by the arrow (X400)

From Table 3, the mean frequency appearance of the oestrous phases is almost evenly distributed in the negative control and the oestrous cycle was normal compared to the treatments of *A. indica* which had disrupted oestrous cycle. In all the doses of treatment, there

was an increase in the frequency appearance of metestrus and diestrus phases. Subsequently there was a significant decrease in estrus and proestrus phases compared to the negative control (ANOVA: $F_{(11,84)} = 35.147$, $P < 0.05$) (Appendix 1). The frequency appearance of metestrus and diestrus phases increased while estrus and proestrus phases reduced with increase in dosage.

Table 3: Mean (Mean ± SE, n=6) frequency appearance of different oestrous cycle phases of mice treated with *A. indica* seed oil

	Concentrations				
Phases	Negative control	0.2ul	0.4ul	0.6ul	0.8ul
Proestrus	4.50 ±0.22	2.83±0.30[b]	2.17± 0.47 [b]	1.83± 0.30 [b]	1.67 ±0.42 [b]
Estrus	4.50± 0.22	2.50± 0.22 [b]	2.50 ±0.56 [b]	2.33± 0.33 [b]	2.00 ±0.58 [b]
Metestrus	4.17 ±0.40	5.83 ±0.30 [b]	5.33± 0.72 [b]	5.33± 0.42 [b]	5.17± 0.40[a]
Diestrus	4.83± 0.48	6.83± 0.30 [b]	8.00 ±0.68 [b]	8.50± 0.56 [b]	9.17 ±0.54 [b]

[a] insignificant response ($P > 0.05$)

[b] significant response ($P < 0.05$)

The effect of *R. communis* on the oestrous phases increased the appearance of diestrus phase and reduced proestrus and estrus appearance with less effect in the metestrus in all the doses which was significantly different compared to the negative control (ANOVA: $F_{(13,82)} = 32.985$, $P < 0.05$) (Appendix 2). Diestrus phase appearance increased and proestrus and estrus appearance reduced with increase in dosage (Table 4).

Table 4: Mean (Mean ± SE, n=6) frequency appearance of different oestrous cycle phases of mice treated with *R. communis* seed oil

	Concentrations				
Phases	Negative control	0.2ul	0.4ul	0.6ul	0.8ul
Proestrus	4.50 ± 0.22	2.67 ± 0.21 [b]	2.17 ± 0.31 [b]	2.17± 0.17 [b]	1.33± 0.42 [b]
Estrus	4.50 ± 0.22	2.00 ± 0.26 [b]	2.17 ± 0.40 [b]	1.50 ±0.22 [b]	1.33 ± 0.42 [b]
Metestrus	4.17 ± 0.40	4.83 ± 0.31[a]	4.50 ± 0.67 [a]	5.00 ± 0.63 [a]	5.50 ± 0.43 [b]
Diestrus	4.83± 0.48	8.50 ± 0.34 [b]	9.17± 0.75 [b]	9.33± 0.33 [b]	9.83± 0.91 [b]

[a] insignificant response (($P > 0.05$)

[b] significant response ($P < 0.05$)

The results of both treatments (*R. communis* and *A. indica*) showed disruption of the oestrous cycle and were not significant (ANOVA: $F_{(1,116)} = 0.846$, $P > 0.05$) (Figure 12; Appendix 3).

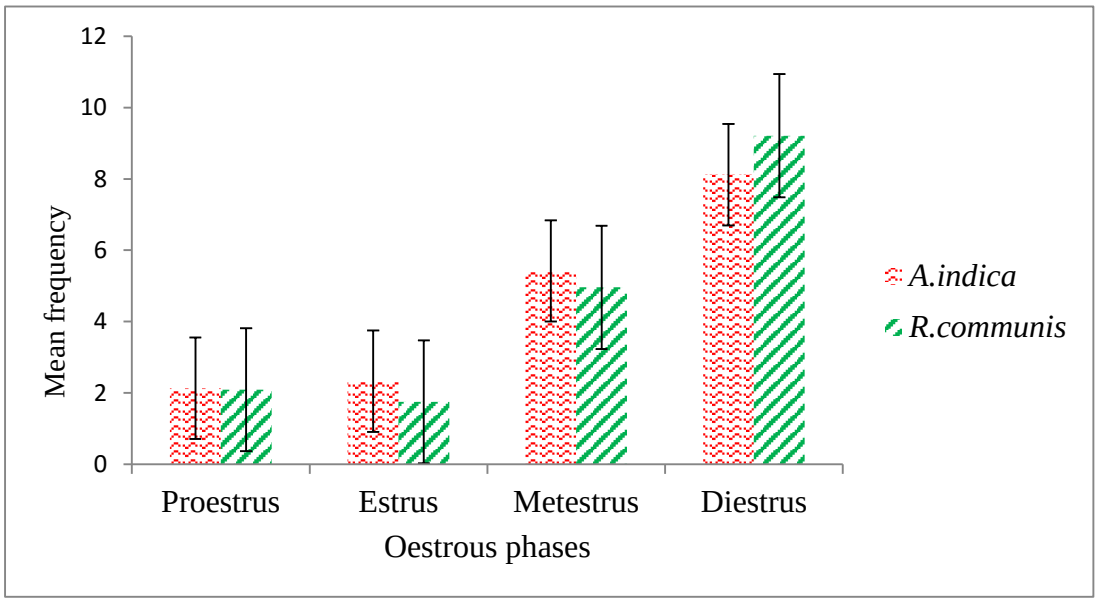


Figure 12: Mean (Mean ± SE, n=24) frequency appearance of each oestrous phase in all the doses of *R. communis* and *A. indica* over a period of 18 days

Both plants caused no significant effect (ANOVA: $F_{(77, 66)} = 1.243$, $P < 0.05$) (Appendix 4) on body weight of the mice throughout the study period (Table 5).

Table 5: Live body weights (g) on day1, 7 and 14 (Mean ± SE, n=6) of mice treated with *A. indica* and *R. communis* seed oils.

	Day 1	Day 7	Day 14
Control			
Negative untreated	31.87±2.00	32.05±1.79	31.78±2.03
Positive (DP)	27.20± 1.49	27.60±2.50	27.15± 2.10
A. indica			
0.2ul	29.30±2.63	29.10±2.52	28.73±2.47
0.4ul	29.90±2.93	29.55±2.94	29.33±2.96
0.6ul	30.32±3.31	30.01±3.31	29.45±3.10
0.8ul	28.10±2.94	27.37±2.73	26.73±2.91
R. communis			
0.2ul	29.43±2.94	29.23±2.79	28.87±2.77
0.4ul	30.13±2.08	30.18±2.21	29.87±1.88
0.6ul	29.20±3.53	28.73±3.79	28.25±3.79
0.8ul	27.75±1.52	26.95±1.32	26.40±1.31

4.3 Mating success in mice post-treatment with *A. indica* and *R. communis* seed oils

Mating success was determined the next day after the introduction of the male. Successfully and unsuccessfully mated mice were recorded as 'YES' or 'NO' respectively within one cycle (Table 6). The effect of *A. indica* and *R. communis* on mating showed a dose dependent response. Data showed that at a low dose of 0.2ul in both plants they had less effect on mating success and the difference was not significant (χ^2, df = 2, $P > 0.05$) (Appendix 5) but as the dose increased from 0.4ul to 0.8ul the mating success was reduced and was significant (χ^2, df= 2, $P < 0.05$) (Appendix 6) compared to the negative control. Higher doses of *A. indica* and *R. communis* caused 67% and 83% reduction in mating success, respectively.

Table 6: The number of mated (YES), unmated mice (NO) and mating success (%) in mice post-treatment with *A. indica* and *R. communis* oil concentrations (ul/kg)

	Mating		
	YES	**NO**	**Success (%)**
Control			
Negative untreated	6	0	100
Positive (DP)	NIL	NIL	NIL
A. indica			
0.2	4[a]	2	67
0.4	3[b]	3	50
0.6	2[b]	4	33
0.8	2[b]	4	33
R. communis			
0.2	4[a]	2	67
0.4	3[b]	3	50
0.6	3[b]	3	50
0.8	1[b]	5	17

Pearson Chi-Square (df= 2, $P < 0.05$).

[a] insignificant response ($P>0.05$)

[b] significant response ($P<0.05$)

4.4 Fertility index, gestation period and number of litters of mice post-treatment with *A. indica* and *R. communis* seed oils

At a concentration of 0.2ul, both *A. indica* and *R. communis* had less effect on fertility index, gestation period and number of litters compared to the negative control. At 0.4ul *A. indica* and *R. communis* at 0.4ul and 0.6ul, there was a significant *(*ANOVA: $F_{(3, 4)} =18.667$; $P<0.05$) (Appendix 7) reduction in fertility index at 50%, at 0.6ul *A. indica* at 33%, 0.8ul *A. indica* and 0.8ul *R.communis* both at 17% (Figure 13). At 0.4ul and 0.6ul *A. indica* there was a significantly ($P < 0.05$) (ANOVA: $F_{(3,44)} =206.888$, $P < 0.05$) (Appendix 8) prolonged gestation period (24-25 days) with reduced litter size compared to the negative control.

Similarly at a dose of 0.4ul *R. communis* had the same effect on gestation period as in 0.2ul *R. communis* with significant (ANOVA: $F_{(3, 4)} =14928.210$; $P < 0.05$) (Appendix 9)

reduction of litter size. At 0.8ul *A. indica*, and *R. communis* at 0.6ul and 0.8ul, mice were mated but none of them littered (Table 7).

Table 7: Fertility index (%), gestation period (Mean ± SE, n=6) and number of litters (Mean ± SE, n=6) of mice post-treatment with *A. indica* and *R. communis* seed oils.

	Fertility index (%)	**Gestation period(days)**	**No. of litters**
Control			
Negative untreated	100	22.33±0.21	9.67±0.88
Positive (DP)	NIL	NIL	NIL
A. indica			
0.2ul	67	23.50±0.29	3.75±0.48
0.4ul	50	24.33±0.33	3.33±0.33
0.6ul	33	25.00±0.00	2.00±0.00
0.8ul	17	NIL	NIL
R. communis			
0.2ul	67	23.25± 0.25	3.00±0.41
0.4ul	50	23.33±0.33	2.67±0.88
0.6ul	50	NIL	NIL
0.8ul	17	NIL	NIL

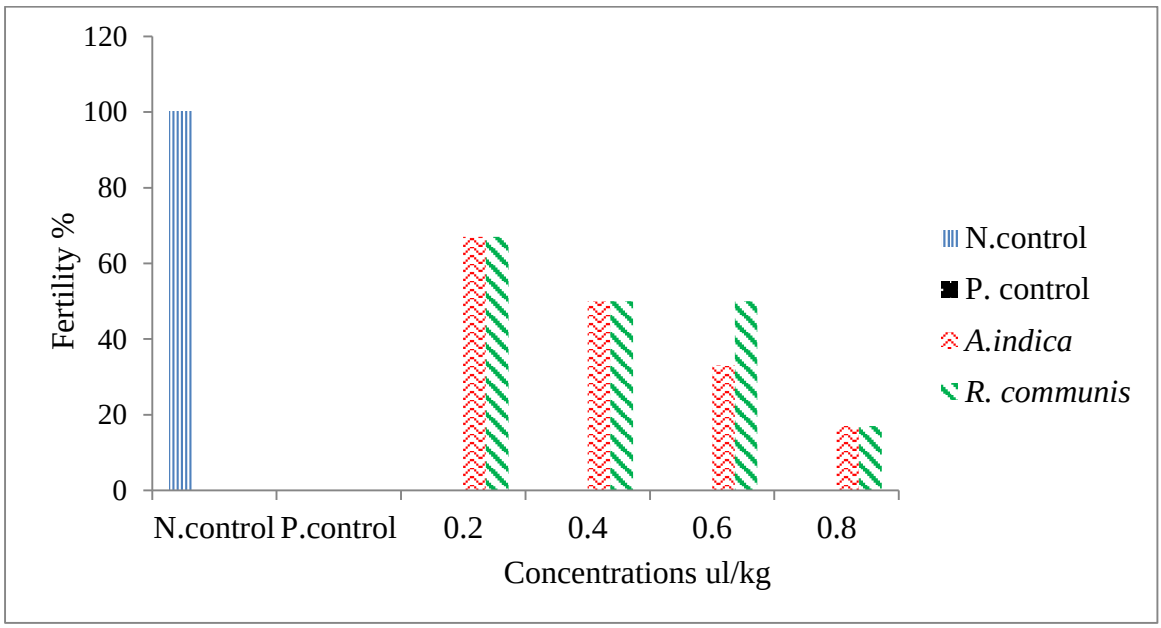
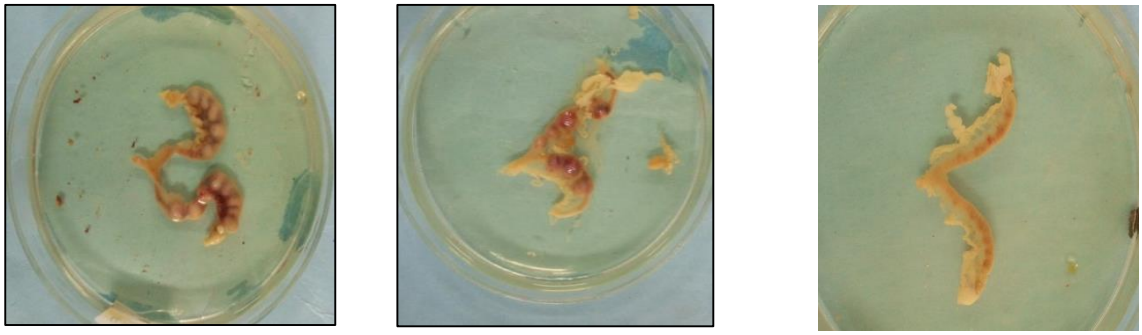


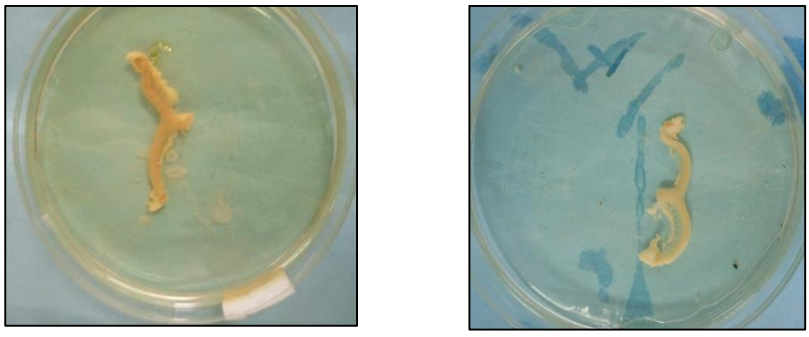
Figure 13: Fertility index of mice post-treatment with *A. indica* and *R. communis* seed oils compared to controls (N=negative; P=positive)

4.5 Embryo implantation of mice post-treatment with *A. indica* and *R. communis* seed oils

Implantation sites in the uterine horns of mice at control and different doses of *A. indica* and *R. communis* are shown in Figure 14 and 15. In the control group, all the mice showed implantation sites at mean of 10.33 while those administered with *A. indica* and *R. communis* had significant (ANOVA: $F_{(7,16)} = 4.030$; $P < 0.05$) (Appendix 10) decrease in implantation sites. At doses of 0.2ul both *A. indica* and *R. communis* had mean implantation of 4.67 and 8 respectively whereas at 0.4ul both *A. indica* and *R. communis* had 2.3 and 4.3 respectively. Both plants at doses of 0.6ul and 0.8ul had no implantation sites (Table 8).

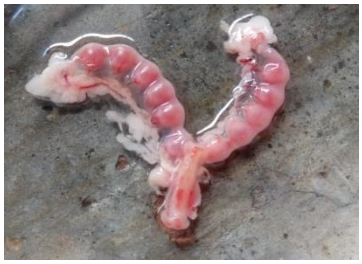


Control (13) 0.2ul (6) 0.4ul (5)



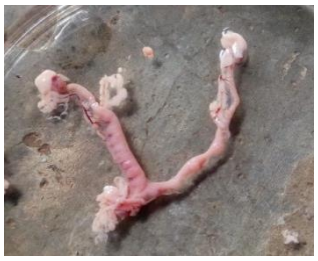
0.6ul (0) 0.8ul (0)

Figure 14: Embryo implantation of mice treated with *A. indica* at magnification X15 (Number in brackets denotes number of implantation sites).



Control (11)

0.2ul (9)



0.4ul (7)

0.6ul (0)

0.8ul (0)

Figure 15: Embryo implantation of mice treated with *R. communis* at magnification X15

(Number in brackets denotes number of implantation sites)

Table 8: Number of implantation and mean implantation (Mean ± SE, n=3) of mice post treatment with A. indica and R. communis seed oils.

	No. of implantation	Mean implantation
Negative control (untreated)	11,11,9	10.33±0.67
A. indica		
0.2ul	0,8,6	4.67±2.40
0.4ul	2,5,0	2.33±1.45
0.6ul	0,0,0	0
0.8ul	0,0,0	0
R. communis		
0.2ul	9,8,7	8.00±0.58
0.4ul	2,4,7	4.33±1.45
0.6ul	0,0,0	0
0.8ul	0,0,0	0

The anti-implantation activity of *A. indica* and *R. communis* at a dose 0.2ul was 42% and 22% respectively, while *A. indica* and *R. communis* at 0.4ul was 77% and 58%, respectively. Both treatments at 0.6ul and 0.8ul showed 100% anti-implantation activity (Figure 16). *Azadirachta indica* and *R. communis* showed that the anti-implantation activity was a dose dependent response compared to the negative control.

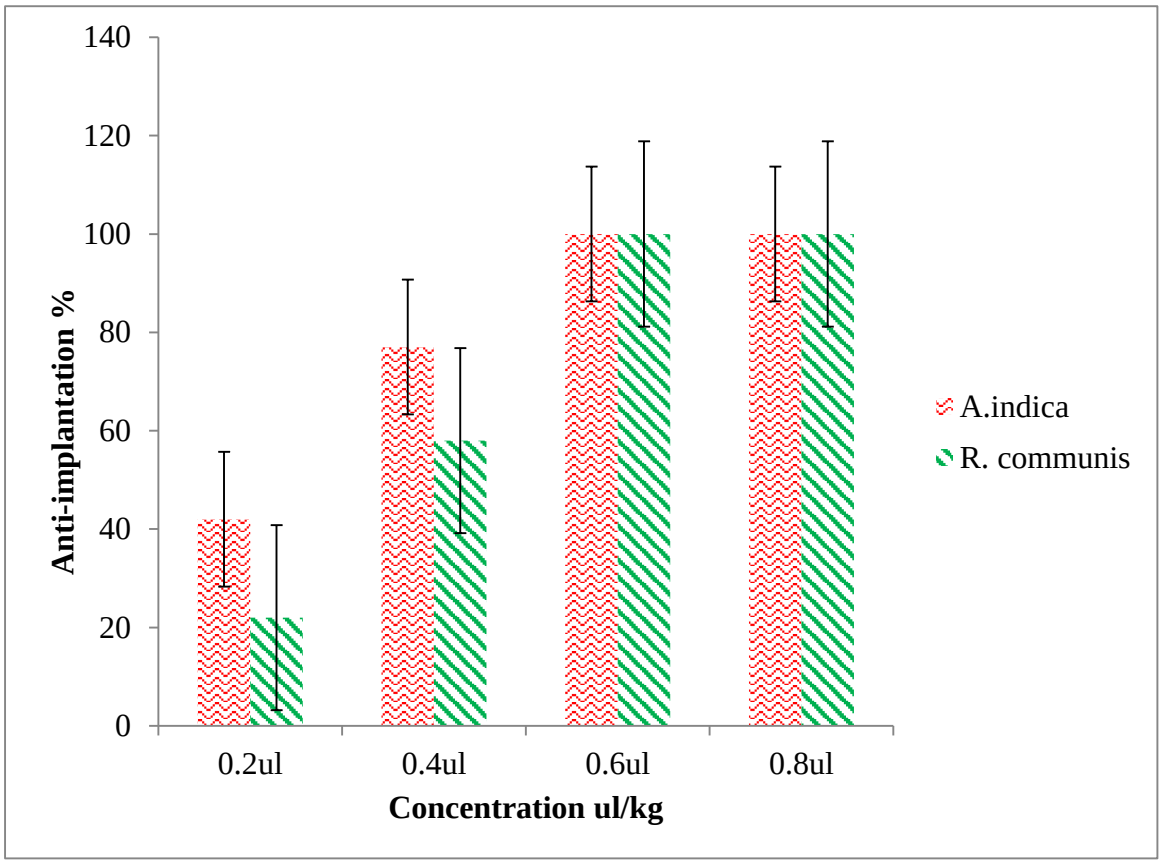


Figure 16: Anti-implantation percent of mice post-treatment with *A. indica* and *R. communis* seed oils.

CHAPTER FIVE

DISCUSSION

5.1 Chemical constituents of *A. indica* and *R. communis* seed oils

Azadirachta indica and *R. communis* seed oils can be extracted by a variety of processes or combination of processes, such as mechanical (hot or cold press), traditional method (boiling) and solvent extraction (Bachmann, 2004; Jimoh and Mohammed, 2006; Oluwole *et al.,* 2012). Seed oils of *A. indica* and *R. communis* were extracted from mature dried seeds by cold pressing and boiling respectively and chemical compositions were determined using Gas Chromatography (GC)-Mass Spectrometry (MS). The GC-MS analysis of *A. indica* and *R. communis* seed oils both revealed the presence of methyl and ethyl esters, cyclic esters, alkanes and alkenes, unsaturated and saturated fatty acids. It is worthwhile to note that the phytochemical constituents of *A. indica* and *R. communis* seed oils from Marigat, Baringo County, Kenya are being reported for the first time.

In similar studies, Jena and Gupta (2012) reported the phytochemical constituents of *R. communis* seed oil of Indian origin to contain fatty acids and esters of n-hexadecanoic acid (1.2%), Octadecanoic acids (0.7%), Octadec-9-enoic acid (3.2%), 9,12-octadecadienoic acid (3.4%), Eicosanoic acid (0.3%), Ricinoleic acid (89.4%), 12- octadecadienoic acid (0.2%) and Methyl ester. Likewise, Martín *et al*. (2010) reported predominant fatty acids of *R. communis* and *A. indica* seed oils of Cuban origin to be Ricinoleic acid (86%) and Octadec-9-enoic acid (44.5%), respectively, which were higher than those reported in the present study. Martín *et al*., (2010) further reported that other minor fatty acids in *R. communis* were n-hexadecanoic acid (1.3%), Octadecanoic acids (1.2%), Octadec-9-enoic acid (3.6%), 9,12-octadecadienoic acid (5.5%) and 12- octadecadienoic acid (0.5%) while *A. indica* seed oils were n-hexadecanoic acid (18,1%), Octadecanoic acids (18.1%), 9,12-octadecadienoic acid (18.3%), Eicosanoic acid (0.8%) and 12- octadecadienoic acid (3.4%).

Ricinus communis and *A. indica* seed oils from the Nigerian origin revealed varying percentage concentration of their chemical constituents. Omowanle *et al*. (2018) reported the constituent of *R. communis* oil to be Ricinoleic acid (74.42%), Hexadecanoic acid (9.25%), 9,12-Octadecadienoic acid (6.55%), Octadecanoic acid (7.60%) and 9-Octadecenoic acid (2.18%) while *A. indica* oil was dominated by 9,12-Octadecadienoic acid (45.56%), Hexadecanoic acid (27.81%) and Octadecanoic acid (19.69%). Similarly, Yusuf *et al*., (2015) findings on *R. communis* oil had presence of Ricinoleic acid (74.10%), 9,12-Octadecadienoic acid (10.32%), 9-Octadecenoic acid (7.55%), Octadecanoic acid (2.81%) and Hexadecanoic acid (2.59%) by cold pressing method of extraction. Djenontin *et al*.

(2012) also reported that *A. indica* oil obtained from Benin had similar fatty acids including Octadec-9-enoic acid (43.5%), Hexadecanoic acid (17.8%) and Octadecanoic acid (17.4%) apart from 9, 12-Octadecadienoic acid (18.7%) which was not found in the current study. In the above findings the presence of (Z)-6-Octadecenoic acid and Ricinoleic acid in *R.communis* oil and Octadecanoic acid, Hexadecanoic acid and Octadec-9-enoic acid in *A. indica* oil are consistent with the results of the current study but differ in percent concentration. The most interesting features are the prominent concentration of 6-Octadecenoic acid (37.33%) of *R. communis* oil and Octadecanoic acid (29.69%) of *A. indica* in the current study which have not been reported elsewhere (Martín *et al*., 2010; Jena and Gupta, 2012; Yusuf *et al*., 2015; Omowanle *et al*., 2018).

The active compounds available in the plant materials may vary considerably across geographical locations and depending on soil and growth limitations which directly influence the metabolism of the plant and the exposure to different biotic components (Brooker and Kleinig, 2006; Chéraif *et al*., 2007; Tran and Hinds, 2013; Bett, 2015). Furthermore, different extracts from the same plant material using different equipment and solvents for extraction may give different responses. For instance, Suryawanshi (2011) reported on the isolation of active compounds in *A. indica* seed oil through steam distillation method and found to be a mixture of 28 components containing sulphur compounds and esters of fatty acids and solvent extraction of *A. indica* seed oil to have triterpenoids such as azadirachtin, nimbin, nimbidin, salannin. Other studies on solvent extraction of *A. indica* seed oil revealed presence of steroids, triterpinoids, reducing sugars, alkaloids, phenolic compounds, flavonoids and tannins (Sharma *et al*. 2013; Azamthulla *et al*., 2015; Tesfaye and Tefera, 2017) while *R. communis* seed oil are alkaloids, saponins, phenols, flavonoids, ricin, ricinine, lectin and tannins (Sharma *et al*. 2013; Azamthulla *et al*., 2015;) which are in contrast to the current seed oils composition. Mechanical extraction of oil was the preferred choice in the current study to avoid solvent residues which may pose health hazards to the rodents and also produce quality oil which is lighter and has milder oduor with majority of phytoconstituents being intact.

Traditional extraction of *R.communis* oil produces pure oil free from the toxic ricin protein. These qualities ensure immediate end-use application of the oil even without refining. Moreover, mechanical extraction was preferred because it is affordable and easily available to small scale farmers compared to use of expensive solvent extraction.

Octadecanoic acid and Tetradecane which was present in *A. indica* seed oil and *R. cummunis* seed oil respectively are reported to have ovary inhibition in insects (Mumoki *et*

al., 2018). *Azadirachta indica* and *R. communis* seed oils have been reported to possess insecticidal activity against *Callosobruchus maculatus* (Lale and Abdulrahman, 1999; Babarinde *et al*., 2016; Hussein *et al*., 2016). Presence of azadirachtin compound in solvent extraction of *A. indica* seed oil was reported to have antifeedant, growth disrupting, insect repellent and ovicidal activity against a variety of insect pests. Also act as anti-fungal and anti-viral (Campos *et al*. 2016; Tesfaye and Tefera, 2017; Yanar, 2019) which was not detected using the current method of extraction. However, insecticidal activity of *R. cummunis* seed is attributed to its major components of protein ricins and alkaloid ricinine which is not present in the current *R. cummunis* seed oil (Hussein *et al*., 2016).

The chemical constituents found in *A. indica* and *R. communis* seed oils in the current study can be applied in different sectors. For example, the presence of Ricinoleic acid in *R. communis* oil makes it unique among vegetable oils as it remains the only commercial source of a hydroxylated fatty acid (Uzoh and Nwabanne, 2016). Removal of hydroxyl group in Ricinoleic acid can be used in the production of alkyd resins for surface coating and biofuel production (Martín *et al*., 2010; Waidee *et al*., 2018). Other fatty acids in the present *A. indica* are Octadecanoic acid and Hexadecanoic acid which are known to be used in the production of detergents, soaps, and cosmetics such as shampoos and shaving cream products (Gunstone, 2004; Mak-Mensah and Firempong, 2011; Warra, 2015).

In therapeutic applications, monounsaturated fatty acid (MUFA) plays an important role in human health and diseases. According to several studies, presence of Octadecenoic acid in the oil composition which was found in both *A. indica* and *R. communis* in the current study can be used in treatment and prevention of cardiovascular and autoimmune diseases, wound injury, immunomodulation, inhibiting platelet aggregation, cancer, prevents endoplasmic reticulum stress, lipoapoptosis and insulin resistance in hepatocytes, decreasing serum low density lipoproteins cholesterol, reducing Thromboxane A_2 (TXA_2) secretion and drug absorption (Sales-Campos *et al*., 2013; Karacor and Cam, 2015; Pardo *et al*., 2015). Warra (2015) reported Octadecenoic acid to be used as pharmaceutical solvent and as moisturizer. In conclusion, the bioactive compounds in *A. indica* and *R. communis* seed oils in the current study can be applied in the agricultural, cosmetics, industrial, reproduction inhibition and pharmaceutical sectors.

5.2 Frequency appearance of oestrous cycle phases in mice treated with *A. indica* and *R. communis* seed oils

Oral administration of *A. indica* and *R. communis* seed oils at concentration (0.2, 0.4, 0.6 and 0.8ul/kg body weight) showed a dose dependent increase in the duration of diestrus and metestrus phases and subsequent reduction of proestrus and estrus phases, causing disruption of the oestrous cycle. The oestrous cycle is composed of a series of endocrine, behavioural and physiological events that typically occur every 4-6 days throughout the reproductive life span unless interrupted by pregnancy, pseudopregnancy or anestrus. The oestrous cycle is regulated by the hypothalamic-pituitary-ovarian (HPO) axis (Hill, 2017). The observed prolonged diestrus and metestrus phases indicate low concentration of oestrogen which is elevated during estrus phase. Cyclic esters present in *A. indica* and *R. communis* mimick the structure of phytoestrogens which may be responsible for the disruption of the oestrous cycle or they may be synergistic with other compounds to inhibit folliculogenesis and oogenesis.

In the current study, *A. indica* and *R. communis* seed oils had similar effects on oestrous cycle. In previous studies, Roop *et al*. (2005) reported that *A. indica* and *M. azedarach* seed extracts inhibit folliculogenesis in female rat. Many plant extracts have also been reported to disrupt the oestrous cycle with prolonged diestrus phase such as *Cissampelos pareira* leaf extract (Ganguly *et al*., 2007), Neem flower extract (Gbotolorun *et al*., 2008), *Tabernaemontana divaricata* leaf extract (Jain *et al*., 2012a), aqueous wood ash extract of *Azadirachta indica* (Auta and Hassan 2016), *Jatropha gossypifolia* leaf extract (Jain *et al*., 2012b), *Croton menyharthii* and *Uvariodendron kirkii* (Kaingu, 2016).

The phytochemical screening of most of these plants have not been explored but Kaingu, (2016) reported aqueous extract of *Croton menyharthii* and *Uvariodendron kirkii* with high concentrations of flavonoids, sterols, saponins, alkaloids, phenols, quinones and terpenoids which were not present in the current findings. The current results of *A. indica* and *R. communis* seed oils act as oestrous cycle disruptors and therefore, they may develop potential biorodenticides in management of rodent population.

5.3 Mating success in mice post-treatment with *A. indica* and *R. communis* seed oils

Successful mating is indicated by the presence of sperm on the vaginal smear and presence of copulation plug in the vagina. Both *A. indica* and *R. communis* seed oils at low doses had less effect on mating success but at high doses they caused 67% and 83% reduction in mating success, respectively, compared to the negative control. Therefore, the effects of both treatments on mating showed a dose dependent response whereby mating success

reduced with increase in dosage. Successfully mated mice ovulated but unsuccessfully mated mice did not ovulate probably due to hormonal imbalance, inhibition of folliculogenesis, oogenesis or anti-ovulatory activity (Pritchett and Taft, 2007).

Mating is controlled by female hormones which are released during proestrus and estrus phases. During these phases the oestrogen concentration is elevated which enters neurons and evokes mating behaviour. Since *A. indica* and *R. communis* seed oils prolonged diestrus and metestrus phases and subsequently reduced proestrus and estrus phases, it led to less mating behavior in the test mice.

Several medicinal plant extracts that inhibit oogenesis, such as tuber of *Dioscorrea bulbifera* contain steroidal sapogenin, diosgenin, sorbitol, lucetin, fibre, fat, auroxanthin, carbohydrates and cryptoxanthin by inhibiting the release of oestrogen hormone. The whole plant of *Butea monosperma* contains tannins, thiamines, leucocyanidin, procyanidin gallic acid, proteins, fat, carbohydrates and glycoside and the seed of *Ficus religiosa* contains phytosterolin, beta-sitosterol, glycoside, albuminoids, carbohydrate and fatty matter which inhibit both the release of ovum from the ovary and gonatropin hormones (Azamthulla *et al*., 2015).

Daniyal and Akram (2015) reported that petroleum extract of the roots of *Polygonum hydropiper* inhibit ovulation upto 60% in rabbits. Inhibition of oogenesis eventually reduces mating success by inhibiting the release of oestrogen hormone and ovum from the ovary. Reduced mating success by *A. indica* and *R. communis* seed oils in the current study may be a great potential for the development of biorodenticides to be integrated in management of pest population of rodents.

5.4 Fertility index, gestation period and number of litters of mice post-treatment with *A. indica* and *R. communis* seed oils

Azadirachta indica and *R. communis* seed oils had a dose dependent response on fertility index, gestation period and number of litters. Fertility index and number of litters reduced and the gestation period was prolonged with increase in the dosage. Reduction of fertility index was contributed by unsuccessful mating in majority of the mice. At higher concentrations of plant extracts few mice were mated and none littered which suggest that the effect could be due to abortion or anti-implantation activity. The study supports the findings of Makonnen *et al*. (1999) that *R. communis* seed extract possess anti-implantation and abortifacient effects in female guinea pigs. Also, Atawodi and Atawodi (2009) found that purified neem seed extract was efficacious in the termination of pregnancy in both rodents

and primates. Quinn and Whittingham (1982) reported that exogenous Octadecenoic acid and Hexadecanoic acid inhibit fertilization of mouse oocytes *in vitro*. Similar results were also observed by Tripathi *et al*. (2015) who reported that Hexadecanoic acid and Octadecanoic acid aggravate an inhibition of maturation rate which leads to comparatively low fertilization, cleavage and blastocyst formation rates. Octadecanoic acid, Octadecenoic acid and Hexadecanoic acid were present in the current compounds of *A. indica* and *R. communis* seed oils which may have caused reduction in fertility rate and subsequent anti-implantation activity. Sharma *et al*. (2013) reported on ethanolic extract of *Curcuma aromatic* rhizomes to have strong anti-implantation activity in female rat and contain alkaloids, carbohydrates, phytosterols, fixed oils, fats, proteins, amino acids, glycosides, flavonoids, saponins and tannins. This finding is quite similar to the current study due to presence of fixed oils. The mated mice prolonged their gestation period which lasted between 24-25 days at high concentrations compared to the negative control. The anti-implantation or abortifacient effects of both *A. indica* and *R. communis* extracts contributed to the reduction of the litter size which was significantly different compared to the negative control. Both plant extracts, however, caused no significant difference in the body weight compared to the negative control which indicates that other physiological functions were normal. This is in agreement with the findings of Raizada *et al.* (2001) who reported the absolute body weight of 90-day-azadirachtin-treated male and female rats were comparable to the controls and no significant differences were found in their values. On the contrary, Salhab *et al*. (1997), reported that female rabbits treated with *R. communis* at the level of 7.5 mg/kg body weight daily for at least ten consecutive days experienced transient mild diarrhea and loss of body weight. The results of the present investigation indicated the reduction in fertility, number of litters and prolonged gestation period which may be used in control of rodent population.

5.5 Embryo implantation of mice post-treatment with *A. indica* and *R. communis* seed oils

After fertilization, the zygote is formed during the first 3 days, blastocyst formation takes place within 5 days and implantation is completed at the end of the 5th day of pregnancy. Successful implantation requires attachment of the embryos with the uterus which is regulated by ovarian steroids (Yoshinaga, 2013). At high doses *A. indica* and *R. communis* extracts caused a significant anti-implantation activity (100%) compared to the negative control. The effects of the treatments may be on the hypothalamic-pituitary-ovarian (HPO) axis which regulates the release of oestrogen and progesterone necessary for embryo

development or implantation process. The findings support the abortive and anti-implantation activity of both treatments as discussed before. Kulkarni *et al.* (2005) reported that loss of implantation caused by fatty acids of plant extracts may be due to anti-zygotic, anti-blastocytotoxic or anti-implantation activity. The findings of this study are also consistent with those of Juneja *et al.* (1996) that showed postcoital fertility blocker in a mouse by injecting 20ul and 40ul *A. indica* surgically into each uterine horn.

The saturated, mono- and di-unsaturated free fatty acids and their methyl esters have been reported to have immunocontraceptive property which initiates a cellular immune response within the uterine compartment leading to blocking of implantation (Garg *et al*., 1998). This also applies to the current study where *A. indica* and *R. communis* seed oils had fatty acids. In conclusion, both *A. indica* and *R. communis* seed oils had no significant difference on reproductive inhibition in female mice due to their quite similar phytochemical constituents. Therefore, they have potential for integration in pest management of rodent population due to their availability and environmental friendliness.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

From the results of the study it can be concluded that:

i) The GC-MS analysis of *A. indica* and *R. communis* seed oils both revealed the presence of methyl and ethyl esters, cyclic esters, alkanes and alkenes, unsaturated and saturated fatty acids.

ii) Both extracts disrupted the oestrous cycle which may be due to inhibition of folliculogenesis and oogenesis, anti-ovulatory or hormonal imbalance

iii) The test seed oils reduced mating success, fertility index, litter size and prolonged gestation period which may be due to presence of Octadecenoic acid, Hexadecanoic and Octadecanoic acid.

iv) The test seed oils caused inhibition of implantation which may be due to its abortifacient activity or anti-implantation activity.

Recommendations

Azadirachta indica and *Ricinus communis* can be referred to as multi-purpose plants since they play vital role in several sectors such as agricultural, industrial, cosmestics and pharmaceutical sector. The study revealed that the plants are effective in the reproductive inhibition in the rodents, hence, they are potential biorodenticides. Therefore there is need to:

i) Formulate *A. indica* and *R. communis* seed oils as rodent baits.

ii) Study on rodent damage, biology and their population dynamics for effective reduction of their final population size.

iii) Further studies on the application of *A. indica* and *R. communis* oil constituents as a human contraceptive.

iv) Encourage the conservation and planting of *A. indica* and *R. communis* in Baringo County, Kenya.

REFERENCES

Adams, R. P. (2007). Identifications of essential oil components by gas chromatography/mass spectroscopy. Allured Publishers, Carol Stream, Illinois (pp. 21-22).

Anjarwalla, P., Belmain, S., Solav, P., Jamnadass, R., and Stevenson, P. C. (2016). Handbook on Pesticidal Plants.World Agroforestry Centre (ICRAF): Nairobi (pp13-22).

Asif, M. (2013). A review on spermicidal activities of Azadirachta indica. Journal of Pharmacognosy and Phytochemistry, 1(5), 18-19.

Atawodi, S. E., and Atawodi, J. C. (2009). Azadirachta indica (neem): a plant of multiple biological and pharmacological activities. Phytochemistry Reviews, 8(3), 601-620.

Auta, T., and Hassan, A. T. (2016). Alteration in oestrus cycle and implantation in Mus musculus administered aqueous wood ash extract of Azadirachta indica (neem). Asian Pacific Journal of Reproduction, 5(3), 188-192.

Azamthulla, M., Rajkapoor, B. and Kavimani, S. (2015). A Review on medicinal plants exhibiting antifertility activity. World Journal of Pharmacy and Pharmaceutical Sciences, 4(3): 243-272.

Babarinde, S. A., Akinyemi, A. O., Odewole, A. F., Yisa, S., Oke, O. O. and Adeyemo, A. O. (2016). Phytochemical composition and insecticidal properties of mechanicaly extracted castor, seed oil against cowpea seed bruchid (Callosobruchus maculatus Fabricius) infesting bambara groundnut. Elixir International Journal, 92, 39086-39092

Bachmann, J. (2004). Oil seed processing for small scale producers. National Sustainable Agriculture Information Service., Fayetteville, Arkansas. 2-12.

Belmain, S. R., Htwe, N. M., Kamal, N. Q. and Singleton, G. R. (2015). Estimating rodent losses to stored rice as a means to assess efficacy of rodent management. Wildlife Research, 42(2), 132-142.

Bett, P.K., (2015). Chemical composition of Cupressus lusitanica, miller and Eucalyptus saligna, smith essential oils and bioactivity against lepidopteran and coleopteran pests of stored grains, Doctoral dissertation, Egerton University, pp 26-36.

Bett, P.K., Deng, A.L., Ogendo, J.O., Kariuki, S.T., Kamatenesi-Mugisha, M., Mihale, J.M., and Torto, B. (2016). Chemical composition of Cupressus lusitanica and Eucalyptus saligna leaf essential oil and bioactivity against major insect pests of stored food grains. Industrial Crops and Products, 82, 51–62.

Bradford, A. (2015). Live Science Contributor Facts About Rats. https://www.livescience.com/52342-rats.htm Retrieved November 17, 2017.

Brooker, M. I. H., and Kleinig, D. A. (2006). Field Guide to Eucalyptus. vol.1. South-eastern

Australia, Third edition. Bloomings, Melbourne.

Campos, E. V., de Oliveira, J. L., Pascoli, M., de Lima, R., and Fraceto, L. F. (2016). Neem oil and crop protection: From now to the future. Frontiers in plant science, 7, 1494

Chéraif, I., Ben J., Jannet, H. (2007). Chemical composition and antimicrobial activity of essential oils of Cupressus arizonica Greene. Biochemical Systematics and Ecology 35 (12): 813–820.

Daniyal, M., and Akram, M. (2015). Antifertility activity of medicinal plants. Journal of the Chinese Medical Association, 78(7), 382-388.

Deb, K., Reese, J., and Paria, B. C. (2006). Methodologies to study implantation in mice. In: Placenta and Trophoblast, Soares, M.J.; Hunt, J.S. (Ed.). Humana Press: Totowa, (pp.9- 34).

Devi, P., Kumar, P., and Dhamija, I. (2015). Antifertility activity of medicinal plants on male and female reproduction. International Journal of Pharmaceutical Sciences and Research, 6(3), 988.

Djenontin, T. S., Wotto, V. D., Avlessi, F., Lozano, P., Sohounhloué, D. K., and Pioch, D. (2012). Composition of Azadirachta indica and Carapa procera (Meliaceae) seed oils and cakes obtained after oil extraction. Industrial Crops and Products, 38, 39-45.

Falkner, F., and Tanner, J. M. (Eds.). (2013). Postnatal Growth Neurobiology. Springer Science and Business Media: New York (pg 460).

Foote, A. L. and Crystal, J. D. (2007). Metacognition in the rat. Current Biology, 17 (6), 551-555

Ganguly, M., Borthakur, M. K., Devi, N., and Mahanta, R. (2007). Antifertility activity of the methanolic leaf extract of Cissampelos pareira in female albino mice. Journal of Ethnopharmacology, 111(3), 688-691.

Garg, S., Talwar, G. P., and Upadhyay, S. N. (1998). Immunocontraceptive activity guided fractionation and characterization of active constituents of neem (Azadirachta indica) seed extracts. Journal of Ethnopharmacology, 60(3), 235-246.

Gbotolorun, S. C., Osinubi, A. A., Noronha, C. C., and Okanlawon, A. O. (2008). Antifertility potential of Neem flower extract on adult female Sprague-Dawley rats. African Health Sciences, 8(3), 168-173.

Gunstone, F. D., and Hamilton, R. J. (2004). The chemistry of oils and fats: Sources. Composition, Properties and Uses, Blackwell Publishing Ltd. United Kingdom. pp 54.

Hamid, H.Y., and Zakaria, Z.A.B. (2013). Reproductive characteristics of the female laboratory rat. *African Journal of Biotechnology*, **12**(19), 2510-2514.

Harding, E. J., Paul, E. S., and Mendl, M. (2004). Animal behaviour: Cognitive bias and affective state. *Nature*. **427** (6972*)*, 312.

Harvey, A. E., Lashinger, L. M., and Hursting, S. D (2011). The growing challenge of obesity and cancer: an inflammatory issue. *Annals of the New York Academy of Sciences*, **1229**(1), 45-52.

Hickman, D. L. (2019). Wellbeing of Alcohol-preferring Rats Euthanized with Carbon Dioxide at Very Low and Low Volume Displacement Rates. Journal of the American Association for Laboratory Animal Science, 58(1), 78-82.

Hill, M.A. (2017). Embryology Reproductive Cycles. https://embryology.med.unsw.edu.au/embryology/index.php/ReproductiveCycles. Retrieved November 7, 2017.

Hinds, L. A., Hardy, C. M., Lawson, M. A. and Singleton, G. R. (2003). Developments in fertility control for pest animal management. ACIAR Monograph Series, 96, 31-36. http://007pestcontrol.co.uk. Retrieved November 7, 2017.

Hussein, H. M., Ubaid, J. M., and Hameed, I. H. (2016). Insecticidal activity of methanolic seeds extract of Ricinus communis on adult of Callosobruchus maculatus (coleopteran: brauchidae) and analysis of its phytochemical composition. International Journal of Pharmacognosy and Phytochemical Research, 8(8), 1385-1397.

Jain, S., Choudhary, G. P., and Jain, D. K. (2012a). Pharmacological evaluation of anti–fertility activity of ethanolic extract of Jatropha gossypifolia leaf in female albino mice. Asian Pacific Journal of Tropical Biomedicine, 2(3), 1671-1674.

Jain, S., Jain, D. K., Balekar, N., and Jain, A. (2012b.). Antifertility effect of chronically administered Tabernaemontana divaricata leaf extract on female albino mice. Asian Pacific Journal of Tropical Disease, 2, 159-162.

Jena, J., and Gupta, A. K. (2012). Ricinus communis Linn: a phytopharmacological review. International Journal of Pharmacy and Pharmaceutical Sciences, 4(4), 25-29.

Jimoh, A., U. G., and Mohammed, A. D. (2006). Extraction, characterization and modification of castor seed oil. Leonardo Journal of Sciences, 8, 43-52.

Jombo, G.T. and Enenebeaku, M. N. (2008). Antibacterial profile of fermented seed extracts of Ricinus communis: findings from a preliminary analysis. Nigerian Journal of Physiological Sciences, 23, 1-2.

Jozefowiez, J., Staddon, J. E. R., and Cerutti, D. T. (2009). Metacognition in animals: how do we know that they know? Comparative Cognition & Behavior Reviews, 4, 29–39.

Juneja, S. C., Williams, R. S., Farooq, A., and Chegini, N. (1996). Contraception potential of neem oil: effect on pregnancy success in the mouse. Journal of Assisted Reproduction and Genetics, 13(7), 578-585.

Kaingu, C. K., Oduma, J. A., Mbaria, J., and Kiama, S. G. (2017). Effects of Croton menyharthii and Uvariodendron kirkii extracts on ovarian corpora lutea and reproductive hormones. Phytomedicine, 1(780), 20-24.

Kaingu, D. C. K. (2016). Evaluation of anti-fertility potential of selected medicinal plants of Tana River County, Kenya. Doctoral dissertation, University of Nairobi, pp 4-20.

Karacor, K., and Cam, M. (2015). Effects of oleic acid. Medical Science and Discovery, 2(1), 125-32.

Keirn, G. (2011). Rodent damage costing agriculture industry millions, APHIS Public Affairs Specialist, USDA. www.westernfarmpress.com/.../rodent-damage-costing-agriculture-industry-millions. Retrieved November 19, 2017.

Koul, O., Isman, M. B., and Ketkar, C. M. (1990). Properties and uses of neem (Azadirachta indica). Canadian Journal of Botany, 68(1), 1-11.

Kumar, R., Mehta, S., and Pathak, S. R. (2018). Bioactive constituents of neem. In Synthesis of Medicinal Agents from Plants (pp. 75-103). Elsevier.

Kulkarni, T. R., Kothekar, M. A., and Mateenuddin, M. O. H. D. (2005). Study of anti-fertility effect of lemon seeds (Citrus limonum) in female albino mice. Indian Journal of Physiology and Pharmacology, 49(3), 305.

Lale, N. E. S., and Abdulrahman, H. T. (1999). Evaluation of neem (Azadirachta indica A. Juss) seed oil obtained by different methods and neem powder for the management of Callosobruchus maculatus (F.)(Coleoptera: Bruchidae) in stored cowpea. Journal of Stored Products Research, 35(2), 135-143.

Lord, M.J., Jolliffe N.A., Marsden C.J., Pateman, C.S., Smith D.C., Spooner R.A., Watson P.D., and Roberts, L.M.(2003). Mechanisms of cytotoxicity. Toxicological Reviews, 22(1)53-64.

Lupo, L. (2017). Why the Difference Between Rats and Mice Matters. https://www.thespruce .com/the-difference-between-rats-and-mice-2656563 Retrieved November 26, 2017.

Makonnen, E., Zerihun, L., Assefa, G., and Rostom, A. A. (1999). Antifertility activity of Ricinus communis seed in female guinea pigs. East African Medical Journal, 76(6), 335-337.

Mala, A. O., Irungu, L. W., Mitaki, E. K., Shililu, J. I., Mbogo, C. M., Njagi, J. K., and Githure, J. I. (2014). Gonotrophic cycle duration, fecundity and parity of Anopheles

gambiae complex mosquitoes during an extended period of dry weather in a semi- arid area in Baringo County, Kenya. International Journal of Mosquito Research, 1(2), 28-34.

Malebane, P. (2017). A systematic review of rodent pest research in Afro-Malagasy small-holder farming systems: Are we asking the right questions? PloS ONE, 12(3).

Mangels, R., Tsung, K., Kwan, K., and Dean, M. D. (2016). Copulatory plugs inhibit the reproductive success of rival males. Journal of Evolutionary Biology, 29(11), 2289-2296.

Marcondes, F. K., Bianchi, F. J., and Tanno, A. P. (2002). Determination of the estrous cycle phases of rats: some helpful considerations. Brazilian Journal of Biology, 62(4A), 609- 614.

Mak-Mensah, E. E., and Firempong, C. K. (2011). Chemical characteristics of toilet soap prepared from neem (Azadirachta indica A. Juss) seed oil. Asian Journal of Plant Science and Research, 1(4), 1-7.

Martín, C., Moure, A., Martín, G., Carrillo, E., Domínguez, H., and Parajo, J. C. (2010). Fractional characterisation of jatropha, neem, moringa, trisperma, castor and candlenut seeds as potential feedstocks for biodiesel production in Cuba. Biomass and Bioenergy, 34(4), 533-538.

McNeil, R.T., Noronha, C.C., Kusemiju, T.O., and Okanlawon, A.O. (2003). The Anti-ovulatory effect of a seed extract of Ricinus communis-linn. Nigerian Journal of Health and Biomedical Sciences, 2 (1), 31- 34.

Meerburg, B. G., Singleton, G. R., and Kijlstra, A. (2009). Rodent-borne diseases and their risks for public health. Critical Reviews in Microbiology, 35(3), 221-270.

Mkenda, P., Mwanauta, R., Stevenson, P. C., Ndakidemi, P., Mtei, K., and Belmain, S. R. (2015). Extracts from field margin weeds provide economically viable an environmentally benign pest control compared to synthetic pesticides. PloS ONE, 10(11), e0143530.

Morovati, M., Mahmoudi, M., Ghazi-Khansari, M., Khalilaria, A., and Jabbari, L. (2008). Sterility and Abortive Effects of the Commercial Neem (Azadirachta indica A. Juss) Extract NeemAzal-T/S® on Female Rat (Rattus norvegicus). Turkish Journal of Zoology, 32(2), 155-162.

Mumoki, F. N., Pirk, C. W., Yusuf, A. A.,and Crewe, R. M. (2018). Reproductive parasitism by worker honey bees suppressed by queens through regulation of worker mandibular secretions. Scientific Reports, 8(1), 1-11

Murphy, R. G., Williams, R. H., Hughes, J. M., Hide, G., Ford, N. J., and Oldbury, D. J. (2008). The urban house mouse (Mus domesticus) as a reservoir of infection for the human parasite Toxoplasma gondii: an unrecognised public health issue? International Journal of Environmental Health Research, 18(3), 177-185.

Nandwa, A. N. (2016). Effect of methanolic extracts of Sida cuneifolia roots on the fertility of albino rats (Rattus norvegicus), Doctoral Dissertation, University of Eldoret, pp 16-24

NPMA (2014). Health Hazards Posed by Rodents. https://www.pestworld.org/news-hub/pest-health-hub/health-hazards-posed-by-rodent. Retrieved June 12, 2018

Ognakossan, K. E. (2017). Assessment of rodents' postharvest losses in on-farm maize storage in Kenya, Doctoral dissertation, Jomo Kenyatta University of Agriculture and Technology, pp 4-5.

Ognakossan, K. E., Mutungi, C. M., Otieno, T. O., Affognon, H. D., Sila, D. N., and Owino, W. O. (2018). Quantitative and quality losses caused by rodents in on-farm stored maize: a case study in the low land tropical zone of Kenya. Food Security, 10(6), 1525-1537.

91-121.

Oluwole, F. A., Abdulrahim, A. T., Aviara, N. A., and Ndahi, N. S. (2012). Traditional method of extracting castor oil. Continental Journal of Engineering Sciences, 7(2), 6-10.

Omowanle, J., Ayo, R. J., Habila, J., Ilekhaize, J., and Adegbe, E. A. (2018). Physico-chemical and Gc-Ms Analysis of some selected plant seed oils; Castor, Neem and Rubber Seed Oils. FUW Trends in Science and Technology Journal 3(2), 644-651

Paccola C. C., Resende C. G., Stumpp T., Miraglia S. M., and Cipriano I. (2013). The rat estrous cycle revisited: a quantitative and qualitative analysis. Animal Reproduction, 10(4), 677- 683.

Pardo, V., González-Rodríguez, Á., Muntané, J., Kozma, S. C., and Valverde, Á. M. (2015). Role of hepatocyte S6K1 in palmitic acid-induced endoplasmic reticulum stress, lipotoxicity, insulin resistance and in oleic acid-induced protection. Food and Chemical Toxicology, 80, 298-309.

Pritchett, K. R., and Taft, R. A. (2007). Reproductive biology of the laboratory mouse. In: The Mouse in Biomedical Research, Lyon, M. F., Rastan,S., Brown,S. M. D (Ed.). Academic Press.3,91-121.

Quinn, P., and Whitfingham, D. C. (1982). Effect of fatty acids on fertilization and development of mouse embryos in vitro. Journal of Andrology, 3(6), 440-444.

Quraishi, H. A., Islam, N., Iqbal, A., Bhat, S. A., Ahmed, J., Ashraf, S. S., and Khan, Q. A. (2018). Therapeutical and medicinal properties of Neem (Azadirachta indica) in context of Unani system of medicine: a review study. Journal of Drug Delivery and Therapeutics, 8(6-s), 394-399.

Raizada, R.B., Srivastava, M.K., Causal, R.A., and Singh, R.P. (2001). Azadirachtin, a Neem biopesticides: sub chronic toxicity assessment in rats. Food Chemical and Toxicology, 39: 477-483.

Raji, Y., Oloyo, A. K., and Morakinyo, A. O. (2006). Effect of methanol extract of Ricinus communis seed on reproduction of male rats. Asian Journal of Andrology, 8(1), 115-121.

Rao, M. V., and Alice, K. M. (2001). Contraceptive effects of Phyllanthus amarus in female mice. Phytotherapy Research, 15(3), 265-267.

Roop, J. K., Dhaliwal, P. K., and Guraya, S. S. (2005). Extracts of Azadirachta indica and Melia azedarach seeds inhibit folliculogenesis in albino rats. Brazilian Journal of Medical and Biological Research, 38(6), 943-947.

Russell, W. (2008). The female rat reproductive cycle: a practical histological guide to staging. Toxicologic Pathology, 36(3), 375-84.

Sales-Campos, H., Reis de Souza, P., Crema Peghini, B., Santana da Silva, J., and Ribeiro Cardoso, C. (2013). An overview of the modulatory effects of oleic acid in health and disease. Mini Reviews in Medicinal Chemistry, 13(2), 201-210.

Salhab, A. S., Issa, A. A., and Alhougog, I. (1997). On the contraceptive effect of castor beans. International Journal of Pharmacognosy, 35(1), 63-65.

Sani, U. M., and Sule, M. I. (2007). Anti-fertility activity of methanol extracts of three different seed varieties of Ricinus communis Linn. (Euphorbiaceae). Nigerian Journal of Pharmaceutical Sciences, 6(2), 78-83.

Sarraj, M. A., and Drummond, A. E. (2012). Mammalian foetal ovarian development: consequences for health and disease. Reproduction, 143(2), 151-163.

Schmutterer, H. (1990). Properties and potential of natural pesticides from the neem tree, Azadirachta indica. Annual Review of Entomology, 35(1), 271-297.

Shareef, M., and Akhtar, M. S. (2018). Neem (Azadirachta indica) and its potential for safeguarding health, prevention and treatment of diseases. Matrix Science Medica, 2, 4-8.

Sharma, R. K., Goyal, A. K., and Bhat, R. A. (2013). Antifertility activity of plants extracts on female reproduction: A review. International Journal of Pharmacy and Biological Sciences, 3(3), 493-514.

Sinha, K. C., Riar, S. S., Tiwary, R. S., Dhawan, A. K., Bardham, J., Thomas, P., Kain, A. K., and Jain, R. K. (1984). Neem oil as a vaginal contraceptive. Indian Journal of Medical Research, 79, 131-6

Suryawanshi, J. A. S. (2011). Neem-natural contraceptive for male and female-an overview. International Journal of Biomolecules and Biomedicine, 1(2), 1-6.

SPSS, (2015) IBM SPSS Statistics for Windows, Version 23.0. IBM Corp. Armonk, New York.

Stevenson, P. C., Kite, G. C., Lewis, G. P., Forest, F., Nyirenda, S. P., Belmain, S. R., Sileshi, G. W., and Veitch, N. C. (2012).Distinct chemotypes of Tephrosia vogelii and implications for their use in pest control and soil enrichment. Phytochemistry, 78, 135-146.

Tesfaye, B., and Tefera, T. (2017). Extraction of essential oil from neem seed by using Soxhlet extraction methods. International Journal of Advanced Engineering, Management and Science, 3(6), 646-650.

Tran, T. T., and Hinds, L. A. (2013). Fertility control of rodent pests: a review of the inhibitory effects of plant extracts on ovarian function. Pest Management Science, 69(3), 342-354.

Tripathi, S. K., Nandi, S., Gupta, P. S. P., and Mondal, S. (2015). Influence of common saturated and unsaturated fatty acids on development of ovine oocytes in vitro. Asian Journal of Animal Science, 9, 420-426.

Umadevi, M., Kumar, S. P. K., Bhowmik, D., and Duraivel, S. (2013). Medicinal plants with potential antifertility activity. Journal of Medicinal Plant, 1(1), 26-33.

Upadhyay, S. N., Kaushic, C., and Talwar, G. P. (1990). Antifertility effects of neem (Azadirachta indica) oil by single intrauterine administration: a novel method for contraception. Proceedings of the Royal Society of London Series B: Biological Sciences, 242(1305), 175-179.

Uzoh, C. F., and Nwabanne, J. T. (2016). Investigating the effect of catalyst type and concentration on the functional group conversion in castor seed oil alkyd resin production. Advances in Chemical Engineering and Science, 6(02),190-200.

Waidee, T., Unpaprom, Y., and Ramaraj, R. (2018). Evaluation of Biodiesel Production from Pre-Heated Castor Oil (Ricinus communis). In International Conference on Renewable Energy, 14, pp15.

Waithaka, P. N., Gathuru, E. M., Githaiga, B. M., and Kimani, S. N. (2017). Control of Passion Fruit Fungal Diseases Using Essential Oils Extracted from Rosemary (Rosmarinus officinalis) and Eucalyptus (Eucalyptus agglomerata) in Egerton University Main Campus Njoro, Kenya. International Journal of Microbiology, pp 1-6

Warra A. A. (2015). Physico-chemical and GC/MS analysis of wild castor (Ricinus communis L.) seed oil. Applied Science Reports, 9(3), 123-128.

Witmer, G. W., and Aaron, B. (2017). Ecology, Impacts, and Management of Invasive Rodents in the United States. Ecology and Management of Terrestrial Vertebrate Invasive Species in the United States CRC Press: Boca Raton, (pp. 193-220).

Yanar, D. (2019). Side effects of different doses of azadirachtin on predatory mite Metaseiulus occidentalis (Nesbitt)(acari: Phytoseiidae) under laboratory conditions. Applied Ecology and Environmental Research, 17, 3433-3440.

Yoshinaga, K. (2013). A sequence of events in the uterus prior to implantation in the mouse. Journal of Assisted Reproduction and Genetics, 30(8), 1017-1022.

Yusuf, A. K., Mamza, P. A. P., Ahmed, A. S., and Agunwa, U. (2015). Extraction and characterization of castor seed oil from wild Ricinus communis Linn. International Journal of Science, Environment and Technology, 4(5), 132-140.

APPENDICES

Appendix 1: ANOVA summary table-Frequency of oestrous phases of mice after exposure to different doses of *A. indica*

Source of variation	**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Corrected Model	98.581[a]	11	8.962	35.147	.000
Intercept	513.095	1	513.095	2012.262	.000
frequency	98.581	11	8.962	35.147	.000
Error	21.419	84	.255		
Total	720.000	96			

Appendix 2: ANOVA summary table- Frequency of oestrous phases of mice after exposure to different doses of *R. communis*

Source of variation	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Corrected Model	100.736[a]	13	7.749	32.985	.000
Intercept	372.394	1	372.394	1585.160	.000
frequency	100.736	13	7.749	32.985	.000
Error	19.264	82	.235		
Total	720.000	96			

Appendix 3: ANOVA summary table- Frequency of oestrous phases of mice after exposure to different doses of *R. communis* and *A. indica*

Source of variation	**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Corrected Model	199.317[a]	25	7.973	32.532	.000
Intercept	697.405	1	697.405	2845.674	.000
frequency	183.478	13	14.114	57.589	.000
plant	.040	1	.040	.164	.686
frequency * plant	2.280	11	.207	.846	.595
Error	40.683	166	.245		
Total	1440.000	192			

Appendix 4: ANOVA summary table - Live body weights of mice post-treatment with *A. indica* and *R. communis* seed oils

	Sum of Squares	df	Mean Square	F	Sig.
Contrast	21.310	77	.277	1.243	.183
Error	14.690	66	.223		

The F tests the effect of Weight. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Appendix 5: Chi-Square Tests of *R. communis* and *A. indica* on mating success at 0.2ul doses

Chi-Square Tests			
	Value	**df**	**Sig.**
Pearson Chi-Square	2.571[a]	2	.276
Likelihood Ratio	3.793	2	.150
Linear-by-Linear Association	1.821	1	.177
N of Valid Cases	18		

Appendix 6: Chi-Square Tests of *R. communis* and *A. indica* on mating success at 0.8ul doses

	Chi-SquareTests Value	**df**	**Sig.**
Pearson Chi-Square	9.000[a]	2	.003
Likelihood Ratio	11.457	2	.001
Linear-by-Linear Association	8.500	1	.004
N of Vali	18		

Appendix 7: ANOVA summary table --Fertility index of mice post-treatment with *A. indica* and *R. communis* seed oils

Source of variation	**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Corrected Model	.373[a]	3	.124	18.667	.008
Intercept	1.830	1	1.830	274.571	.000
Fertility	.373	3	.124	18.667	.008
Error	.027	4	.007		
Total	2.400	8			

Appendix 8: ANOVA summary table - Gestation period of mice post-treatment with *A. indica* and *R. communis* seed oils

Source of variation	**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Corrected Model	101.765[a]	3	33.922	206.888	.000
Intercept	118.259	1	118.259	721.264	.000
Gestation	101.765	3	33.922	206.888	.000
Error	7.214	44	.164		
Total	159.000	48			

Appendix 9: ANOVA summary table -- Litter size of mice post-treatment with *A. indica* and *R. communis* seed oils

	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	6019.752[a]	3	2006.584	14928.210	.000
Intercept	8197.850	1	8197.850	60988.833	.000
Litters	6019.752	3	2006.584	14928.210	.000
Error	5.914	44	.134		
Total	9034.000	48			

Appendix 10: ANOVA summary table - Number of implantation of mice post-treatment with *A. indica* and *R. communis* seed oils

Source of variation	**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Contrast	.766	7	.109	4.030	.010
Error	.434	16	.027		

The F tests the effect of No. of implantation. This test is based on the linearly independent pairwise comparisons among the estimated marginal means

Appendix 11: Research Permit

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

Telephone:+254-20-2213471, 2241349,3310571,2219420
Fax:+254-20-318245,318249
Email: dg@nacosti.go.ke
Website : www.nacosti.go.ke
When replying please quote

NACOSTI, Upper Kabete
Off Waiyaki Way
P.O. Box 30623-00100
NAIROBI-KENYA

Ref: No. **NACOSTI/P/19/10332/28244**

Date: **21st February, 2019**

Ann Jepkorir Kiplagat
Egerton University
P.O. Box 536-20115
NJORO

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"Reproductive inhibition properties of Azadirachta Indica A. Juss and Ricinus Communis Linn. seed oils in female mice"*** I am pleased to inform you that you have been authorized to undertake research in **Baringo County** for the period ending **21st February, 2020.**

You are advised to report to **the County Commissioner and the County Director of Education, Baringo County** before embarking on the research project.

Kindly note that, as an applicant who has been licensed under the Science, Technology and Innovation Act, 2013 to conduct research in Kenya, you shall deposit **a copy** of the final research report to the Commission within **one year** of completion. The soft copy of the same should be submitted through the Online Research Information System.

BONIFACE WANYAMA
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioner
Baringo County.

The County Director of Education
Baringo County.

Appendix 12: Research Authorization from the County Commissioner, Baringo County, Kenya

OFFICE OF THE PRESIDENT

Telephone. 053-21285
Fax. (053)-21285
E-Mail:
baringocountycommissioner@yahoo.com
baringocountycommissioner@gmail.com

When replying please quote:

MINISTRY OF INTERIOR AND CO-ORDINATION OF NATIONAL GOVERNMENT

COUNTY COMMISSIONER'S OFFICE,
BARINGO COUNTY,
P.O. BOX 1 - 30400
KABARNET.

REF.NO: **ADM.18/1 VOL.II/118**

23RD AUGUST, 2019

All Deputy County Commissioners
BARINGO COUNTY

RE: RESEARCH AUTHORIZATION

Reference is made to a letter No.NACOSTI/P/19/10332/28244 dated 21st February, 2019 from the Director General/CEO NACOSTI.

This is to confirm that **Ann Jepkorir Kiplagat** of **Egerton University** has been authorized to carry out research on **"Reproductive inhibition properties of Azadirachta Indica A. Juss and Ricinus Communis Linn Seed oils in female mice",** for the period ending **21st February, 2020.**

Please accord her the necessary support.

HENRY WAFULA
COUNTY COMMISSIONER
BARINGO COUNTY

COUNTY COMMISSIONER
BARINGO COUNTY
P. O. Box 1 - 30400, KABARNET

Appendix 13: Research Authorization from the County Director of Education, Baringo County, Kenya

REPUBLIC OF KENYA

MINISTRY OF EDUCATION
STATE DEPARTMENT OF EARLY LEARNING & BASIC EDUCATION

OFFICE OF THE COUNTY DIRECTOR
(**BARINGO COUNTY**).

Our Email:countyedubaringo@gmail.com
Tel / Fax: 053/21282

P.O. BOX 664
KABARNET

REF: NACOSTIP/19/103328244/Vol 195

23/08/2019

Ann Jepkorir Kiplagat
Egerton University
P. O. Box 536-20115
NAKURU.

RE : RESEARCH AUTHORIZATION

Reference is made to your request letter Ref. **No. NACOSTI/P/19/10332/28244/ dated** 21/02/2019 on the above subject.

I am pleased to inform you that you have been authorized to carry out research on ***"Reproductive inhibition properties of azadirachta indica A. Juss and Ricinus Communis linn. Seed oils in female mice", Baringo County Kenya."*** *for* a period ending **21th February,** 2020.

The authorities concerned are therefore requested to give maximum support so that this research is completed within schedule.

I take this opportunity to wish you well during this research in our county.

For: COUNTY DIRECTOR OF EDUCATION
BARINGO
P. O. Box 664 - 30400
KABARNET

Karati Moses N.
County Director of Education
Baringo County

Appendix 14: Ethical clearance

UNIVERSITY OF NAIROBI
FACULTY OF VETERINARY MEDICINE
DEPARTMENT OF VETERINARY ANATOMY AND PHYSIOLOGY
P.O. Box 30197,
00100 Nairobi,
Kenya.

Tel: 4449004/4442014/ 6
Ext. 2300
Direct Line. 4448648

REF: FVM BAUEC/2019/236

Ms. Anne Jepkorir Kiplagat
Egerton University
Dept. of Biological Sciences
13/08/2019

Dear Ms. Anne,

RE: Approval of Proposal by Biosafety, Animal use and Ethics committee

Reproductive inhibition properties of *Azadirachta indica* A. Juss and *Ricinus communis* LINN seed oils in female mice.

By Ms. Anne Jepkorir Kiplagat SM21/ 14431/2015

We refer to your MS.c proposal submitted to our committee for review and your application letter dated 10th August 2019.

We have reviewed your proposal and are satisfied that the proposed husbandry, handling and in vitro protocols of laboratory mice meets acceptable minimum standards of the faculty ethical regulation guidelines. The numbers of mice to be used also meets the 3R principle.

We have also noted that a registered veterinary surgeon KVB 1373 will supervise the work.

We hereby give approval for you to proceed with the project as outlined in the submitted proposal.

Yours sincerely

Kaluwa

Dr. Catherine Kaluwa, BVM, MSc, Ph.D
Chairperson,
Biosafety, Animal Use and Ethics Committee
Faculty of Veterinary Medicine

Appendix 15: Research publication

EISSN: 2707

East African Journal of Science, Technology and Innovation, Vol. 1 (2): 2020.

Chemical composition of oils of *Azadirachta indica* A. Juss and *Ricinus communis* Linn seed in Marigat, Baringo County, Kenya

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[1]Department of Biological Sciences, Egerton University, P.O Box 536-20115, Njoro, Kenya
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Abstract

All parts of *A. indica* (neem) and *R. communis* (castor) plants have mostly been used as natural remedies in the control and treatment of several ailments, control of pests and insects, animal feeds and production of industrial products globally. The seed oils of *A. indica* and *R. communis* are known to have antidiabetic, anti-helminthic, antifertility, antioxidant, antibacterial, anti-inflammatory, anti-cancer, insecticidal and mosquitocidal activity. This study reports for the first time the chemical composition of *A. indica* and *R. communis* seed oils from Marigat, Baringo County, Kenya. Seed oils of *A. indica* and *R. communis* were extracted from mature dried seeds through cold pressing and boiling respectively and chemical composition determined using Gas Chromatography (GC)-Mass Spectrometry (MS). The constituents of both seed oils were dominated by saturated and unsaturated fatty acids, cyclic esters and methyl esters. The predominant constituents of *R. communis* were (Z)-6-Octadecenoic acid (37.33%), Ricinoleic acid (30.22%) and 13-Hexyloxacyclotridec-10-en-2-one (26.67%) while those of *A. indica* were 2-hexyl-1-decanol (30.97%), Octadecanoic acid (29.69%) and Oxalic acid, 6-ethyloct-3-yl ethyl ester (15.55%). Oils contained Hexadecanoic acid and Octadecanoic acid which are used in the manufacture of several products such as candles, soaps, lotions, perfumes and cosmetics. Octadecenoic acid is important in control of human diseases and Ricinoleic acid in production of alkyd resins for surface coating and biofuel. From the results, *A. indica* and *R. communis* seed oils constituents have potential in the agricultural, industrial, comestics and pharmaceutical sectors but require further fractionation to isolate the bioactive compounds.

Keywords: *Azadirachta indica; chemical constituents; GC-MS, Ricinus communis; seed oil*

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Introduction

Natural seed oils from plants are in high demand for the development of new products in therapeutics, agriculture, cosmetics and industrial sector compared to synthetic products. Synthetic products have several limitations such as high cost, toxicity to non-targeted species, poor absorption, develop resistance, low bio-availability, pollutants to the environment and adverse side effects (Abdul *et al.*, 2018). The use of plant-based products in therapeutics, agriculture, cosmetics and industrial sector as alternative to synthetic-based products could be a better option because they are biodegradable, inexpensive, have less side effects and are readily available (Mkenda *et al.*, 2015). Some of these seed oil plants are *Azadirachta indica* A., *Ricinus communis* L., *Celosia argentea* L., *Aesculus indica* C., *Sisymbrium irio* L., *Abies pindrow* R., *Ulmus wallichiana* P., *Nigella sativa*, *Cuminum cyminum* L., *Cassia abbreviate*, *Moringa oleifera* Z., *Annona squamosa* L. and *Pangium edule* R. which have been shown to