FIELD EVALUATION OF *PIPER GUINEENSE* AND *SPILANTHES MAURITIANA* POWDER AS MOSQUITO LARVICIDES IN KILIFI DISTRICT, KENYA

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University or any other award

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We as University supervisors confirm that the work reported in this thesis was carried out by the candidate

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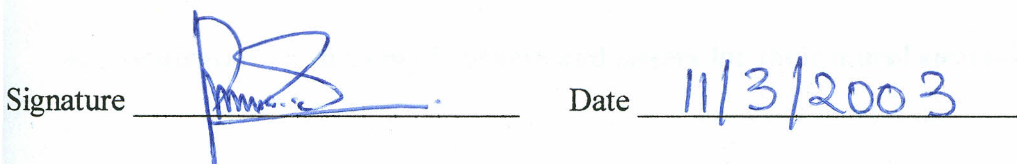

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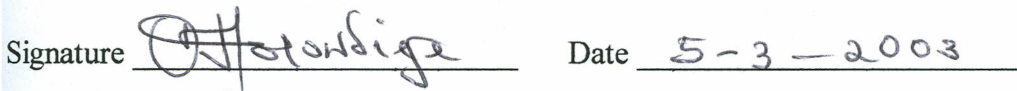

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DEDICATION

To my parents, Boaz Allan and Jannet Ohaga, for their love, financial support and dedication; and to my brothers and sisters for their moral support

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LIST OF ABBREVIATIONS

DDT: Dichlorodiphenyltrichloroethane

DO: Dissolved Oxygen

EIR: Entomological inoculation rates

GoK: Government of Kenya

ITNs: Insecticide treated nets

LD_{50} & LD_{90}: The lethal doses that kill 50% and 90% of the treated larvae, respectively

LT_{50}: The lethal time taken to kill 50% of the treated larvae

MARA: Mapping Malaria Risk in Africa

s.l.: sensu lato

s.s.: sensu stricto

WHO: World Health Organization

ABSTRACT

Field tests were conducted in Kilifi District on field populations of anopheline and culicine mosquitoes using *Piper guineense* and *Spilanthes mauritiana* powder. A total of 36 aquatic habitats containing mosquito larvae were sampled and the larval populations determined by standard dipping technique before and after the application of the plant powder. Four doses (8.571, 5.714, 2.875 and 1.429 g/l) were used in the field trials and larval mortality monitored after 24, 48 and 72 hours. Emerging adult mosquitoes were trapped by modified "Saliternick" mosquito cages and wing length measured by occular microscope. Phenotypic changes were noted. The effects of the plant material on larval habitat temperature, conductivity, dissolved oxygen and predatory non-target organisms were also monitored.

Spilanthes mauritiana and *P. guineense* powder at 8.571 g/l induced highest overall larval mortality of 97.4±0.58, 96.2±0.34%, in anophelines and 100, 96.2±0.32% in culicines. However, at the lowest tested dose (1.429 g/l) the overall larval mortality reduced substantially to 22.4±0.72, 18.5±0.82%, and 19.9±0.51, 25.1±0.68%, for anophelines and culicines, respectively. The LD_{50} values were calculated at 2.74 and 2.26 g/l for *S. mauritiana* and *P. guineense,* respectively, for anophelines. Similarly, LD_{50} of 2.32 and 1.64, respectively, for culicines were determined. There was no significant difference between the mean wing length of emergent adults from the treated pools ($F_{1, 40} = 0.771$; $p = 0.91$) and the controls ($F_{1, 60} = 1.183$; $p = 0.13$). Both powders significantly affected the amount of dissolved oxygen in the treated pools as compared to the controls ($F_{1,22} = 10.57$, $p = 0.0036$). In general, there was no significant difference in the mean water conductivity between the treated and control pools ($F_{1,45} = 0.965$; $p = 0.576$). Both plants showed no significant difference in the mean water temperatures between the treated and control pools ($F_{1, 37} = 0.738$; $p = 0.782$). The plant materials did not show any negative effect on non-target predatory organisms that also contribute in controlling mosquito larval population densities. The two plants exhibited high larval toxicity and may offer an alternative tool for the control of mosquitoes.

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1. Malaria

Malaria is a febrile illness caused by protozoa of the class Sporozoa and genus *Plasmodium* (Farr, 1988). There are four *Plasmodium* species that infect humans: *Plasmodium falciparum, P. ovale, P. malariae* and *P. vivax*. However, *P. falciparum* is the most virulent species and predominates in sub-Saharan Africa, Asia, Oceania, and the Amazons (WHO, 1998a). The parasite is transmitted from one human to another through the bites of infected female *Anopheles* mosquitoes. In endemic regions where transmission is high, people are continuously infected although some may develop immunity to the disease (Allison, 1984). Malaria is diagnosed by the clinical symptoms and microscopic examination of the blood smears. The symptoms first appear 10 to 16 days after the infectious mosquito bite and coincide with the rupturing of infected red blood cells (RBCs) (WHO, 1998a).

1.1.1 Global distribution

It is estimated that more than 40% of the world's population live in areas with high malaria risk and the great majority are found in sub-Saharan Africa (WHO, 2000). The geographical area affected by malaria has shrunk considerably over the past 50 years due to the successful eradication and cessation of transmission in large areas of North America, southern Europe, the former Soviet Union, some countries in Asia and South America (WHO, 1998a). The disease is now confined to poorer tropical areas of Africa, Asia and Latin America (WHO, 1998a). The disease is one of the biggest impediments to economic progress in Africa. It is the biggest killer in the continent, and 90% of global malaria deaths occur in Africa. It accounts for 9% of the disease burden to Africa, and is responsible for one in four deaths for children below the age of five (WHO, 1998a).

In Kenya, malaria claims 30,000 children under 5 years of age every year; accounting for 40% of childhood illnesses, 30% of outpatient, 20% of inpatient attendance and 13% of deaths in public hospitals (GoK, 2001). Malaria is highly endemic in some parts of Coast, Nyanza and Western provinces. Recently, some districts in Kenya have been

affected by outbreaks of "highland" malaria. These districts include, Kisii, Nyamira, Gucha, Kericho, Nandi, Narok, Turkana and Transmara (GoK, 2001). Since malaria transmission is unstable at these high altitudes and the human population has little or no immunity; the highlands are more prone to explosive outbreaks than the lowlands especially when the density of anopheline mosquitoes increases and weather conditions favour transmission (Lindsay and Martens, 1998; Lindblade *et al.*, 2000; Hay *et al.*, 2002).

1.1.2 Factors affecting the distribution of malaria

Several factors significantly affect the distribution of malaria in space and time, between persons, and the resulting morbidity and mortality. Some of these factors include; the natural environment through its vector populations, interaction between vector and parasite, parasite determinants and some of its genetically controlled characteristics, host-biological factors, behavioural, social and economic elements. Factors pertaining to the natural environment include, the availability of the breeding habitats for malaria vectors that influences the distribution of malaria in an area. Rainfall produces temporary pools favoured for breeding by most malaria vector species such as *An. gambiae s.s.* and *An. arabiensis*. The slope of the land and the nature of the soil are some of the other environmentally related factors that affect the type of surface water available and its persistence and subsequently the increase of local malaria vector populations. The optimal temperature range and the relative humidity for most malaria vectors are 20-30°C and 70-80%, respectively (Wernsdorf and McGregor, 1988). Increases in the temperature enhance the growth of vector population by shortening the interval from oviposition to adult emergence and vice versa. Biological factors such as immune response and genetics, as well as socio-economic status, living and working conditions, exposure to vectors, human behaviour and differential attractiveness all play a critical role in determining a persons risk of malaria infection and hence illness. Greenwood (1989) reported that climatic and topographic features determine the ecology of both human and arthropod hosts as well as their contacts. Many other environmental factors have been found to influence the level of exposure of an individual resident in a malaria endemic area to the mosquitoes. These include place and type of housing, the use of anti-

mosquito measures and the position of the house relative to the breeding sites (Greenwood, 1989).

1.2 Malaria vectors

Out of more than 400 described species of *Anopheles* (White, 1977) some 45 of them have been implicated in the transmission of malaria. Different species of *Anopheles* are responsible for the transmission of malaria in specific geographic areas. The density of the adult mosquito population is dependent on larval ecology. Irrigation schemes (particularly used for growing rice and sugar cane) are preferred breeding sites for *An. gambiae s.l.* and *An. funestus* (Mutero *et al.*, 1984). *Anopheles balabacensis* and *An. dirus* are abundant in the forested areas (Muirhead-Thomson, 1951). *Anopheles merus* have extensive breeding sites within the tidal limits of the Kenyan coastal line (Bryan, 1983). The vectors play an important role in the transmission of *Plasmodium spp* parasites in Kenya.

1.2.1 Distribution of *Anopheles* mosquitoes in Kenya

The primary malaria vectors in Kenya are *An. funestus* complex and three members of *An. gambiae* complex: *An. gambiae s.s., An. arabiensis* and *An. merus* (Coluzzi *et al.*, 1985; Petrarca and Beier, 1992; Collins and Besansky, 1994; Mbogo *et al.*, 1996). *Anopheles gambiae s.s.* and *An. arabiensis* are often closely associated with humans and represent the major vectors of malaria (Highton *et al.*, 1979). The distribution of these two species overlap and they occur sympatrically in large areas of tropical Africa (Petrarca *et al.*, 1992). In Kenya, *An. gambiae s.l.* and *An. funestus* are predominantly found along the coast and western regions around the lake basin (Mutero *et al.*, 1984; Beier *et al.*, 1988; Petrarca *et al.*, 1992; Githeko *et al.*, 1994).

Anopheles funestus has been shown to be an important malaria vector, in some cases playing a more important role than *An. gambiae* s.s. and *An. arabiensis* (Fontenille *et al.*, 1997). *An. funestus* is a member of a species complex comprising at least nine members, the adults of which are not easily distinguished on the basis of morphological characteristics (Gillies and De Meillon 1968; Gillies and Coetzee, 1987) although some

species may be distinguished using larval characteristics. The members of this complex are *An. funestus s.s. An. vaneedeni, An. parensis, An. aruni, An. confuses, An. lessoni, An. brucei, An. rivolorum*, and *An. fuscivenosus* (Gillies and De Meillon 1968; Kamau *et al*., 2002). Of the nine species in the complex, *An. funestus s.s.* has the widest distribution. It is also highly anthropophagic (Gillies and De Meillon, 1968).

1.2.2 Feeding and resting behaviour of adult *Anopheles* mosquitoes

Eighty percent of female *Anopheles* mosquitoes feed on any large mammal that is available (Gillies, 1972). The host preference by a particular species of mosquitoes is also likely to be influenced by environmental conditions and availability of the host (Gillies, 1972).

Some of the mosquitoes are strictly zoophilic while others are anthropophilic. Of the 3 species of *An. gambiae* complex, *An. arabiensis* and *An. merus* are partially zoophilic and partially endophilic (White, 1974; Mosha *et al.,* 1983; Mutero *et al.,* 1984). Studies in western Kenya have shown that *An. arabiensis* has a lower proportion (in terms of frequency) of human blood meals, which reflects a higher degree of exophily (Joshi *et al.,* 1975; Highton *et al.,* 1979; Githeko *et al.,* 1994). Petrarca *et al* (1992) found that a significant proportion of *An. arabiensis* fed on cattle but were collected indoors, this supports earlier observations that *An. arabiensis* are partially zoophilic and endophilic. Meaning that they feed on cattle outdoors but always rest inside the houses. *Anopheles arabiensis* is generally easier to be diverted to cattle feeding than *An. gambiae s.s* (Githeko *et al*., 1994). *Anopheles gambiae s.s.* is primarily endophilic and endophagic whereas *An. arabiensis* and *An. merus* show some degree of exophily and zoophagy (White, 1974; Coluzzi *et al.,* 1979; Gillies and Coetzee, 1987). Studies along Kenyan coast by Mwangangi *et al*., (2002) showed that *An. gambiae s.s.* and *An. merus* fed predominantly on humans. This implies that *An. gambiae s.s.* is anthropophillic and efficient vectors of malaria. However, studies by Mbogo *et al*., (1996) with ITNs trials showed that there was a greater tendency toward exophagy rather than the typical endophagy of most anthropophagic *An. gambiae s.s.* in the villages where ITNs were introduced. The adult mosquitoes that emerge from larvae exposed to the chemicals may

be efficient vectors of malaria. In this study, emerging adults were collected from the treated pools. Therefore, understanding of feeding and resting behaviour of adult mosquitoes may help to determine whether these emerged adults may be of higher vectorial capacity in terms of their source of mosquito blood meal and hence their capacity for parasite transmission.

1.2.3 The life cycle, morphology and behaviour of *Anopheles* mosquitoes

Under optimum conditions, the complete cycle from egg to adult takes 7-14 days or slightly less depending on the environmental conditions (Service, 1996).

Eggs. After mating and feeding on blood, the female *An. gambiae* lays some 50-200 brownish or blackish boat shaped eggs. The eggs are laid singly on the water surface and measure 1 mm in length. Viable eggs hatch into larvae within 2 to 3 days in the tropics, but in cooler temperate regions they may not hatch until after 4 to 7 days or longer (Service, 1986).

Larvae. These are aquatic, metapneustic and pass through four larval instars. While on the water, they lie parallel to the surface to allow water intake and surface feeding. Mosquito larvae can be distinguished from all other aquatic insects by being legless and having a bulbous thorax that is wider than the head and the abdomen. All mosquito larvae require water in which to develop. No mosquito has larvae that can withstand desiccation although they may be able to survive short periods in wet mud (Service, 1996).

Anopheline larvae lack a siphon, with the result that when they are at the water surface they lie parallel to it and not subtended at an angle like the Culicinae (Service, 1996). The larvae feed and spend most of their time at the water surface. The abdomen has small, brown sclerotized plates (tergal plates) on the dorsal surface of abdominal tergites number 1- 8. In addition, most or all of these segments have a pair of well-developed palmate hairs (float hairs) (Service, 1996). On either side of abdominal segment number eight, there is a group of spines that are joined together at the base to form a structure

called the pectin (Service, 1996). Culicine larvae can also be distinguished from the anophelines by the following characteristics; all culicine larvae possess a siphon, which may be long or short. They hang upside down and at an angle from the water surface when they are getting air. There are no abdominal palmate hairs or tergal plates as seen in anophelines (Service, 1996). At mean water temperatures of 25-28^{0}C, the larvae undergo four moults (instars) within 6-9 days to reach the pupal stage (Service, 1996).

Pupae. They are unable to feed. Being less dense than water, they normally spend most of their time at the water surface breathing through the paired respiratory trumpets. The pupal coat splits dorsally and the adult emerges. Pupal duration is determined by temperature; in tropical countries, it is usually 2 –3 days (Service, 1996).

Adults. The newly emerged adults inflate its wings, and separate grooming its head appendages before flying away (Kettle, 1992). When the progeny of any one egg batch emerge as adults, the males emerge first and become sexually competent within 24 hours after emergence. By the time the females emerge, the males are ready for mating. The females require a blood meal for ovarian development followed by the maturation and oviposition of a batch of eggs (Gillies, 1955).

The percentage of the eggs, larvae and pupae that survive to the adults is unknown. However, there is usually heavy mortality, especially among larvae due to predators, disease, drought and flood among other factors (Service, 1996). Larval loss due to predation is one of the factors that reduce the numbers that develop into adults. In some instances, predatory *Culex (Lutzia) tigrepes* colonizes the same pools as *An. gambiae*, causing a dramatic reduction in larval density (Haddow, 1942). In permanent wells in Tanzania, Christie (1958) concluded that predation pressure was so intense that few larvae survived to pupae. Notonectidae were among the most important predators in the wells. It is possible that the same pressures exist in other types of permanent waters, thus limiting the productivity for *An. gambiae*. It is noted that the activity displayed by *An. gambiae* larvae, in contrast to species such as *An. funestus*, would tend to increase their

vulnerability to attack by predators (Service and Oguamah, 1958). The understanding of the life cycle is important as it can help one to know the crucial stage to target for control.

1.2.4 The body size of adult female *Anopheles* mosquitoes

Adult mosquito body size, usually quantified by wing length, has been reported to influence the degree of parousness and other bionomic factors in mosquito populations (Nasci, 1987). For instance, Nasci (1990) showed that for several mosquito species, blood feeding success and survival rate rose with increasing female body size. Briegel (1990) found that blood meal size and fecundity were positively correlated with the body size in *An. albimanus*, *An. gambiae* s.l and *An. stephensi*. Since larger mosquitoes were efficient fliers than smaller individuals, the size is likely to affect the prospects for females becoming inseminated (Nayar, 1969). Studies by Ameneshewa and Service (1996) revealed differences in the parous rate between three classes of *An. arabiensis* indicating that the probability of a female becoming parous under field conditions is significantly proportional to its wing length, and presumably its overall body size. Adult mosquito size is also regarded as important factor in influencing fecundity (Reisen, 1975; Steinwascher, 1982) and blood meal size (Briegel, 1990; Kitthawee *et al.*, 1990). The significant correlation observed between wing length of *An. arabiensis* and insemination rate indicates that larger females have a better chance of mating than smaller ones (Wing *et al.*, 1985). This might be attributed to the better flight capacity. However, Wing *et al* (1985) observed no relationship between size of female *An. quadrimaculatus* and susceptibility to malaria infection.

Several studies have indicated that variations in adult body size among vector mosquito populations are epidemiologically important because reproductive success, longevity, blood meal capacity and frequency of feeding, as well as parasite infection rates, all increase with the adult mosquito size (Reisen, 1975; Steinwascher, 1982; Wing *et al.*, 1985; Briegel, 1990). The current study investigated the effect of the plant materials on emergent adults body size. If the factors yielding smaller sized adult mosquitoes were to be identified and integrated into malaria control programmes, then the production of smaller and less virile adults that are less efficient disease vectors could be enhanced.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Malaria control

The strategy for malaria control is based on breaking the chain of transmission of the parasites between humans and mosquitoes. There are presently two approaches: control of parasites by chemotherapy, chemoprophylaxis; or vaccination and breaking the contact between host and vectors by controlling the vector population by using insecticides, repellents, screens such as bed nets, window gauzes and curtains, or environmental management.

2.1.1 Chemoprophylaxis

Various synthetic anti-malarial drugs have been used for curative or prophylactic purposes. Use has been made of drugs such as quinine, chloroquine, mefloquine, proguanil, 4-aminoquinolines, halofantrin, artemisin, pyrimethamine and sulfadoxine. However, increased resistance to some of these drugs by *Plasmodium species* has been reported (Leornado *et al.*, 1995). The efficacy of chloroquine in malaria chemotherapy has been compromised with the development of resistance to the drug by malaria parasites. In many parts of Africa, the drug is no longer used alone for management (Brasseur *et al.*, 1998). Malaria parasite resistance to alternative anti-malarial drugs such as 4-aminoquinolines, antifolics, quinine, fansidar and halofantrin has also been reported (Figgit *et al.*, 1992; Watkins and Mosobo, 1993; Leonardo *et al.*, 1995; Rosenthal, 2001; Ridley, 2002; Sachs, 2002). The two first line drugs, chloroquine and Fansidar, are no longer effective in many parts of East Africa where chloroquine resistance is rampant (Rosenthal, 2001; Ridley, 2002; Sachs, 2002). The synthetic anti-malarial drugs have therefore not achieved the main goal of malaria eradication due to increased cases of drug resistance, high costs, high failure rates and side effects being reported in many parts of the world (Leornado *et al.*, 1995; Rosenthal, 2001; Ridley, 2002; Sachs, 2002).). For prophylaxis, other drugs including amodiaquine, pyrimethamine, proguanil, and sulfadoxine may be used in areas where chloroquine resistance is high. The antibiotic

deoxycyline (a tetracycline derivative) is also an effective prophylactic when used in combination with other drugs. However, WHO discourages the use of these second line drugs for prophylaxis, because long-term usage may contribute to resistance development (WHO, 1998b).

Chemoprophylaxis as a control strategy has been attempted and is still being debated (WHO 1998b). Treatment is considered necessary for pregnant women, but prophylaxis for small children is debatable because of the risk of long-term side effects and danger of increased selection for resistant parasite strains (Carnevalle and Mouchet, 1987). In addition, drug administration can prevent development of natural immunity or simply delay disease development until children are older (Carnevalle and Mouchet, 1987). Furthermore, a countrywide prophylaxis exercise is expensive and requires strong organization. Therefore, there is need to change focus from treatment to prevention from infective bites or the disease through vector control and vaccination.

2.1.2 Vaccine development

An effective malaria vaccine has been a goal of researchers for more than 30 years. Repeated exposure does result in high degree of natural immunity, primarily to the erythrocytic or blood stages. Immunity does not prevent infection, but death and morbidity are reduced and infections in adults are mild or asymptomatic. The search is underway for pre-erythrocytic stages (Butcher, 1992; Carnevale and Mouchet, 1987; Carlson *et al.*, 1994). A pre-erythrocytic vaccine would protect against the infectious form injected by a mosquito (sporozoite) and inhibit parasite development in the liver (Hoffman, 1996). It has been demonstrated that in a previously unexposed individual, if a few parasites were to escape the immune defenses induced by a pre-erythrocytic vaccine, they could eventually multiply and result in full blown disease (Hoffman, 1996).

Many attempts at malaria prevention have been directed towards pre-erythrocytic vaccine. The synthetic molecule SPf66, developed in Colombia (Patarroyo *et al.*, 1987) to prevent *P. falciparum* malaria, is one of the anti-malaria vaccines currently being "tested" in human populations. SPf66 is both blood stage and sporozoite vaccine,

however, it is usually directed at blood stage parasites (Graves, 2001). The peptide has been tested in animal models and human volunteers subjected to experimental challenge (Patarroyo *et al.,* 1987; 1988) as well as in individuals from endemic areas in Colombia and other countries in America and Africa (Valero *et al.*, 1993; Sempertegui *et al.*, 1994; Teuscher *et al.,* 1994). SPf66 has been shown to induce production of specific IgG antibodies against the peptide in approximately 60% of vaccinees once immunization is completed (Sempertegui *et al.,* 1994; Teuscher *et al.,* 1994)). Initial results in children and adults residing in relatively low endemic regions of South America and a highly endemic area of Tanzania suggested that this vaccine might delay the time to a first clinical malaria episode, but subsequent trials in the Gambia and Tanzania, children in Thailand and adults in Brazil failed to show efficacy (Graves, 2001). There is no evidence of efficacy for SPf66 in Africa but it has caused a modest reduction of malaria attacks in South America (Graves, 2001). The first pre-erythrocytic vaccine to show significant protection against natural *P. falciparum* infection is RTS, S. In conjunction with a new adjuvant, ASO2, the vaccine was reported to be partially effective against natural infection with *P. falciparum* in semi immune men in the Gambia (Bojang *et al.*, 2001). Protection was partial and short lived, but the vaccine could have potential in epidemic areas with low endemicity or short transmission seasons (Bojang *et al.*, 2001). These vaccines need to be long lasting or boosted by natural reinfections as well as being cheap to produce. Nevertheless, even if a highly effective vaccine is available, problems of poverty, lack of public infrastructure, management constraints and community mobilization may contribute to incomplete coverage of the populations most at risk (Hopkins, 1985).

2.1.3 Vector control

Vector control includes activities that reduce the number of infective or infectious bites of the vector by reducing the vector density, longevity and preventing human-vector contact. The principal aim of vector control is the reduction of disease morbidity and mortality by reducing the level of transmission. It involves the use of methods targeted at controlling the mosquito population at larval or adult stages of their life cycle. Vector control is assumed to be one of the effective methods for malaria control since it is easier

to control mosquito populations within a given geographical area than giving vaccines for protection or administration of prophylactic drugs to individual persons.

2.1.3.1 Control of adult *Anopheles* mosquitoes

Insecticides such as pyrethrum extracts have been used extensively in mosquito control, but the quick bio-degradability and high costs of isolation of natural pyrethrins reduced their use (Sukumar *et al.,* 1991). This accelerated the development of affordable and persistent synthetic pyrethrins like permethrin and allethrin. Synthetic pyrethroids such as allethrin and permethrin have been effective insecticides (Shidrawi, 1990; Chandre, 2001). These compounds have however been found to be toxic to many non-target organisms. Moreover, increased vector resistance has been reported against synthetic pyrethroids (Shidrawi, 1990). Household spraying and residual insecticides is highly effective in reducing malaria in some parts of Africa (Chandre, 2001). However, their effectiveness is already under threat as a result of the emergence of pyrethroid resistance in *An. funestus* in Mozambique and *An. gambiae* in West Africa (Chandre, 2001). The reported resistance to the synthetic pyrethrins calls for use of other more effective insecticides mostly from the plant kingdom. Vector resistance to synthetic insecticides is a recurring theme and a major problem in malaria control programmes (Shidrawi, 1990; Chandre, 2001). By 1985, at least 117 mosquito species had been reported to be resistant to one or more of insecticides with 67 of these in the genus *Anopheles* (Pant, 1988; Shidrawi, 1990; Chandre, 2001). The best evidence of resistance to pyrethroids in the *An. gambiae* complex is the 5.8-fold tolerance to bioallethrin seen in a strain of *An. gambiae* from Burkina Faso (Malcolm, 1985; Pant, 1988). A population of *An. gambiae* in an area of western Kenya showed a 2.5 - fold increase in the LT_{50} one year after a permethrin-impregnated bed net study was implemented (Vulule *et al.*, 1994). Organophosphate pesticides are alternatives to pyrethrins since they have short persistence. However, resistance has been reported to these insecticides as well (Lines, 1988). Organocarbamate insecticides, specifically carbaryl and propoxur have been used in the chemical control of *Ae. aegypti* during out breaks of dengue fever or when the vector is detected during surveillance activities (WHO, 1997). The data collected indicate that there is some amount of reduced sensitivity to carbaryl in the populations of adult *Ae.*

aegypti mosquitoes (WHO, 1997). Malathion resistance has been recorded in *An. arabiensis* in the Gezira District of Sudan (Lines, 1988). The widespread use of persistent insecticides facilitates resistance development, especially when selection pressure is applied against a large proportion of the population. DDT and other organochlorine insecticides exemplify this. The evolution of resistance to most insecticides by the vector has prompted the need to develop new tools for vector control.

Repellents have also been used in vector control. Synthetic repellents such as dimethyl phthalate and 2-ethyl-1, 3-hexanediol have not provided a great impact in controlling the rate of inoculation and transmission of malaria parasite since most of these repellents are highly volatile and thus provide only short lived protection against the vector. The most common mosquito repellent formulations available on the market contain DEET (N, N-diethyl-tuolamide), which has shown excellent repellency against mosquitoes and other biting insects (Yap, 1986; Coleman *et al.,* 1993; Walker *et al.,* 1996). The use of plants as traditional natural repellents has been documented from many areas (Curtis *et al.*, 1990), but most of the products from plants have not been analyzed. Citronella products are used in India and are effective against anopheline mosquitoes. However, their protective effects do not last long (Sukumar *et al.*, 1991). In Tanzania, the smoke from burning plants provided some protection (Trigg, 1996). However, the effectiveness of these methods is probably limited and will depend on both the biology of the local vectors and the intensity of the transmission. In China, *Eucalyptus* spp and *Artemisia* spp have been used for years as traditional repellents for mosquitoes (Trigg, 1996). The active principle, p–methane-3, 8-diol has been isolated and is now used commercially as a repellent, Mosiguard® for personal protection (Trigg, 1996).

The use of insecticide treated bed nets (ITNs) and curtains with pyrethroids seems to be the most promising available method of controlling malaria in endemic tropical countries. Although Vulule *et al.* (1994) have observed increased tolerance, several studies have shown that the use of ITNs is effective in reducing morbidity and mortality due to malaria (Lengeler, 2000). A trial of permethrin-impregnated bed nets in Gambia resulted in a 70% reduction in clinical cases of malaria in children who slept under nets (Alonso *et*

al., 1991). A series of impregnated bed net studies in Kenya documented a reduction in incidence of infections in children under 6 years during both the high and low transmission seasons (Mbogo *et al.*, 1996). Entomological inoculation rates (EIR) declined by 50% during the high season. Nevertheless, acquisition of new infections still occurred at a very high rate during the high transmission season, and it was estimated that 100% of the children would have been infected with *P. falciparum* within 13.6 weeks in the bed net villages and within 10.6 weeks for the controls (Beach *et al.*, 1993). Mbogo *et al.* (1996) observed that permethrin impregnated bed nets exert a major impact upon abundance of the indoor-resting principal vectors of *P. falciparum* in the coastal villages of Kenya. Densities of *An. gambiae s.l.* and *An. funestus* were 9 times lower in the houses where ITNs were in use as compared to households where no nets were used (Mbogo *et al.*, 1996). The behaviour of the vectors and human host largely affect the success of the control method. During the high transmission season, substantial numbers of vectors may be feeding outdoors - during the early evening before the usage of bed nets (Mbogo *et al.*, 1996). Thus bed nets may be most useful in areas where transmission is less stable, seasonal or of low intensity but not in high transmission areas and in places where vector populations may be high. ITNs are now regarded as the "panacaea" to malaria control. This is due to its encouraging effects in reducing both morbidity and mortality among children. However, it has been observed that, there is a tendency of behaviour change by vector species in the areas where bed nets are in use (Mbogo *et al.*, 1996). Studies in Kilifi District showed that, a significant proportion of malaria vectors appeared to bite earlier in the evening in houses where ITNs were used, with greater tendency towards exophagy rather than the typical endophagy of most anthropophilic *An. gambiae s.l.* (Mbogo *et al.*, 1996). This change in behaviour renders the use of ITNs less effective, as mosquitoes will often bite when bed nets are not in use. The use of ITNs in Kenya is not as widespread as might be desired (GoK, 2001). This is because; neither the nets nor the required insecticides are widely available or affordable to most communities. Other problems include non-compliance in the proper use of nets and failure to maintain the insecticide treatment rhythm (GoK, 2001). In the face of these limitations, there is need to think beyond ITNs and refocus on larval control which reduces the number of

emerging adults and consequently the vector populations which carry and transmit malaria parasites.

2.1.3.2 Control of larval *Anopheles* mosquitoes

The various means that are used for the control of malaria epidemics have not achieved much in the control of malaria parasite transmission in Kenya. A potential target of malaria control is the anopheline larva. This is because the life cycle can be interrupted before the emergence of adults that bite and transmit malaria parasites. Source reduction through modification of larval habitats was the key to malaria parasite eradication efforts in the United States, Italy and Israel (Kitron and Spielman, 1989). It is therefore rational that appropriate management of larval habitats in the sub-Saharan Africa may also help to suppress vector densities and malaria transmission rates (WHO, 1998b). The control of mosquito larvae may be one of the efficient and economical means of controlling malaria epidemics.

The classical method that has been used to kill mosquito larvae involves the application of oil on water. The oil contains poison that presumably affects the nervous system (Wigglesworth, 1976). *Anopheles* larvae below such film at 24°C die in 2 to 3 hours. The mosquito larvae may also die from suffocation, the oil also reduces the surface tension hence the larvae cannot come out of the water for air. However, the oil film on the water surface is likely to prevent free exchange of oxygen between the water surface and the free air thus leading to suffocation of other non-target aquatic organisms. This factor has prompted the employment of other means of controlling mosquito larval populations. These methods include: environmental management, biological control, natural organic larvicides and botanicals or use of plant materials.

2.1.3.2.1 Environmental management

This involves practices that create unfavorable habitats for larval survival. It may also involve the elimination of aquatic habitats. A simple approach is to fill with rubble, sand, and earth larval habitats of different sizes (Service, 1996). Other environmental modifications include the removal of overhanging vegetation to reduce breeding by shade

loving mosquitoes such as *An. dirus* (Service, 1996). Deforestation can also eliminate the malaria vectors by destroying adult mosquito resting habitats. Planting vegetation along streams and reservoirs make habitats inimical to sun loving *An. gambiae*. However, this approach has not achieved much because it is impossible, to fill in all the scattered, small and temporary collections of water (Service, 1996). Secondly, the environmental changes such as agricultural irrigation schemes, creation of dams for water reservoirs and road construction or mining sites may favour the breeding of other species that were previously present in only small numbers or absent altogether (Service, 1996). Besides, the approach is labour intensive and costly thus untenable. There is, therefore, need to focus on more practical larval control methods such as biological control and natural larvicides.

2.1.3.2.2 Biological control

Biological control implies the use of predators, parasites or entomo-pathogens to reduce the population of other organisms. Due to insecticide resistance and the adverse environmental impact of insecticide use, considerable resources have been devoted to the search for biological control agents. Several attempts have been made to control mosquito larvae by biological means. To date, only larvivorous fish have been used successfully in malaria control projects, but these cases are few. The use of North American fish *Gambusia affinis* successfully reduced malaria incidences in Italy and Greece, where malaria transmission was unstable (Wickramasinghe and Costa, 1986). Prior to this, other fishes such as Armagosa pupfish (*Cyprinoden nevadensis armagosae*) and Guppies (*Poecillia reticulata*) were used (Moyle, 1976). These species reduced the number of mosquito larvae by almost half in most of the larval habitats during the entire study period (Moyle, 1976). The use of larvivorous fish, however, has its own disadvantages. The mass rearing and the restocking programmes required in the approach is very expensive. Besides, the fishes may not survive in some temporary breeding sites. Invertebrate predators such as coleopterans, dipterans and hemipterans have also been considered as biological control agents but are difficult to rear *en* mass; feed non-specifically, and do not persist once vector target densities are reached (Rishikesh *et al*., 1988).

Rishikesh *et al.*, (1988) have summarized efforts to identify useful pathogens and parasites including viruses, bacteria, fungi, nematodes and sporozoa. The main pathogens include: the fungi *Coleomomyces* spp, *Culicinomyces clavosporus*, *Metarhizium anisopliae* and *Lagenidium gigantium* that have demonstrated little or no adverse effects on populations of invertebrate and vertebrate non-target organisms (Lawrence and Cynthia, 1990). Other control agents include the protozoan *Nosema algerae* and the mermithid nematode, *Romanomermis culicivorax* (Rishikesh *et al.* 1988). None of these agents have shown any promise for wide scale larval control, having proven difficult to rear and store, as well as being unstable or inefficient under the field conditions.

The bacterial endospore toxins produced by various strains of *Bacillus* species such as *B. thuringiensis israelensis* H-4 and *B. sphaericus* have also been used as larvicidal agents (De Berjac and Sutherland, 1989; Davidson and Yousten, 1990). Their most attractive feature in vector control stems from the purported failure to induce mechanisms of resistance that confer cross-resistance to other classes of insecticides. They can also be produced on a local level with far less capital outlay than would be required for traditional insecticides. Unfortunately, the *Bacillus* toxins are still relatively expensive. Since they have no residual activity, they either require frequent application or are only suitable for environments where a one-time control measure produces a valuable outcome. Resistance of *An. gambiae* mosquitoes against the delta-endotoxins of *B. thuringiensis* has been demonstrated (Rao *et al.*, 1995). Although no resistance against the delta-endotoxin complex of *B. thuringiensis israelensis* has been detected so far, its long term use could reveal first signs of resistance (Rao *et al.*, 1995). The mechanisms of resistance to *B. sphaericus* are not yet defined (Rodcharoen and Mulla, 1996) but more than one mechanism seems to be involved (Rodcharoen and Mulla, 1996). Resistance to *B. thuringiensis* has resulted from reduced binding of the toxin to the brush border in the lumen of the insect gut (Rodcharoen and Mulla, 1996) or by enhanced binding of the toxin by gut proteases (Rodcharoen and Mulla, 1996).

2.1.3.2.3 Synthetic larvicides

Vector control by synthetic chemical larvicides has been implemented in some circumstances, especially when the use of residual adulticides was not effective or too expensive. The choice of such larvicides for mosquito control has been based on the species and behaviour of the mosquitoes, effects to domestic animals, wildlife, fish, other aquatic organisms, environmental pollution, presence of insecticide resistant mosquitoes and cost factors (Michael *et al.*, 1991). Paris green dust has been used to control larvae (Service, 1986). However, this compound is expensive due to its high copper content. However, there have been no instances of mosquito larvae developing resistance to most of these larvicides (Service, 1996). This may be explained by their limited use. Most of the inorganic larvicides are highly toxic to aquatic organisms and plants because of the relatively large amounts of water-soluble arsenic acid in them that pose environmental pollution problems. Synthetic organic chemicals have also been used in mosquito larval control. Use of emulsions or granular formulations of DDT, dieldrin, heptachlor, or lindane has been widely applied (WHO, 1984). Where resistant strains are encountered, parathion or baytex has been used (Metcalf *et al.*, 1962). However, most of these larvicides are organochlorines, organophosphates or carbamates. They are toxic and have cumulative environmental effects and persistence in the ecosystem due to their resistance to enzymatic degradation by soil and other environmental micro-organisms and chemical reactions (Charlese *et al.*, 1995). Temephos (Abate), an organophosphate of very low mammalian toxicity, has also been used to treat portable waters to control *Aedes aegypti* breeding in water storage pots (Service, 1996). Widespread use of the same pesticides for the control of agricultural pests has led to rapid resistance development in vector populations. Organocarbamates insecticides such as carbaryl, methomyl, carbofuran, and propoxur have also been used in the treatment of larval habitats during routine inspections in the Carribbean (Strickman, 1985). The use of carbaryl specifically for mosquito larval control has recorded a high degree of success (WHO, 1997). However, it is not recommended for use because the larval populations have developed resistant genes passed on from the adults (WHO, 1997).

For most malaria vectors, reducing mosquito population densities by means of larvicide application may be an efficient way of reducing malaria transmission especially when a large proportion of the larval habitat can be easily identified and targeted. The behaviour and ecology of the target vector determines the efficacy of the larvicide. For example, *An. gambiae* often breeds in small temporary rain pools. The number and wide distribution of these small pools may present insurmountable difficulties in control efforts using larvicides except in circumstances such as the eradication campaigns where the introduced species occurred in a limited geographic region (Laird and Miles, 1985).

The use of insect growth regulators (IGR) to control mosquitoes has also been attempted. IGR are chemicals which inhibit/disrupt growth of the insects. Most of these compounds have been grouped as: juvenile hormone mimics or chitin synthetase inhibitors (Laird and Miles, 1985). These compounds generally have no toxicity to other non-target organisms. They are relatively specific to the insect and primarily active against the immature stages of mosquitoes. Currently, the most widely used IGR is Altosid® (Laird and Miles, 1985). It has no remarkable effects on non-target aquatic organisms but it is not recommended for use in drinking water sources. However, there is a great desire to obtain larvicides or IGR from inexhaustible natural sources such as plants that can be cultivated, extracted and bio-degradable compounds obtained to avoid environmental pollution (WHO, 1996).

2.1.3.2.4 Natural organic larvicides

Various natural organic chemicals have been extracted from plants and bioassays carried out to determine their effectiveness as larvicides. One of the earliest reports of the use of plant extracts against mosquito larvae is credited to Campbell *et al.* (1933) who found that plant alkaloids like nicotine, anabasine, methyl-anabasine, and lupinine extracted from the Russian weed *Anabasis aphylla* killed larvae of *Culex pipiens, Cx. quinquefasciatus*, and *Cx. territans*. Haller (1940) noted that extracts from Amur cork tree fruit *Phellodendron amurense*, yielded a quick acting mosquito larvicide. The chemicals can be extracted from either whole plants or specific parts of the plants such as leaves, fruits, roots, and bark depending on the activity of the derivatives.

It has been shown that some limonoids (azadirachtin), quinones (plumbagin), alkaloids, flavonoids, terpenoids, polyacetylenes, and butyl-amides extracted from plants show a high degree of larvicidal activity against mosquito larvae (Kubo *et al.*, 1994). For instance, piperine and wisanine are alkaloids that were isolated from *Piper guineense* and found to be very active on *Aedes aegypti* larvae (Addae-Mensah and Achieng, 1986). The same extract has been shown to have larvicidal activity against *An. gambiae* in the laboratory (Okinyo, 2002). Limonoids such as azadirachtin from *Azadirachta indica* and terpenoids such as 5-E-ocimenone from *Tagetes minuta* have been reported to possess larvicidal activity against mosquito larvae (Maradufu *et al.*, 1978). Larvicidal activity of long chain fatty amides such as N-isobuyl-2E, 4E, 8Z, 10Z-dodeca-2, 4, 8, 10-tetraenamide isolated from *Spilanthes mauritiana* have been reported (Jondiko, 1989). The amides from *Zanthoxylum gilleti* (*Fagara macrophylla*) have also been reported as larvicides against *Culex* species (Kubo *et al.*, 1994). Their efficacy against *Anopheles gambiae* in the laboratory has since been demonstrated (Okinyo, 2002). Phenolics like 2-hydroxy-4-methoxybenzaldehyde from *Mondia whytei* have been reported to have larvicidal activity against *An. gambiae* (Mahanga, 2002). The same compound was reported to have tyrosinase inhibition activity (Kubo and Hammond, 1999) and flavouring activity (Mukonyi and Ndiege, 2001). Other plants that have been successfully tested for larvicidal activity include amongst others: *Vernonia ammophila, Swartzia madagarensis, Pogestemon cablin, Sium suave, Datira candida, Achryrolcline satureoides, Petiveria alliacea,* and *Gardenia lutea.* (Michael *et al.*, 1991). The efficacy of most of these plant extracts as potential larvicides have only been tested under laboratory conditions. However, their efficacy under natural field conditions against anopheline larval populations has not been investigated. As much as these investigations have not been done, their potential in mosquito control is thought to be high. This project examined the activity of *P. guineense* (Piperaceae), and *S. mauritiana* (Compositae), powder as potential mosquito larvicides under field conditions in Kilifi, Kenya.

2.2 Plant species

Almost all plants contain secondary metabolites that are toxic to certain organisms. These compounds are believed to play specific roles in plant defense against microbes, parasites, pests and predators (Jacobson 1989). Coincidentally, they exhibit bioactivity against other organisms that may not have any relation to the plants such as the animal disease vectors (Jacobson 1989). Plants and plant products having insecticidal or acaricidal properties are used traditionally by local communities in many different areas of the world against medically or agriculturally harmful insects, ticks and mites (Jacobson 1989; Curtis *et al.*, 1991). Several plants and products are traditionally used to kill or repel mosquitoes. A small number such as pyrethrum and citronella have become commercially important (Curtis *et al.*, 1991). For many plant species used traditionally against blood sucking insects there is lack of scientifically reliable data demonstrating their efficacy under uncontrolled field conditions. Many plants produce chemicals with anti-feedant, insecticidal or IGR properties, which could potentially be exploited to provide larvicides that are safer and considerably less environmentally damaging than synthetic chemicals. Among the most promising plants are *Piper guineense* and *Spilanthes mauritiana.*

2.2.1 *Piper guineense*

This is a dicotyledonous plant that belongs to the wider family of Piperaceae. It is known by its common names such as West Africa black pepper, Ashanti pepper, climbing black pepper and Kakamega forest climber. It is a wild slender plant common in the rain forests. It is a soft shrubby hairless climber to at least 10 m by means of adventitious roots near the nodes; stems are corky ridged near the base with solitary spikes. The leaves are elliptic or ovate in shape, with the base cuneate to subcordinate (often unequal), apex acuminate, 7-17 by 3-9 glabrous; the leaves are aromatic in smell when crushed. Flowers are minute, yellowish, in spikes 2-9 cm long. The fruits appear in clusters and are reddish brown when ripe and black when dry. In Kenya, the plant is common around Kakamega and Bukura forests (Kokwaro, 1993). The roots are used for the treatment of sore throat. The insecticidal and medicinal properties of the plant have

been known for centuries (Mbata and Ekpendu, 1992). Among other things, the seeds are traditionally used to protect grain from insect attack (Mbata and Ekpendu, 1992).

The fruits appear in abundance in the months of March, April and May when the rain is fairly heavy. *P. guineense* was chemically analyzed by Addae-Mensah *et al.* (1977) and found to contain various alkaloids as well as lignans and sterols. Some of the constituents of this plant have been found to have anti-microbial, hypotensive, sedative, and insecticidal properties (Ivbijaro, 1990; Mbata *et al.,* 1995).

2.2.2 *Spilanthes mauritiana*

This plant belongs to the family Compositae. It is a trailing herb with ovate toothed leaves under 5 cm; their heads are small of rather bright orange yellow florets with noticeable rays; phyllaries are 6-7 mm long, often a half as long as the head. *S. mauritana* is perennial in the tropics and sub-tropics, but may be grown as an annual plant in temperate regions. The plant is usually common in riverine grassland and lawns in the upland districts of 600-2000 m a.s.l. Such areas in Kenya include Nairobi, Machakos, Nanyuki, Narok, Mumias, Kitale and Mt. Elgon regions. Medicinally, the plant has been shown to induce lactation in virgins or older women (Richo, 1996). According to Richo (1996) the entire plant (root, stem, leaf and flower) is pharmacologically active and non-toxic to humans and most invertebrates. Native to the tropics of both Africa and South America, the plant must be ancient (Richo, 1996). People of traditional cultures independently discovered the utility of this plant for treating toothaches, as a powerful urinary antiseptic and as a prophylactic against malaria (Richo, 1996). The plant owes its activity to the antiseptic alkaloid spilanthol (present at a concentration of as much as 1.25% in the flowers) (Richo, 1996) as well as immune stimulating alkylamides. Spilanthol is effective at very low concentrations against blood form malaria parasites. This is the explanation for its utility against specifically malaria spirochetes either as prophylactic or as a treatment for malarial paroxysms (Richo, 1996). The herb is also a strong anti-bacterial agent. Studies show strong *in vitro* activity of *S. mauritiana* extracts against such common pathogens as *Eschereria coli* and *Klebsiella pneumoniae* (Richo, 1996). The leaves of *S. mauritiana* may be used as a salad

ingredient, but very sparingly. Jondiko (1989) investigated the larvicidal constituents of this plant and identified N-isobuyl-2E, 4E, 8Z, 10Z-dodeca-2, 4, 8, 10-tetraenamide as the bio-active principle.

2.3 Mode of action of plant extracts

The way that a particular insecticide affects its target is referred to as mode of action. Various modes of action exist, for example, stomach poisons affect the vector when they are ingested by the larvae during feeding and absorbed into the digestive tract. Some stomach poisons can also be applied directly to the vector or as a systemic to the host. The systemic insecticides are sometimes incorporated into the soil around ornamentals or bedding plants (Russell, 1972). The effectiveness of plant extracts against mosquitoes in most experiments is measured by their ability to delay the mosquitos' development time (Supavarn, *et al.,* 1996). In studies by Supavarn *et al.,* 1996, plant extracts had an effect on mosquitoes by delaying development, especially at the pupal stage. The reason for this phenomenon is not known, but previous reports by Russell, 1972 suggested that certain plants contain an insect hormone like substance that may inhibit insect development. The results of these tests also indicate that some plants used in the tests may have contained a similar substance. A few plant extracts have also been shown to affect larval development. For instance, sage significantly delayed larval development of *Aedes aegypti* (Supavarn, *et al.,* 1996).

2.4 JUSTIFICATION

Following increased incidences of malaria parasite resistance to majority of existing drugs in the market, there is need to control anopheline densities in the residential areas. Many governments in the developing world spend a lot of money treating people suffering from malaria. This has caused increased economic loses in terms of work time and low productivity with high costs of maintenance of a "sick nation". The use of insecticide sprays and repellents to control adult mosquito bites has not been efficient due to the increased reports of resistance to the synthetic insecticides or low protection times for repellents. In addition, the synthetic insecticides play a role in environmental

pollution. In some cases, certain mosquitoes have simply changed their cycle of activity and will attack at odd times for example, just before bedtime, early in the morning and sometimes during daytime when these insecticides are not being used. Indeed, most insecticides have also become too expensive for the poor communities. Due to the toxicity and high cost of anti-malarial drugs in the market coupled with their increasing ineffectiveness due to the development of resistance, there is need to find alternative effective vector control tools. Proper use of bed nets in the rural areas where majority of population lives has not been adopted as most people in the village cannot afford them. Global warming due to industrialization has also led to epidemics in areas previously malaria free. All these factors have contributed to the increase of malaria cases.

These factors call for effective control of mosquitoes in their early stages of development before they mature into adults that can bite and transmit malaria among other diseases. Therefore, there is an urgent need for research, development and formulation of effective mosquito larvicides that do not affect non-target aquatic life. Such compounds have been reported in various plant families. The extraction, isolation, and identification of the active compounds to be used for effective control of mosquitoes and hence control of malaria have been carried out. Various chemicals derived from plants have been tested for larvicidal activity against mosquito larvae and their ability to inhibit growth and development of mosquito larvae have also been documented. However, the efficacy of these larvicides has only been tested under controlled laboratory conditions using the laboratory strains of anopheline larvae. Therefore, there is need to test for the efficacy of the same larvicides under uncontrolled natural conditions using the natural populations of anopheline larvae. A comparison of the efficacy of the larvicides under laboratory and natural conditions will provide a stronger basis for their use in mosquito control programmes. The plant kingdom is therefore a rational choice of potential source of larvicides since the plants can be cultivated and pose no danger to environmental degradation and biodiversity depletion. These may provide an alternative economic lifeline to the rural farming communities by introducing new high value cash/ health crops.

2.5 HYPOTHESIS

Piper guineense and *Spilanthes mauritiana* powder does not have larvicidal activity against natural populations of mosquito larvae under field conditions.

2.6 OBJECTIVES

2.6.1 General objective

To examine the larvicidal activity and efficacy of *P. guineense* and *S. mauritiana* powder against natural population of mosquitoes under field conditions.

2.6.2 Specific objectives

1. To determine the larvicidal activity of *P. guineense* and *S. mauritiana* powder under field conditions.
2. To determine the relative time taken to cause 100% larval and pupal mortality.
3. To determine the relative doses at which the larvicides are most effective.
4. To determine the effect of the powders on the dissolved oxygen content, conductivity and temperature of the larval habitats.
5. To identify which of the two plant powders is most effective against anopheline and culicine larvae.
6. To determine the effect of the plant powders on morphological characteristic and body size of emergent adult mosquitoes.
7. To determine the effects of larvicidal powder of *P. guineense* and *S. mauritiana* on non target aquatic organisms.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

The study was carried out in Kilifi District in the Coast Province of Kenya (figure 1). The study area has been previously described for malaria epidemiological, demographic and entomological surveys (Mbogo *et al.,* 1993; Snow *et al*., 1993; 1994). Kilifi District lies between the latitudes 2° and 4° South and between 39° and 40° East (GoK, 1997). The District has a population of 544,303 according to the 1999 population and housing census (GoK, 2000). It borders Taita-Taveta to the west, Malindi to the north, Mombasa and Kwale districts to the south.

The average annual rainfall ranges from 400 in the hinterland to 1100 mm on the coastal belt. The District has two main rain seasons in a year. The long rains start from April to June with a peak in May while the short rains fall from October to December. The mean annual minimum and maximum temperatures in the district are 21.1 and 35.1^{0}C, respectively (GoK, 1997). The district is usually hot with temperatures of up to 34^{0}C and humid all year round with relative humidity more than 60% in the coastal belt (GoK, 1997). Kilifi District has four major topographical features: coastal plain, creeks and estuaries, foot plateau and Nyika Plateau. There is a strong correlation between topography and the soils in the area. The several soil types in the area differ widely in depth, texture, physical and chemical properties (GoK, 1997). Most of these soils are of low fertility. Seasonal rivers and streams form the drainage example.

Malaria is the leading cause of morbidity and mortality constituting 42-48% of all clinically diagnosed illnesses at Kilifi District Hospital (Snow *et al*., 1993). The prevalence of malaria parasites ranges between 24.4 and 90.0%. Most malaria cases occur in July following the long heavy rainy periods (Mbogo *et al*., 1993).

The ethnic communities in Kilifi district are the Giriama, Kauma and Chonyi that constitute some of the 9 sub-tribes of the Mijikenda at the Kenyan coast. The inhabitants

of this area are mainly peasants, growing maize and cassava for subsistence and coconuts, mangoes and cashew nuts as cash crops. Goats, cattle and occasionally sheep are kept for domestic consumption. Most houses have walls made of mud and thatched with palm leaves (makuti).

3.1.1 Study sites

The study was conducted a long Jaribuni stream of Jaribuni site (Figure 1) in Kilifi district. Jaribuni site is one of the 3 sites (Mtepeni, Majajani and Jaribuni) that have been used for extensive larval ecology studies along the Kenyan coast (Mbogo, Personal communication). This particular site was selected from a sub set of 3 sites previously examined for field collection of larval and adult mosquitoes (Mbogo, Personal communication). The criteria used for selection of the site included: (i) known aquatic habitats of anopheline mosquitoes based on preliminary surveys in the area (ii) presence of relatively high larval and adult populations and (iii) the relative permanence of aquatic habitats in the area. The stream pool commonly called Jaribuni River (Plate 2) by the locals lies 03^0 36.81^0S and 03^0 949.28^0E, at an altitude of 160 m and the nearest household from the stream being 140 m away.

3.2 Plant collection and preparation

Green leaves of *Piper guineense* and *Spilanthes mauritiana* were collected between August 2001 and March 2002 from Kakamega Forest (Western Province), Kenya and dried under shade for 30 days. The dry dark leaves were separated from the leaf stalks and ground into fine powder form by motor driven hammer mills. The powdered material was further filtered through a series of sieves with small (1 µm) mesh sizes to give the material for bioassay. The plant powders obtained from *P. guineense* and *S. mauritiana* were used for the field trials.

3.3 Preliminary laboratory bioassay

The preliminary bioassays were carried out in the laboratory (ICIPE, Nairobi, Kenya) as follows: To 1000 ml of distilled water in plastic trays, 25 third or fourth instar *An. gambiae s.s.* larvae were transferred into each tray and a known amount of the plant

powder added. Control trays received no treatment. The larvae in both trays were fed on ground fish meal. Larval mortality was recorded after 24, 48 and 72 hours, respectively. The doses used were, 1.0, 3.0, 5.0 and 6.0 g/l, respectively.

3.4 Larval sampling/ Source of larvae

Mosquito larvae were collected from aquatic habitats along Jaribuni stream pool in Kilifi District between July 2001 and April 2002. Larval sampling was carried out weekly. Each habitat was first inspected for the presence of mosquito larvae. The mosquito larvae and pupae were sampled by a standard dipping technique (Service, 1993) (Plate 2). This technique involved immersing a mosquito dipper (enamel bowl, with a long handle) in the breeding pools at an angle of 45^0. The surface water containing larvae and pupae would flow into the bowl. The collected larvae in the bowl were picked by pipettes and counted. The developmental stages (1st to 4th instar) of each larva were recorded. There was an interval of 2-3 minutes between each dip to allow stage 3rd, 4th instar larvae and pupae to return to the surface.

3.5 Larvicidal/ pupicidal assays

A total of 36 circular pools of 35 cm in diameter and depth of 15 cm were dug 1m from the edge of the stream. To prevent the effect of water run off from the stream due to the rains, all the pools flooded with water during the rains were considered incomplete experiments and thus were not included during the analysis. Thirty six plastic wash basins (35 x 13 cm) with a capacity of 3500 ml smeared with mud to mimic the natural aquatic mosquito larval soil habitats found in the area were inserted into each pool. Water (3500 ml) from the river was introduced into each pool and 24 of the pools treated with a known amount of the plant-derived powder and 12 used as controls. The stream water introduced into the basins was first thoroughly checked to confirm the absence of any first instar larvae. The control pools were not treated with the larvicide. Into each of the artificial habitats, a known number (50-100) of anopheline and culicine larvae of various instars and 30 pupae were introduced. The introduced larvae and pupae were left in the artificial habitat for one hour to get acclimatized to the new environment before the plant powder was applied. The pools were covered with modified "Saliternick" mosquito

cages to prevent the escape of emerging adults. Fish food (Tetramin® Baby) was added into the water 24 hours after the introduction of the larvae and pupae. This addition was necessary as a supplement to the natural food contained in the water previously collected from the breeding sites. This solved the problem of larval deaths due to reduced food content. There were four replicate experiments conducted for each powder and dose.

3.6 Field application of the plant powder

To 3500 ml water in each basin containing mosquito larvae and pupae, a known amount of the plant powder was added giving a known concentration. The plant powder was evenly sprinkled on the water surface and gently stirred to accelerate mixing. The doses used were: 8.571, 5.714, 2.857 and 1.429 g/l, respectively.

3.7 Pupal and larval mortality assessment

Pupal and larval mortality for each of the four instars was assessed by counting all the surviving/ living larval instars and pupa of the 50 – 100 larvae and 30 pupa introduced into the pools. The surviving larvae were sampled by dipping technique. Larvae were considered dead if they showed no movement after being agitated for five seconds. Pupal and larval mortality for each instar was monitored after 24, 48 and 72 hours, respectively, and the % larval mortality calculated indirectly using Abbott's formula, taking into account mortality in the controls (Abbott, 1925). This formula takes care of the "natural" larval mortality expected in both the control and experimental pools.

$$P_T = \frac{P_O - P_C}{100 - P_C} X100$$

Where, P_T = Corrected % mortality

P_O = Observed % mortality

P_C = Control % mortality

Figure 1: Study site

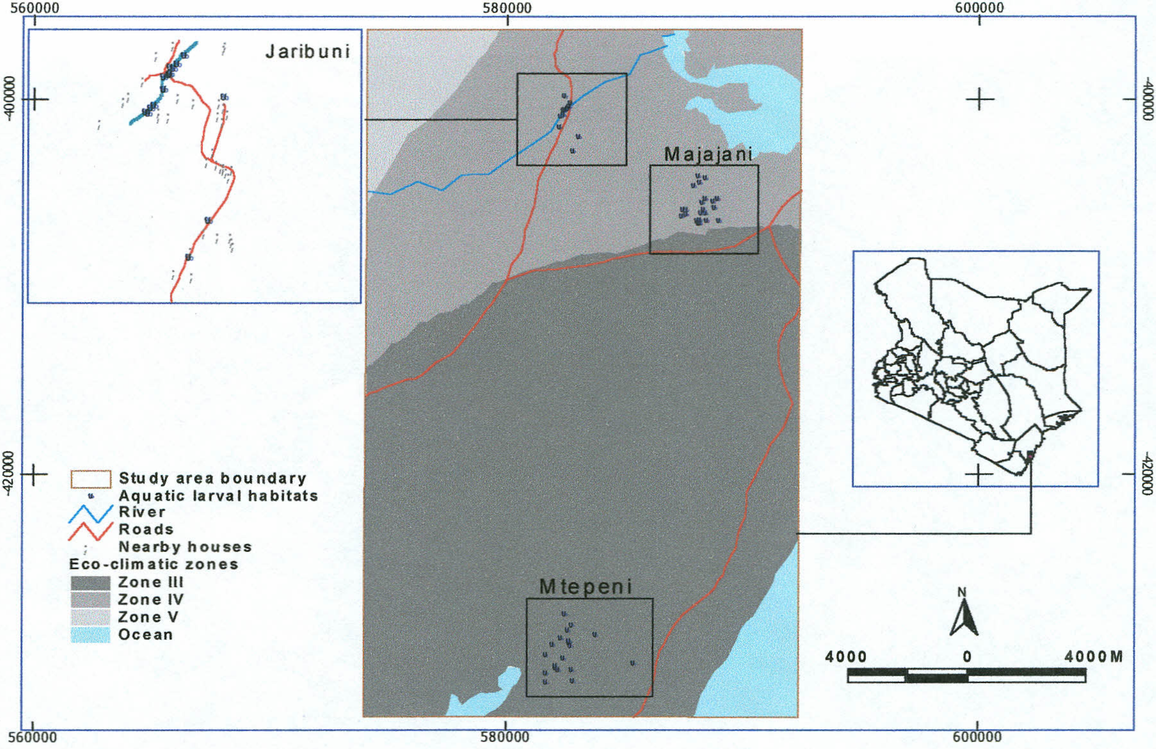


Plate 1: Jaribuni River

Plate 2: Larval sampling by standard dipping technique

3.8 Emergence of adult mosquitoes

The adult mosquitoes that emerged from the pupal stage at each site were trapped by use of modified "Saliternick" mosquito cages (Plate 3) used to cover the pools (Service, 1993). The cages were also used to prevent the entry of any external mosquito larvae or predators and to stop any external adult mosquito from laying eggs in the artificial habitats. The trap consisted of a galvanized framework covered with mosquito netting with the base left open and provided with a cord handle. A sleeve on one side of the cage enabled emerging mosquitoes to be removed by an aspirator. The trap had a length, width and a height of 45 cm. The emerging adults from the sites were caught and removed after 24, 48 and 72 hours, respectively. The emergent mosquitoes were collected by use of aspirator (WHO, 1975) and placed in a paper cup with 6% sucrose solution (w/v). The sucrose solution was to act as source of food for the adults since they had to be transported to the laboratory alive for identification. The collected mosquitoes were transported to the KEMRI laboratory in Kilifi for identification and wing length measurement.

3.9 Characterization of adults

The emergent adults were identified as either culicines or anophelines using known morphological characteristics (Gillies and Coetzee, 1987). The adult anopheline mosquitoes were further identified under a dissecting microscope as *An. gambiae s.l.*, *An. funestus* and other *Anopheles* by use of taxonomic keys (Gillies and Coetzee, 1987).

3.10 Wing size

The wings were removed from both female and male individual mosquitoes by use of forceps. They were placed on a microscope slide using DPX mountant and measured from the axial incision to the wing tip, excluding the fringe scales, using an occular micrometer mounted on a compound microscope. The remaining carcass of the adult *An. gambiae s.l.* were transferred to new vials and preserved in 100% ethanol for future PCR analysis to determine the sibling species. *Anopheles funestus* complex could not be speciated due to logistic reasons.

Plate 3: Emergent mosquito cages placed over pools of water

3.11 Water parameters

On every occasion the artificial habitats were visited (before and after larvicidal application), three parameters were recorded in each pool. These included water temperature, conductivity and dissolved oxygen. Portable hand held field meters were used for the measurement of water temperature (Meter model 5996-70, Corning Inc, New York) and conductivity (Cole Palmer 33,YSI meter, Chestertown, MD). Standard chemical kits (La Motte Chemical, Vernon Hills, IL) were used for determination of dissolved oxygen concentration. These were special instruments that were inserted into each pool. After duration of 2-3 minutes the instrument (meters) automatically recorded the parameters (dissolved oxygen, conductivity or temperature) in the pool.

3.12 Non target aquatic invertebrates and vertebrates (predators).

Six taxons of aquatic invertebrates and two vertebrates were used for bioassays during the study. These included: damsel fly nymph (Zygoptera), dragon fly nymph (Anisoptera), notonectids (Notonectidae), macrodytiscids (Dystiscidae), microdysticidae (Dystiscidae), shrimps (Crustacea), tadpoles (Amphibia), and *Tilapia* (Fish). The taxonomic determinations were made with the help of John Carlson of the Department of Tropical Medicine, Tulane University, USA. These taxons were chosen because of the following reasons: (i) abundance at Jaribuni study site (ii) their representation of different orders and classes (iii) previous identification as predators of mosquito larvae that play a great role in larval population regulation (Service, 1977) and (iv) ease of identification under field conditions.

All aquatic non-target organisms tested were collected from the aquatic habitats along the river. The test organisms were collected on each day by the use of standard dipping technique or aquatic net as already outlined. The specimens were placed in white plastic trays for the identification of the organisms with individuals of the species to be tested being removed manually using pipettes and sieves and then placed in the basins.

Forty eight circular pools (35 x 15 cm) dug 1 m from the edge of the stream were fitted with plastic wash basins (35 x 13 cm) with a capacity of 3500 ml, smeared with mud to mimic the natural habitats found in the area. Water (3500 ml) was introduced into each

pool and 32 of which were treated with a known amount of the plant-derived powder while 16 were left untreated and served as controls.

The powders of *P. guineense* and *S. mauritiana* were used for the trials and the highest and lowest doses previously tested in larvicidal assays used. The forty eight plastic basins filled with water were divided into 8 groups, each comprising 6 basins. Each group consisted of four treatment and two control basins. The collected organisms were introduced into each basin. Into each group, two doses (1.429 and 8.571 g/l) of each plant (*P. guineense* and *S. mauritiana*) powder were applied. Non-target organisms mortality was assessed by counting all the surviving/ living organisms of the total number of individuals introduced into each pool. The surviving organisms were sampled by dipping technique. Mortality was recorded after 24 and 48 hours. Test species were considered dead if they showed no movement after being agitated for 5 seconds. In the experimental set up, 15 damsel fly nymphs, 15 macrodystiscids, 5 dragon fly nymphs, 10 notonectids, 40 microdystiscids, 15 shrimps, 5 tadpoles and 15 fish per basin were added to each of the treated and control basins. There were four replicates for each dose.

3.13 Data processing and management

The data collected were entered onto data sheets (appendices 1 &2) in the field and then double entered into Excel spreadsheets on the day of collection. The data was analyzed by use of SPSS for Windows® (Version 10.0) (CDC, Atlanta, USA) and Microsoft Excel. Data was transformed using the log transformation (Log_{10} (1 + n) before statistical analysis to normalize distribution. The variables transformed were water temperature, conductivity, and dissolved oxygen. One-factor analysis of variance (ANOVA) was used to examine the significance of the effectiveness of the two plant powders and water parameters (Sokal and Rohlf, 2001). Tukey test (Sokal and Rohlf, 2001) was employed to evaluate the significance of the treatments. Chi square test was used to determine the significance between the mortality of non-target organisms between the hours of sampling (Sokal and Rohlf, 2001). Log probit analysis (Finney, 1981) was used to determine log dose regression lines for the mosquito larval mortality in relation to the plant powder dosage.

CHAPTER FOUR

4.0 RESULTS

4.1 Preliminary laboratory study results

The corrected mean percent larval mortality obtained after the application of *P. guineense* powder against *An. gambiae* are shown in Table 1a. Probit analysis was performed on the results obtained from the powder and from the regression analysis and probit transformations; LD_{25}, LD_{50}, LD_{75} and LD_{90} values were calculated (Table 1b).

At the lowest dose (1 g/l), mean mortalities of 15, 45 and 71% were recorded after 24, 48 and 72 hours of exposure, respectively (Table 1a). Mean mortalities of 52, 67 and 85% were recorded after 24, 48 and 72 hours of exposure, respectively at a dose of 3 g/l. Relatively higher mean mortalities 62, 84 and 92% were recorded at 24, 48 and 72 hours of exposure, respectively at a dose of 5 g/l. When these larvae were exposed to the highest dose (6 g/l) high mean mortalities of 77 and 97% were recorded after 24 and 48 hours, respectively. At 72 hours, mean mortality of 100% was recorded at the same dose. In general, as expected, there were high mean mortalities at the highest dose compared to the lowest dose. In addition, larval mean mortality increased with time, higher larval mortalities were recorded after 72 hours compared to 24 hours of exposure.

The $LD_{25,\ 50,\ 75\ \&\ 90}$ were determined by probit analysis (Table 1b). The LD_{25} values were in the range 0.26-1.53. At LD_{50}, values recorded were 3.08, 1.35 and 0.61 after 24, 48 and 72 hours, respectively. The LD_{75} values recorded ranged between 1.40 and 6.21. At LD_{90}, the values recorded were 11.76, 5.56 and 2.99 after 24, 48 and 72 hours, respectively. In general there was a reduction in the LD values with time of exposure with less powder needed to cause larval mortality after 72 hours as compared to 24 hours.

A dose of 5.75 g/l of *P. guineense* was able to cause mortalities of more than 90% of *An. gambiae s.s.* larvae within 48 hours after treatment. It is on the basis of these observations that the relative doses for field studies were selected. Assuming that a dose of 5.7 g/l (20 g/ 3500 ml) would kill 90% of the larvae from the preliminary

results, doses 2.857 g/l (10 g/ 3500 ml) and 8.571 g/l (30 g/ 3500 ml) were selected since they were below and above the tested dose (5.7 g/l), respectively. The 1.429 g/l (5 g/ 3500 ml) was selected to check on the level of effectiveness of the powder at a very low dose.

4.2 Larval mortality

4.2.1 Effect of *Piper guineense* on anopheline larvae

The toxicity of the plant powder against all instars of anopheline larvae is presented in figures 2a, b and c. The powder produced substantial mortalities in all the anopheline instars at the highest dosage (8.571 g/l). Anophelines were highly susceptible to the powder with more than 99% mortality rates at 8.571 g/l, 72 hours after treatment. At the lowest dosage (1.429 g/l), the powder was relatively less effective against the anophelines.

Figure 2a shows the mean percentage larval mortality of 1st- 4th instar anophelines 24 hours after exposure to the powder. At the lowest dose (1.428 g/l), there were low mortalities of 12 and 25% in the second and fourth instars, respectively. However, at the highest dose (8.571 g/l), mortalities of 92 and 99% were realized in the first and fourth instars, respectively. The mean % larval mortalities for all the instars were 18% at the lowest dose, this was not significant ($F_{3,12} = 1.261$; p= 0.332). At the highest dose, non-significant ($F_{3,24} = 1.252$; p= 0.313) mean % larval mortalities for all the instars were 96%.

There was observed reduction of larval mortality in the first instars in three doses except 1.429 g/l after 48 hours compared to 24 hours after treatment (Figure 2b). At 48 hours after treatment, there were reduced mortalities of anophelines to 81 and 94% in the first instars at 2.857 and 5.714 g/l, respectively. Mortalities of 41 and 95% were observed for first and fourth instars, respectively at the lowest (1.428 g/l) and highest (8.571 g/l) doses, respectively.

Mean mortalities for all the instars after 48 hours were 48 and 98% for the lowest and highest dose, respectively. Non-significant mean mortalities of 97% ($F_{3,24} = 2.156$; p= 0.120) were recorded at a dose of 5.714 g/l. The mean % larval mortalities for all

the instars were 87% at a dose of 2.857 g/l, this was not significant ($F_{3,24} = 0.781$; $p= 0.516$). Nearly 100% mortality was observed for the highest dose for all the instars of anopheline larvae (Figure 2c) at 72 hours post exposure, however at the lowest dose (1.428 g/l), mortalities of 64 and 74% were recorded in the first and second instars, respectively. The mean % larval mortalities for all the instars were 67% at the lowest dose, this was not significant ($F_{3,12} = 1.491$; $p= 0.268$). At the highest dose, non-significant ($F_{3,24} = 0.692$; $p= 0.566$) mean % larval mortalities for all the instars were 99% for anopheline larvae after 72 hours.

Mortality after 24 hours was higher but not significant ($F_{1,14} = 0.279$; $p=0.605$) in early stages of anopheline mosquitoes than the late instars. However, this decreased with time in the highest dose causing lower mortality after 72 hours in early instars compared to late instars. There was no significant difference between the mortalities at 24 and 72 hours ($F_{1,46} = 0.189$; $P = 0.665$).

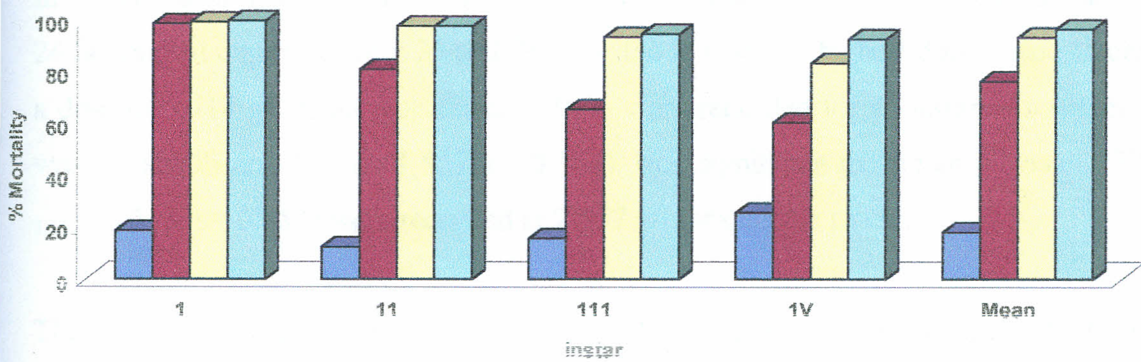
Table 1a: Corrected mean % larval mortality and S.E of *Piper guineense* powder

Dose (g/l)	**Corrected mean % larval mortality ± S.E**		
Time (hours)	24	48	72
1.00	14.70 ± 0.27	45.17 ± 0.42	71.41 ± 0.56
3.00	51.93 ± 0.50	66.80 ± 0.42	84.91 ± 0.22
5.00	62.04 ± 0.41	83.85 ± 0.87	91.80 ± 0.85
6.00	77.00 ± 0.58	96.99 ± 0.75	100.00 ± 0.00

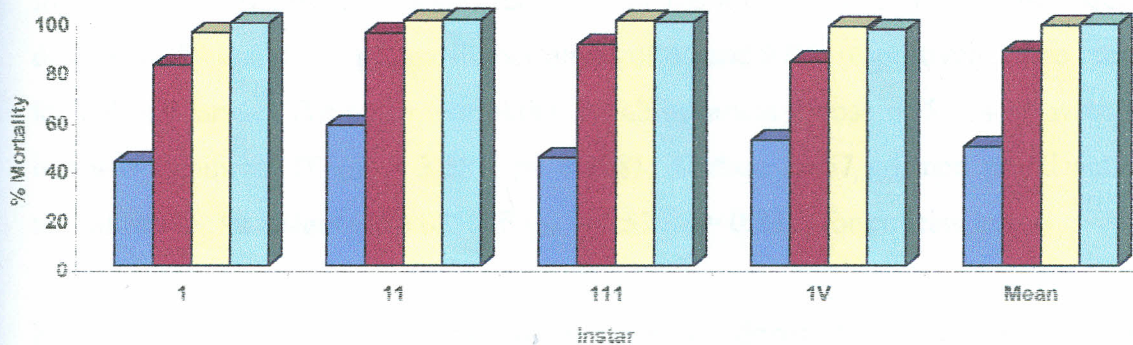
Table 1b: Lethal Dose (LD) values of *Piper guineense* powder

Time (Hours)	**Lethal dose (LD) in (g/l)**			
	25	**50**	**75**	**90**
24	1.53	3.08	6.21	11.76
48	0.65	1.35	2.84	5.56
72	0.26	0.61	1.40	2.99

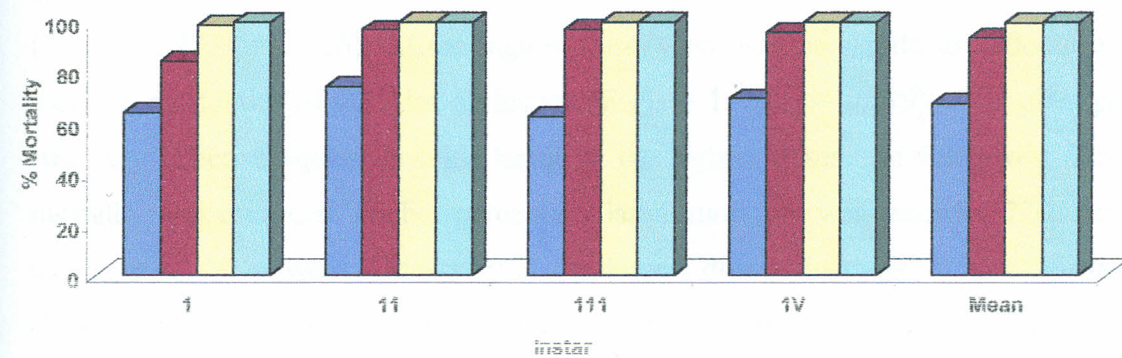
Figure 2a: Mean % larval mortality of anophelines in pools treated with *Piper guineense* after 24 hours



2b: after 48 hours



2c: after 72 hours



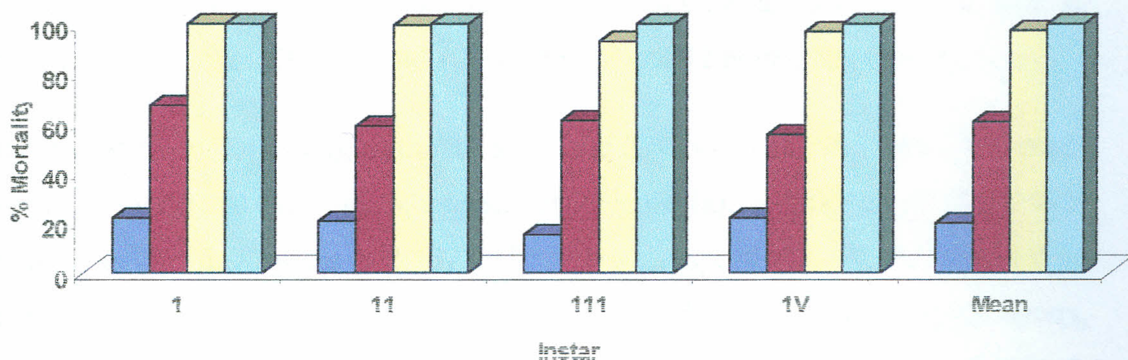
4.2.2 Effect of *Piper guineense* on culicine larvae

The toxicity of the *P. guineense* powder against all instars of culicines is shown in figures 3a, b and c. At 24 hours after application of the plant powder, percentage mortalities at the lowest dose (1.428 g/l) were 17 and 36% for third and fourth instars, respectively (Figure 3a). At the highest dose (8.571 g/l), 100 and 97% mortality was observed for first and fourth instars, respectively. The mean mortalities recorded for culicine larvae at 24 hours post exposure were 25 and 96% for the lowest and highest dose, respectively. At a dose of 5.714 g/l mean mortalities of 96% were recorded for all instars. However, this was not significant ($F_{3,24} = 1.857$; p= 0.164). Non significant mean mortalities of 93% ($F_{3,24} = 0.218$; p= 0.883) were recorded at 2.857 g/l for culicine larvae.

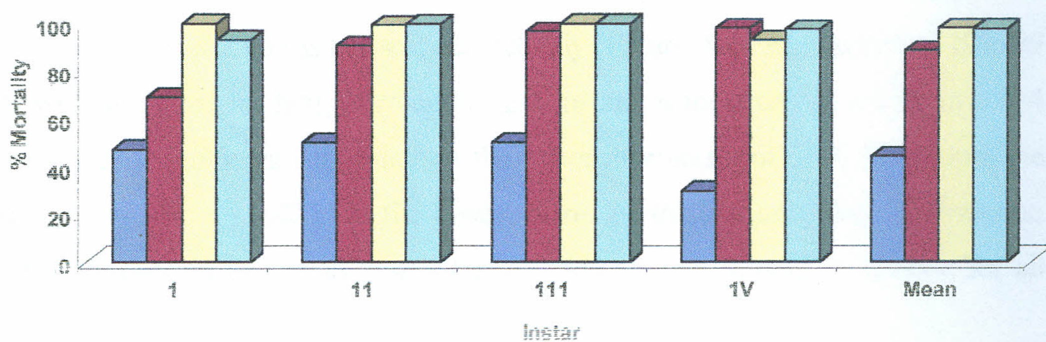
There was a reduction in larval mortality at 48 hours after exposure compared to 24 hours for the first instars at doses 2.857 and 5.714 g/l. After 48 hours of exposure, mortality of fourth and first instar larvae at the lowest dose was 29 and 72%, respectively (Figure 3b). At the highest dose (8.571 g/l), mortality of first instars of culicine larvae reduced to 93 from 99%. Mortality of 97% was recorded for the fourth instars at the same dose. At a dose of 1.429 and 8.571 g/l, mean mortalities of 44 and 97%, respectively, were recorded for culicine larvae. The mean mortalities for all instars at a dose of 5.714 g/l were 98%, this was significant ($F_{3,24} = 3.587$; p= 0.028). At dose 2.857 g/l, non significant mean mortalities for all instars were 88% ($F_{3,24} = 1.530$; p= 0.232) for culicine larvae

Mortality of 100% for third instars was recorded for doses of 5.714 and 8.571 g/l at 72 hours after exposure (Figure 3c). At the lowest dose, mortalities of 64 and 74% were recorded for first and second instars, respectively. There was no significant difference in the mean % mortalities of 63% for all the larval instars after 72 hours at the lowest dose ($F_{3,12} = 2.433$ p= 0.116). At the highest dose, there was no significant difference in the mean % mortalities of 98% for all instars ($F_{3,24} = 1.294$; p= 0.299). The plant powder was very effective against culicine larvae at the highest doses. At the lowest dose, low mortality was recorded. Higher percentage larval mortality was recorded 72 hours after exposure to the powder. Mortality at the lowest dosage increased with exposure time (from 24 to 72 hours). It increased remarkably for the first instars. There was no significant difference in mortalities between the hours of sampling ($F_{1,28}$=0.957; p=0.336). There was no significant difference in mortalities between the early and late instars ($F_{1,46}$ = 0.045; p= 0.831).

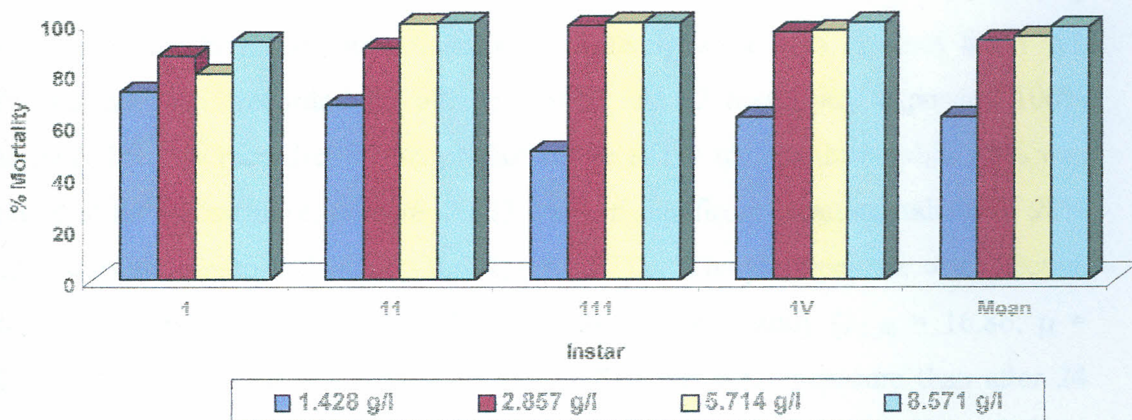
Figure 3a: Mean % larval mortality of culicines in pools treated with *Piper guineense* after 24 hours



3b: after 48 hours



3c: after 72 hours



4.2.3 Effect of *Spilanthes mauritiana* on anopheline larvae

The plant powder exhibited high larvicidal activity against all instars of anophelines at high doses (Figure 4a, b and c). Exposure of first instar larvae to the highest doses (8.571 g/l) resulted in 100% larval mortality 72 hours after treatment. The powder was highly effective larvicide against first instars (99.85%) for anophelines at a dose of 2.857 g/l after 24 hours, 99.77% and 100% after 48 and 72 hours, respectively.

At the lowest dose (1.428 g/l), mortality of 11 and 27% was observed after 24 hours of exposure to fourth and first instars, respectively (Figure 4a). Mortalities of 34 and 98% were recorded for first instars at 2.857 and 5.714 g/l, respectively. At the highest dose (8.571 g/l), mortality of 99 and 92% was recorded for first and fourth instars, respectively. The mean mortalities for anopheline larvae were 46% at dose 2.857 g/l, this was not significant ($F_{3,20}$ = 2.069; p= 0.137). At the highest dose, non-significant mean mortalities of 97% ($F_{3,20}$ = 1.450; p= 0.245) were recorded for all the instars. After 48 hours of exposure to the plant powder, mortality of first instars increased to 43 and 98% for 1.428 and 2.857 g/l doses, respectively (Figure 4b). Mortalities of 100, 99 and 97% were recorded for first, third and fourth instars, respectively at a dose of 5.714 g/l. There was a significant difference in the mean mortalities of 56% for anopheline larvae ($F_{3,12}$ = 4.326; p= 0.028) at the lowest dose. At the highest dose, there was no significant difference in the mean mortalities of 98% ($F_{3,20}$ = 0.343; p= 0.794) for all instars.

The percentage mortality at 72 hours post exposure of anopheline larvae to the powder is shown in figure 4c. At the lowest dose (1.428 g/l), mortalities of 93 and 79% were observed for first and fourth instars, respectively. At 2.857 g/l, 100 and 95% mortality was recorded for first and fourth instars, respectively while at 5.714 and 8.571 g/l, 100% mortality was recorded for all the instars. At 72 hours post exposure, 100% mean mortality was recorded for anopheline larvae at the highest dose, while 88% was recorded at the lowest dose. At dose 2.857 g/l, non significant mean mortalities of 98% for all instars were recorded ($F_{3,20}$ = 0.682; p= 0.573). The plant powder was effective against the anopheline larvae at the highest dose. Significantly ($F_{1,30}$ = 16.86; p = 0.002) higher larval mortality was observed at 72 hours after exposure than after 24 hours. There was no significant difference ($F_{1,46}$ = 0.940; p = 0.337) in the mortalities between the early and late instars.

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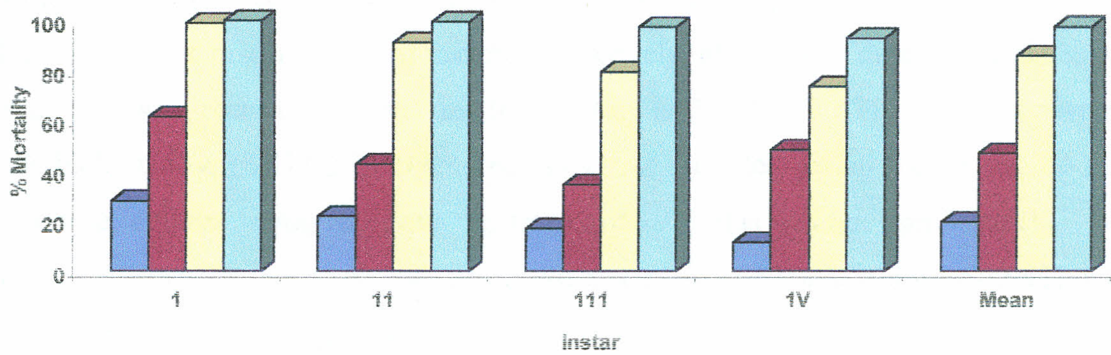
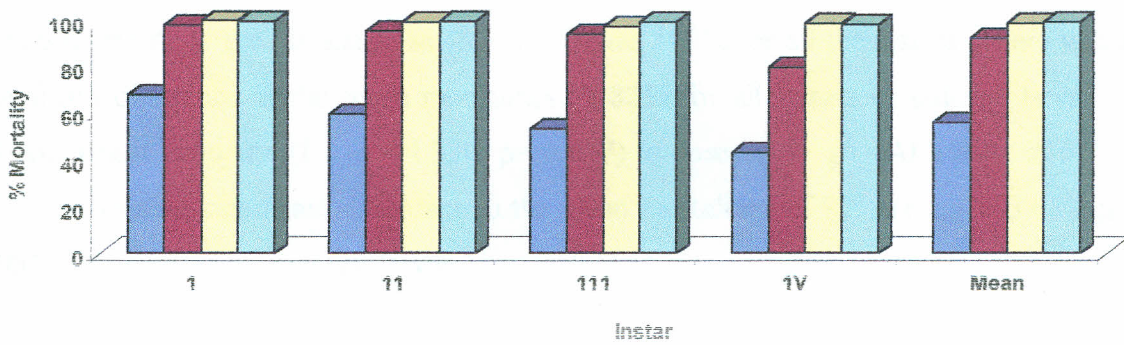
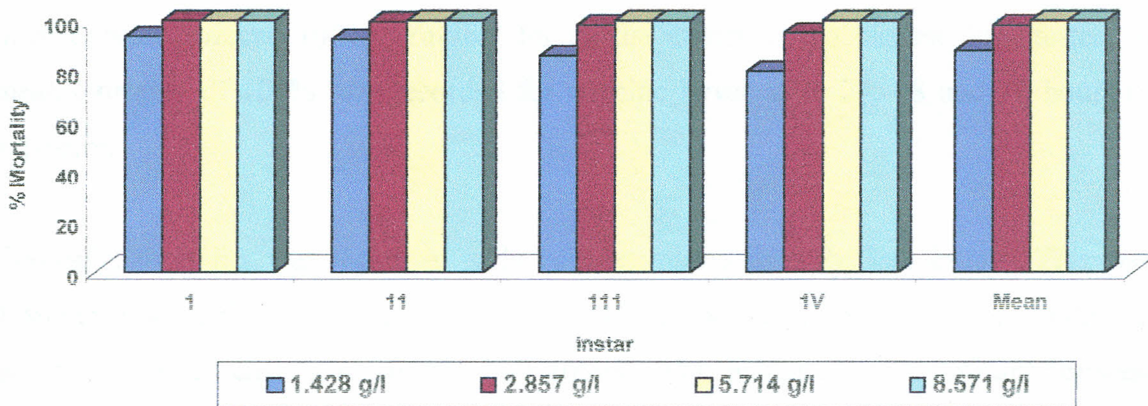
Figure 4a: Mean % larval mortality of anophelines in pools treated with *Spilanthes mauritiana* after 24 hours



4b: after 48 hours



4c: after 72 hours



4.2.4 Effect of *Spilanthes mauritiana* on culicine larvae

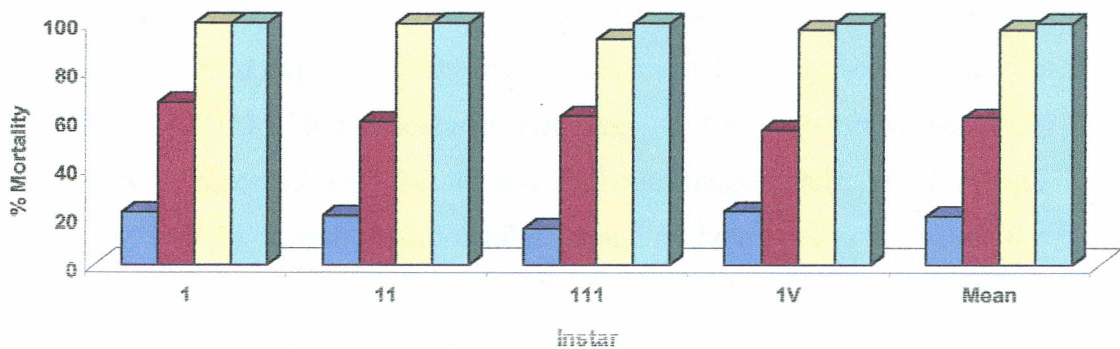
The larval mortality induced by the powder of *S. mauritiana* against all the instars of culicine larvae is shown in figures 5a, b and c. The larval mortality at 24 hours after exposure to the powder is shown in figure 5a. At the lowest dose (1.428 g/l), mortalities of 15 and 22% were recorded for third and fourth instars, respectively. Mortalities of 20 and 21% were for first and second instars, respectively, at the same dose. Mortalities of 66 and 93% were recorded for first and third instars at 2.857 and 5.714 g/l, respectively. At the highest dose (8.571 g/l), 100% mortality was recorded for all the instars. At the lowest dose, culicine larvae had mean mortalities of 19%, this was not significant ($F_{3,12}$ = 0.106; p= 0.955). The mean mortalities for all instars were 61% at a dose of 2.857 g/l. This was not significant ($F_{3,20}$ = 0.787; p= 0.515).

Percentage larval mortality at 48 hours post exposure to the plant powder at the lowest dose (1.428 g/l) was 52 and 68% for third and first instars, respectively (Figure 5b). At 2.857 g/l, mortality of 98 and 75% was recorded for first and fourth instars, respectively. Larval mortality at the highest dose (8.571 g/l) was 100% for all the instars. There was a significant difference in the mean mortalities of 82% for all instars of culicine larvae at 48 hours post exposure ($F_{3,20}$ = 4.330; p= 0.017) at dose 2.857 g/l. At a dose of 5.714 g/l, there was no significant difference in the mean mortalities of 97% ($F_{3,20}$ = 3.088; p= 0.051) for all instars of culicine larvae.

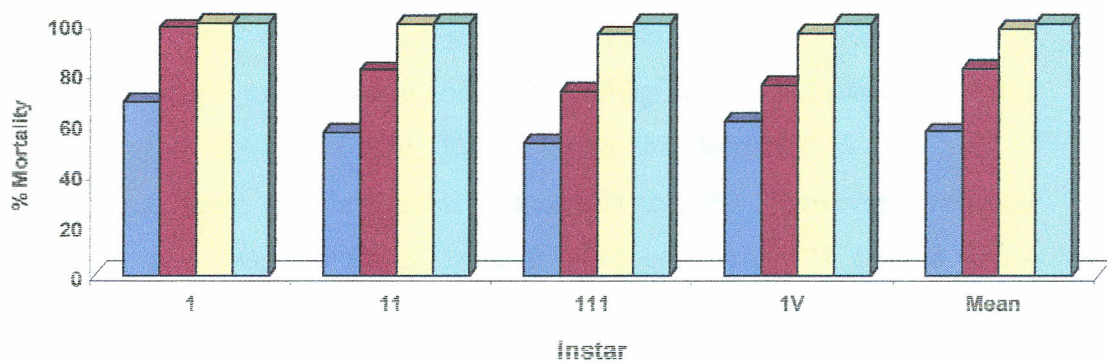
After, 72 hours of exposure, larval mortalities at the lowest dose (1.428 g/l) increased to 80 and 91% for the fourth and first instars, respectively (Figure 5c). At 2.857 g/l, mortalities of 98 and 79% were recorded for the first and fourth instars, respectively. A mortality of 100% was recorded for all the instars at the highest dose (8.571 g/l). The plant powder induced 100% mortality for all the instars at the highest dose tested. A mean mortality of 100% was recorded for culicine larvae after 24, 48 and 72 hours of exposure.

Generally, mortality was higher in early culicine instars than the late instars. This was however, non-significant ($F_{1,46}$ = 0.485; p = 0.489). Increasing exposure time resulted in an asymptotic increase in mortality of all instars. This increase was significant between 24 and 72 hours of sampling ($F_{1,30}$ = 6.998; p = 0.012).

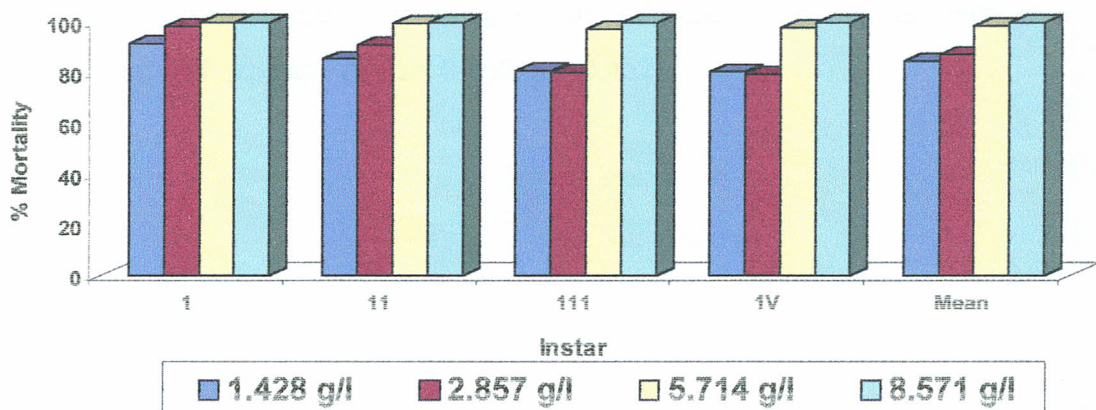
Figure 5a: Mean % larval mortality of culicines in pools treated with *Spilanthes mauritiana* after 24 hours



5b: after 48 hours



5c: after 72 hours



4.3 Pupal mortality

4.3.1 Effect of *Piper guineense* on anopheline and culicine pupae

The pupae could not be identified into anophelines and culicines due to similarities in morphological characteristics. The effect of *P. guineense* against pupae is presented in figure 6a. This plant exhibited substantial pupal mortalities in all the doses tested. At the lowest dose (1.429 g/l), the powder induced mortalities of 5, 79 and 81% after 24, 48 and 72 hours post exposure, respectively. The mortality increased significantly ($F_{1,14} = 8.669$; $p = 0.011$) with dose and exposure time. At 2.857 g/l, mortalities of 67, 92, and 95% were recorded after 24, 48 and 72 hours, respectively. At 5.714 g/l mortalities of 69 and 91% were recorded after 24 and 48 hours respectively. At the highest dose, mortality of 93% was recorded after 24 hours. Highest mortality (100%) was recorded after 72 and 48 hours at a dose of 5.714 and 8.571 g/l, respectively.

4.3.2 Effect of *Spilanthes mauritiana* on anopheline and culicine pupae

The percent mortality of pupae in the pools treated with *S. mauritiana* is presented in figure 6b. At the lowest dose of 1.429 g/l, no pupal mortality was recorded after 24, 48 and 72 hours. Pupal mortality at a dosage of 2.857 g/l remained fairly low. At this dose mortality of 35% was recorded after 24 hours, this decreased to 16% after 48 hours, at 72 hours there was an increase in mortality to 24%. However, substantial pupal mortality was recorded for 5.714 and 8.571 g/l. After 24 and 48 hours, mortalities at 5.714 g/l were 89 and 94% respectively, and increased to 95% after 72 hours. The highest dose (8.571 g/l) resulted in mortality of 99, 63 and 75% after 24, 48 and 72 hours, respectively. The pupae were less susceptible to *S. mauritiana* with no mortality observed with the lowest dose and a decrease in mortality with time observed at higher doses. There was no significant difference in mortalities between the 24 and 72 hours of exposure ($F_{1,14} = 0.560$; $p = 0.467$).

Figure 6a: Mean % pupal mortality in the pools treated with *Piper guineense*

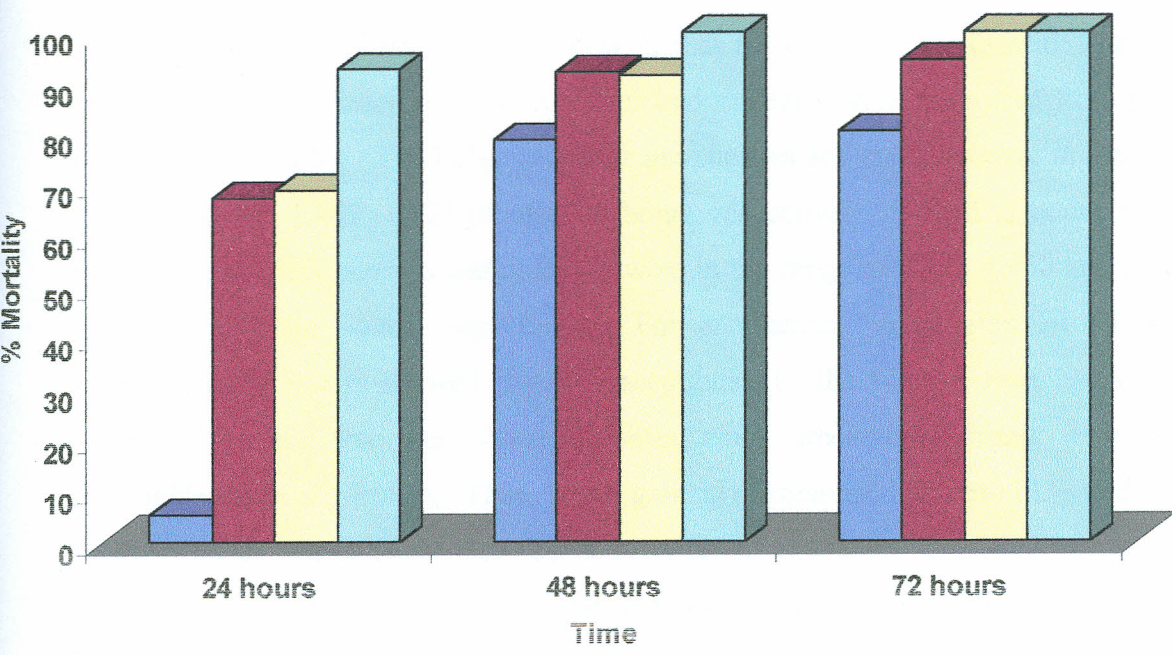
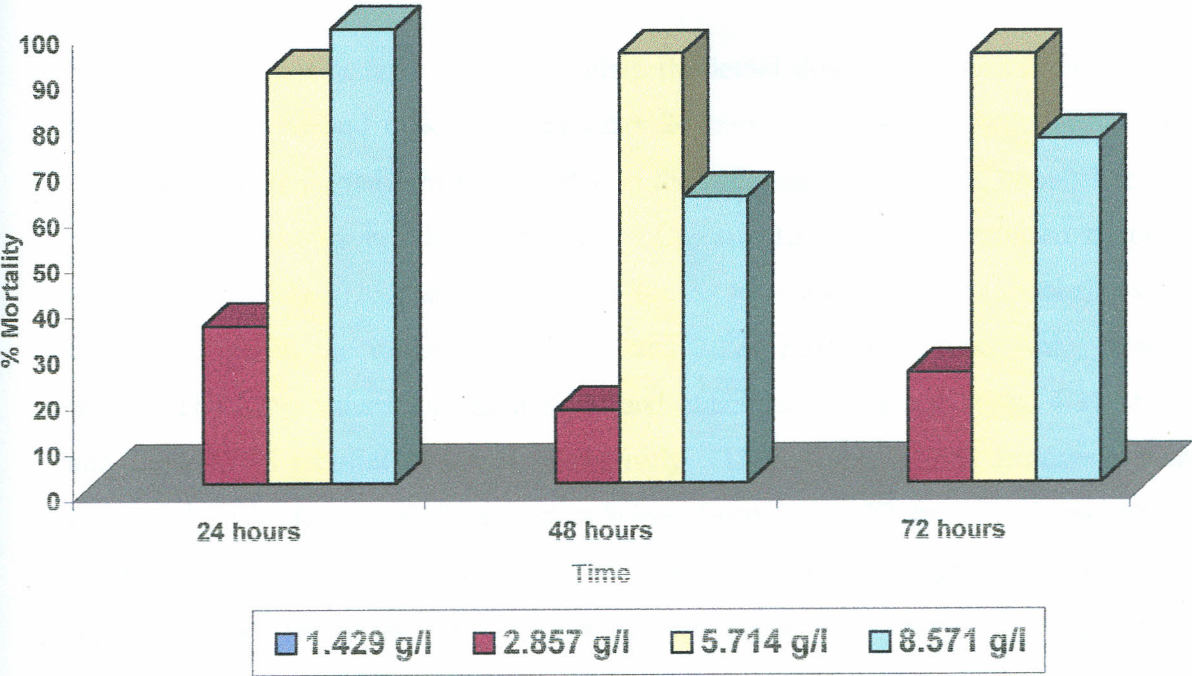


Figure 6b: Mean % pupal mortality in the pools treated with *Spilanthes mauritiana*



4.4 Lethal doses (LD) 50 and 90

4.4.1 *Piper guineense*

Results from probit regression line used to calculate the lethal doses of *P. guineense* powder against anopheline and culicine larvae after 24 hours of exposure is presented in figures 7a-b. It is from the line of best fit that the equation was drawn. The LD_{50} and $_{90}$ for the *P. guineense* for anophelines and culicines were calculated from probit analysis (Table 2a-b). The LD_{50} values for anophelines and culicines were in the range 1.627-2.463 and 1.442 -1.825 g/l after 24 hours, respectively. The LD_{90} values against all the instars of anophelines and culicines were in the range of 3.413-4.450 and 2.742-4.691 g/l after 24 hours, respectively. For anophelines larvae after 24 hours of exposure, early instars showed higher susceptibility to the powder than late instars. However, the converse was observed for culicines, where, late instars were more susceptible than early instars. There was a general decrease in the mean LD_{50} and LD_{90} for anophelines and culicines from 24 to 72 hours of exposure. The same observation was made for the culicines except for the third and first instars that showed an increase. There was no significant difference in the susceptibility of culicines and anophelines to the *P. guineense* powder ($F_{1, 13} = 1.026$; $p = 0.069$). Between the two plants examined, *P. guineense* exhibited a significantly ($F_{1,46} = 29.77$; $p < 0.0001$) higher toxicity to the larval stages than the *S. mauritiana* powder.

4.4.2 *Spilanthes mauritiana*

The probit regression line used to calculate the lethal doses of *S. mauritiana* powder against anopheline and culicine larvae after 24 hours is shown in figures 8a-b. The comparative toxicity (LD) of the powder to the different instars of the anophelines and culicines is as shown in table 3a-b. The LD_{50} and LD_{90} were determined by probit analysis (Table 3a-b). The LD_{50} values for *S. mauritiana* against anophelines and culicines were in the range 2.118-3.297 and 2.224-2.504 g/l, respectively, after 24 hours. The LD_{90} values for anophelines and culicines were in the range 4.376-8.199 and 3.892-5.064 g/l after 24 hours, respectively. There was a general decrease in mean LD_{50} and LD_{90} for anophelines and culicines from 24 to 72 hours. *S. mauritiana* exhibited a higher toxicity to early than to the late instars of anophelines. Log-probit regression analysis showed that culicines were significantly more susceptible to *S. mauritiana* than anopheline larvae ($F_{1, 12} = 31.457$; $p = 0.013$).

Figure 7a: The probit regression line for anopheline larval mortality after 24 hours treatment with *P. guineense* powder

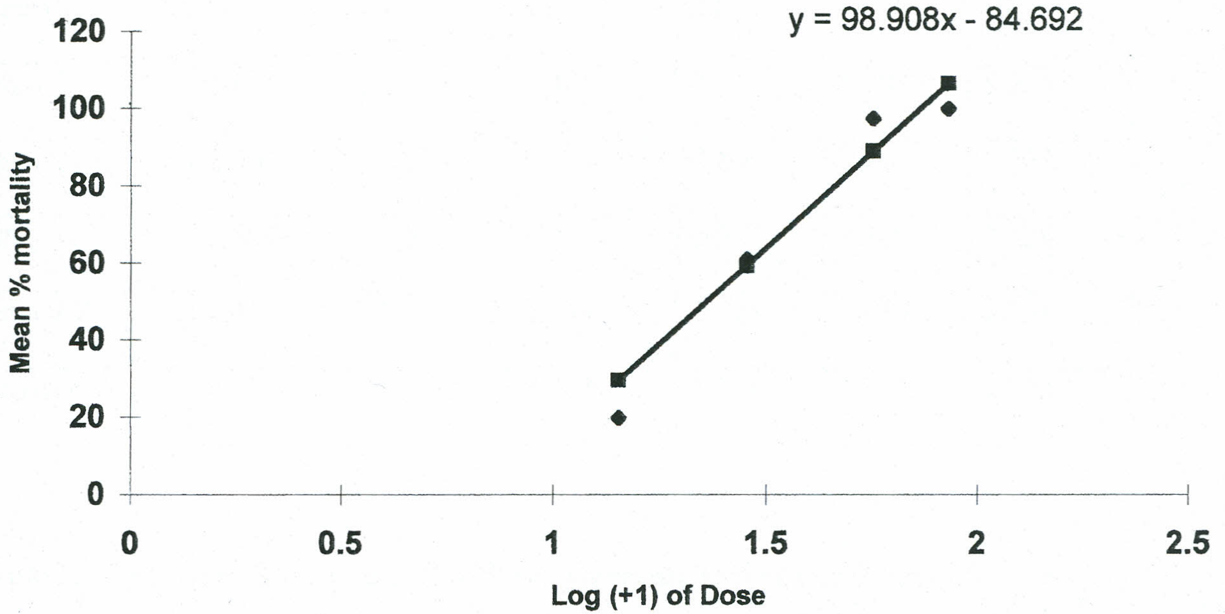


Figure 7b: The probit regression line for culicine larval mortality after 24 hours treatment with *P. guineense* powder

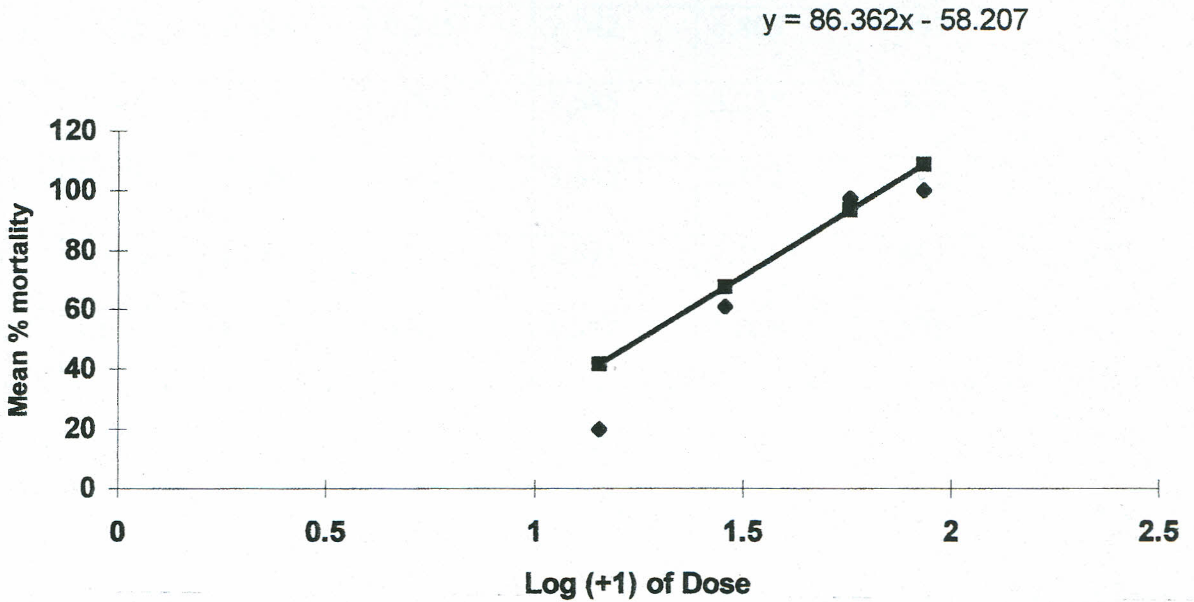


Table 2a: The lethal dose 50 and 90 of *Piper guineense* against anophelines (g/l)

	LD_{50}			LD_{90}		
Time (h)	24	48	72	24	48	72
Instar 1	1.627	1.569	1.304	3.413	4.480	3.514
Instar 11	2.301	1.216	0.958	4.801	2.745	2.131
Instar 111	2.528	1.366	1.047	5.940	3.549	2.520
Instar 1V	2.463	1.254	1.054	7.523	4.450	2.296
Mean (g/l)	**2.256**	**1.309**	**0.849**	**6.244**	**3.849**	**2.703**

Table 2b: The lethal dose 50 and 90 of *Piper guineense* against culicines (g/l)

	LD_{50}			LD_{90}		
Time (h)	24	48	72	24	48	72
Instar 1	1.825	1.583	0.225	2.742	6.863	9.697
Instar 11	1.848	1.265	0.941	3.685	3.235	2.886
Instar 111	1.791	1.429	1.443	3.642	2.372	2.211
Instar 1V	1.442	1.384	0.822	4.691	4.691	2.867
Mean (g/l)	**1.636**	**1.331**	**0.817**	**4.397**	**3.889**	**3.531**

Figure 8a: The probit regression line for anopheline larval mortality after 24 hours treatment with *S. mauritiana* powder

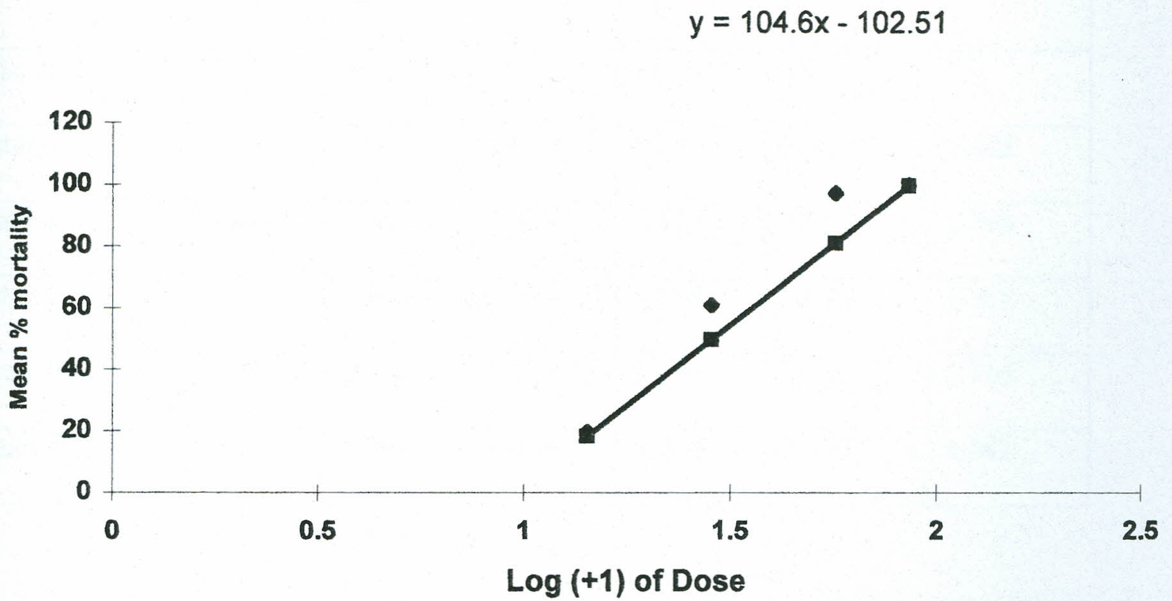


Figure 8b: The probit regression line for culicine larval mortality after 24 hours treatment with *S. mauritiana* powder

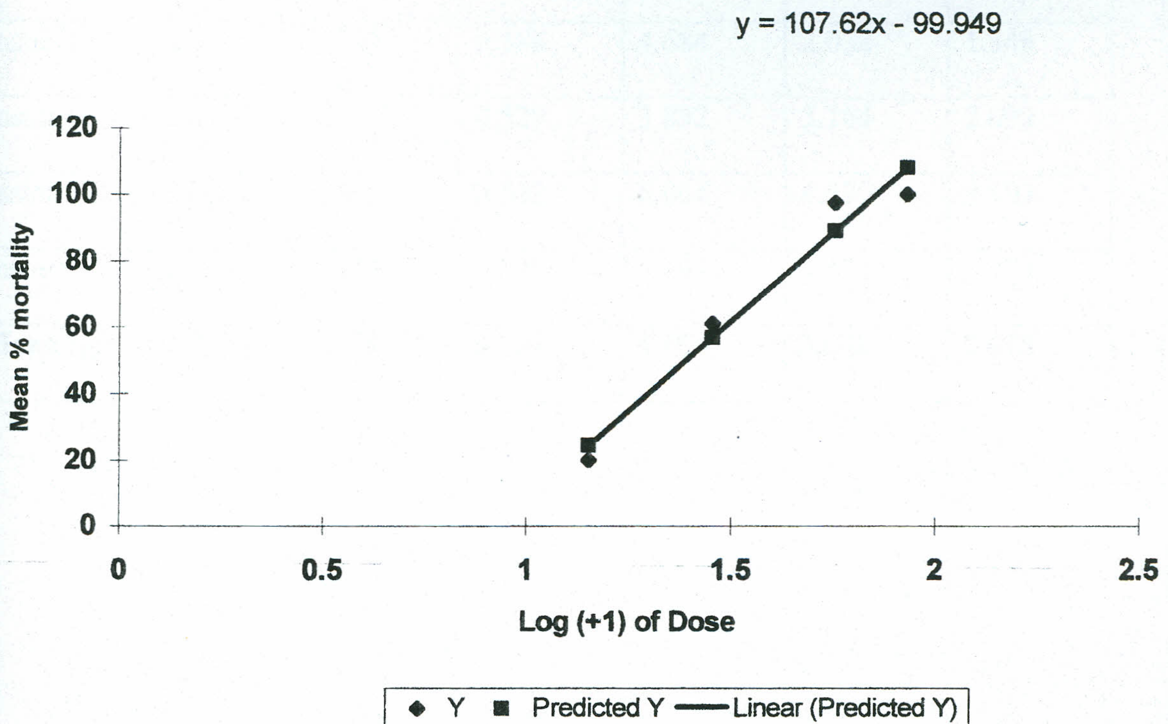


Table 3a: The lethal dose 50 and 90 of *Spilanthes mauritiana* against anophelines (g/l)

	LD_{50}			LD_{90}		
Time (h)	24	48	72	24	48	72
Instar 1	2.118	0.615	0.179	4.376	2.309	0.603
Instar 11	2.645	0.955	0.439	5.332	2.797	1.296
Instar 111	3.068	1.103	0.670	6.878	2.993	1.685
Instar 1V	3.297	1.566	0.727	8.199	4.186	2.116
Mean (g/l)	**2.742**	**1.096**	**0.558**	**6. 313**	**3.175**	**1.547**

Table 3b: The lethal dose 50 and 90 of *Spilanthes mauritiana* against culicines (g/l)

	LD_{50}			LD_{90}		
Time (h)	24	48	72	24	48	72
Instar 1	2.277	1.163	0.386	4.684	2.038	1.348
Instar 11	2.224	1.402	0.529	3.892	3.164	2.092
Instar 111	2.504	1.465	0.542	5.064	4.470	3.193
Instar 1V	2.302	1.234	0.596	4.497	4.127	3.279
Mean (g/l)	**2.318**	**1.271**	**0.464**	**4.409**	**3.610**	**2.475**

4.5 Emergent mosquitoes

4.5.1 Effect of *Piper guineense* on emergence rate

A total of 564 mosquitoes emerged from the cages of which 42.02% (n = 237) were anophelines and 57.78% (n = 327) culicines (Table 4a). Compositions of *Anopheles* species that emerged were 221 *An. gambiae s.l.* and 16 *An. funestus*. At the lowest dose, of the introduced 2840 anopheline larvae and pupae, 3.77% emerged as adults (n = 107). At 8.571 g/l, of 2717 larvae and pupae, 0.18% emerged as adults (n = 5). Out of 2621 larvae and pupa introduced into the control pools, 4.92%; n =129 anophelines emerged as adults. There was a significant difference in the number of emerged adults in the controls and the highest dose (χ^2 = 17.01, df = 1, p, <0.001). The number of emerging adults decreased with an increase in the dose of plant material. More mosquitoes emerged from the lowest dose (1.429 g/l) (70.96%; n = 215) than highest dose (8.571 g/l) (4.62%; n = 14). At doses 2.857 and 5.714 g/l adults that emerged were 46 (15.18%) and 28 (9.24%), respectively. The high mortality of the larval and pupal stages in the highest dose might have caused the low emergence rates observed for *P. guineense*. In the treated pools, more culicines (53.8%; n= 163) than anophelines (46.2%; n = 140) emerged from the pools.

4.5.2 Effect of *Spilanthes mauritiana* on emergence rate

A total of 637 mosquitoes emerged from the cages. Out of this, 365 (57.3%) were anophelines, while culicines comprised of 42.7% (n = 272) (Table 4b). Of the emergent anophelines 351 (55.1%) were *An. gambiae s.l.* and only 14 (2.2%) *An. funestus*. At the lowest dose, of the introduced 3152 anopheline larvae and pupae 4.35% emerged as adults (n = 137). At 8.571 g/l, of introduced 2126 anopheline larvae and pupae were, 0.42% emerged as adults (n = 9). In the control pools, 164 (5.27%) anopheline adults emerged from the 3112 larvae and pupae. There was a significant difference in the number of emerged adults in the controls and the highest dose (χ^2 = 17.31, df = 1, p<0.001). Expectedly, more adults emerged from the cages in the pools treated with the lowest dose (1.429 g/l) (66.38%; n = 235) than the highest dose (8.571 g/l) 14 (3.95%) confirming low larval and pupal mortality in the lowest dose. At doses 2.857 and 5.714 g/l, the emergent adults recorded were 31 (8.76%) and 74 (20.90%), respectively. There was no significant difference in the emergence rate of anophelines and culicines (χ^2 = 1.31, df = 1, p = 0.25).

Table 4a: Emergent mosquitoes after treatment with *Piper guineense* (n)

Species		Dose (g/l)			
	Controls	1.429	2.857	5.714	8.571
Anophelines	4.92%(129)	3.77%(107)	0.56%(16)	0.46%(12)	0.18%(5)
An. gambiae s.l.	121	101	16	10	5
An. funestus	8	6	0	2	0
Culicines	5.46%(164)	4.31%(108)	1.42%(30)	0.83%(16)	0.42(9)
Total	293	215	46	28	14

n = number of emerging mosquitoes

Table 4b: Emergent mosquitoes after treatment with *Spilanthes mauritiana* (n)

Species		Dose g/l			
	Controls	1.429	2.857	5.714	8.571
Anophelines	5.27%(164)	4.35%(137)	0.59%(15)	1.25%(40)	0.42%(9)
An. gambiae s.l.	155	137	12	38	9
An. funestus	9	0	3	2	0
Culicines	5.30%(119)	4.39%(98)	1.09%(16)	2.18(34)	0.90%(5)
Total	283	235	31	74	14

n = number of emerging mosquitoes

4.6 Wing length of the emergent mosquitoes

4.6.1 Effect of *Piper guineense* on emergent adult wing length

The wings of emergent anophelines were mounted on a glass slide and measured using ocular micrometer with the aid of a microscope. Analysis on the wing length of *An. funestus* was not performed due to the low numbers of emerged adults. Wing lengths were measured in 192 *An. gambiae s.l.* and 16 *An. funestus* (Table 5a). No *An. funestus* emerged from the pools treated with 2.857 and 8.571 g/l of *P. guineense.* There was no significant difference in the mean wing length of *An. gambiae s.l.* from the lowest and highest dosage treatment ($F_{1, 28} = 0.888$; $p = 0.633$). The mean wing length of *An. gambiae s.l.* ranged from 2.90 ± 0.019 mm for the lowest dosage to 2.96 ± 0.060 mm for the highest dose. The difference between the lowest and the highest mean wing length in all the doses was 1.22 mm. In the controls, a mean wing length of 2.86 ± 0.024 and 2.73 ± 0.177 mm for *An. gambiae s.l.* and *An. funestus* respectively, were recorded. There was no significant difference in the wing length of emergent adults from treated and control pools ($F_{1, 40} = 0.771$; $p = 0.801$).

4.6.2 Effect of *Spilanthes mauritiana* on emergent adult wing length

The wings of emergent *Anopheles* spp were mounted on glass slide and measured using ocular micrometer with the aid of a microscope. Wing lengths were measured in 309 *An. gambiae s.l.* and 19 *An. funestus* (Table 5b). No *An. funestus* emerged from pools treated with 1.429 and 8.571 g/l of *S. mauritiana.* Due to very low numbers of emergent *An. funestus,* no analysis was performed on their wing length. The minimum and the maximum wing lengths of mosquitoes from the treated pools were 2.0 and 3.4 mm, respectively. A non significant ($F_{1, 43} = 9.141$; $p = 0.254$) difference in the mean wing length for the lowest and the highest dose (2.85 ± 0.024 and 2.86 ± 0.052 mm, respectively) was observed for *An. gambiae s.l.* For the controls, mean wing lengths of 2.81 ± 0.024 and 2.95 ± 0.071 mm were recorded for *An. gambiae s.l.* and *An. funestus,* respectively. There was no significant difference between the mean wing length of emergent adults from the control and treated pools ($F_{1, 60} = 1.183$; $p = 0.237$).

Table 5a: The emergent mosquito wing lengths (mm) after treatment with *Piper guineense*

Dose g/l	Species	N	Minimum winglength	Maximum winglength	Mean±S.E (mm)
Controls	*An. gambiae s.l.*	82	2.00	3.00	2.86 ± 0.024
	An. funestus	10	2.00	3.35	2.73 ± 0.177
1.429	*An. gambiae s.l.*	85	2.00	3.00	2.90 ± 0.019
	An. funestus	3	2.00	3.00	2.71 ± 0.200
2.857	*An. gambiae s.l.*	10	2.20	3.10	2.70 ± 0.111
5.714	*An. gambiae s.l.*	10	2.30	2.90	2.64 ± 0.084
	An. funestus	2	2.00	3.10	3.02 ± 0.080
8.571	*An. gambiae s.l.*	5	2.90	3.20	2.96 ± 0.06

Table 5b: The emergent mosquito wing lengths (mm) after treatment with *Spilanthes mauritiana*

Dose g/l	Species	N	Minimum	Maximum	Mean ± S.E (mm)
Controls	*An. gambiae s.l.*	139	2.15	3.30	2.81 ± 0.024
	An. funestus	6	2.50	2.95	2.95 ± 0.071
1.429	*An. gambiae s.l.*	122	2.10	3.40	2.85 ± 0.024
2.857	*An. gambiae s.l.*	9	2.00	3.00	2.76 ± 0.084
	An. funestus	2	2.46	2.95	2.71 ± 0.245
5.714	*An. gambiae s.l.*	30	2.28	3.30	2.79 ± 0.048
	An. funestus	2	2.25	2.55	2.40 ± 0.15
8.571	*An. gambiae s.l.*	9	2.61	3.05	2.86 ± 0.052

4.7 Effect of application of larvicidal plants on water parameters

4.7.1 Water conductivity

4.7.1.1 Effect of *Piper guineense* on water conductivity

Water conductivity was measured before and after plant powder application in both the treated and control pools. The variation in mean water conductivity with time in the pools is shown in figure 9a. There was an increase in conductivity in all the treatments upto 72 hours. Significantly ($F_{1,25} = 4.67$; $p = 0.007$) higher mean conductivity (5.44 Ms/cm) was recorded after 72 hours post exposure to the highest dose than before treatment (4.15 Ms/cm). At the lowest dose, there was no significant ($F_{1,19} = 0.252$; $p = 0.983$) increase in mean conductivity (4.66 Ms/cm) 72 hours after treatment compared to before (4.07 Ms/cm). In the control pools, there was no significant difference ($F_{1,10} = 1.329$; $p = 0.594$) in conductivity between the hours of sampling. There was no observed significant difference in water conductivity between the pools treated with the lowest doses and the controls ($F_{1, 32} = 1.487$; $p=0.139$).

4.7.1.2 Effect of *Spilanthes mauritiana* on water conductivity

For *S. mauritiana,* variation in mean water conductivity is shown in figure 9b. There was a general increase in conductivity with time in all the doses up to 72 hours. Significantly, higher ($F_{1, 23} = 5.592$; $p = 0.020$) mean conductivity (5.44 Ms/cm) was recorded after 72 hours of exposure to the highest dose compared to before (4.38 Ms/cm). There was no significant difference ($F_{1, 21} = 0.495$; $p = 0.842$) in the mean conductivity (4.89 Ms/cm) at the lowest dose 72 hours and before post exposure (4.38 Ms/cm). In the control pools, a non-significant ($F_{1,48} = 0.789$; $p = 0.374$) difference in mean water conductivity (4.19 and 4.33 Ms/cm) was observed at 0 and 72 hours, respectively. There was no observed significant difference ($F_{1,45} = 0.965$; $p = 0.576$) in the mean water conductivity between the pools treated with the low doses and the controls. There was no observed significant difference in water conductivity between the pools treated with the lowest doses and the controls ($F_{1, 86} = 0.111$; $p = 0.333$).

Figure 9a: Variation in water conductivity for *Piper guineense*

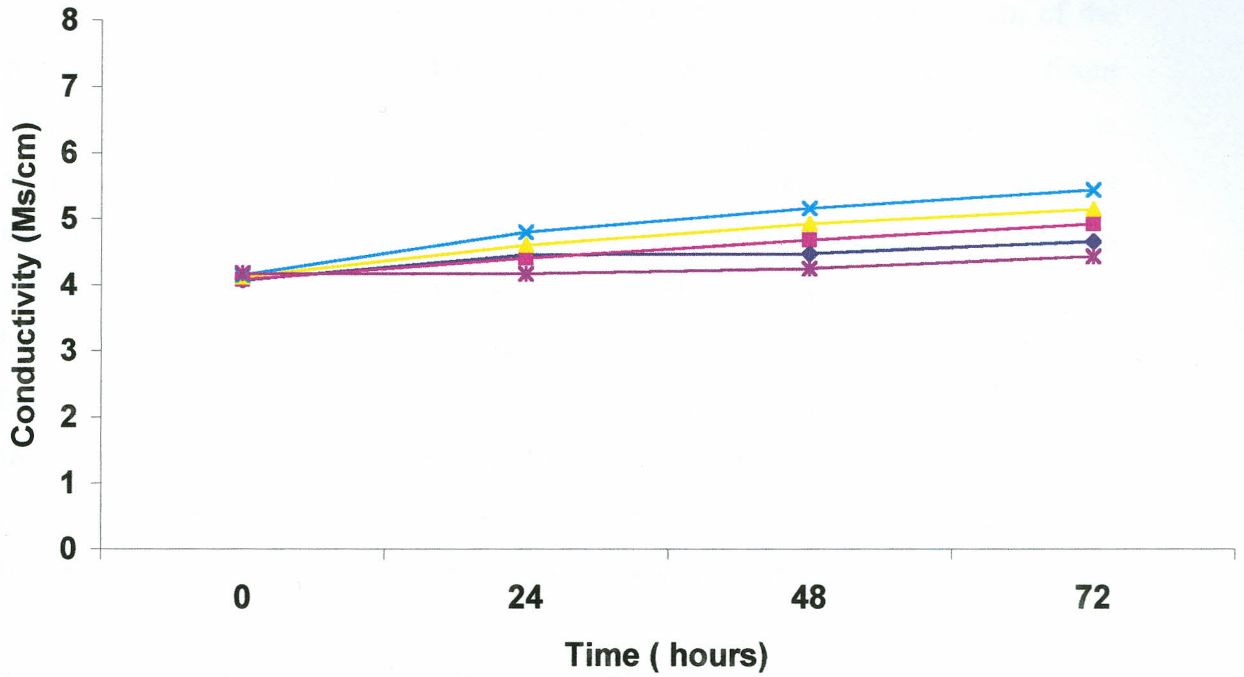
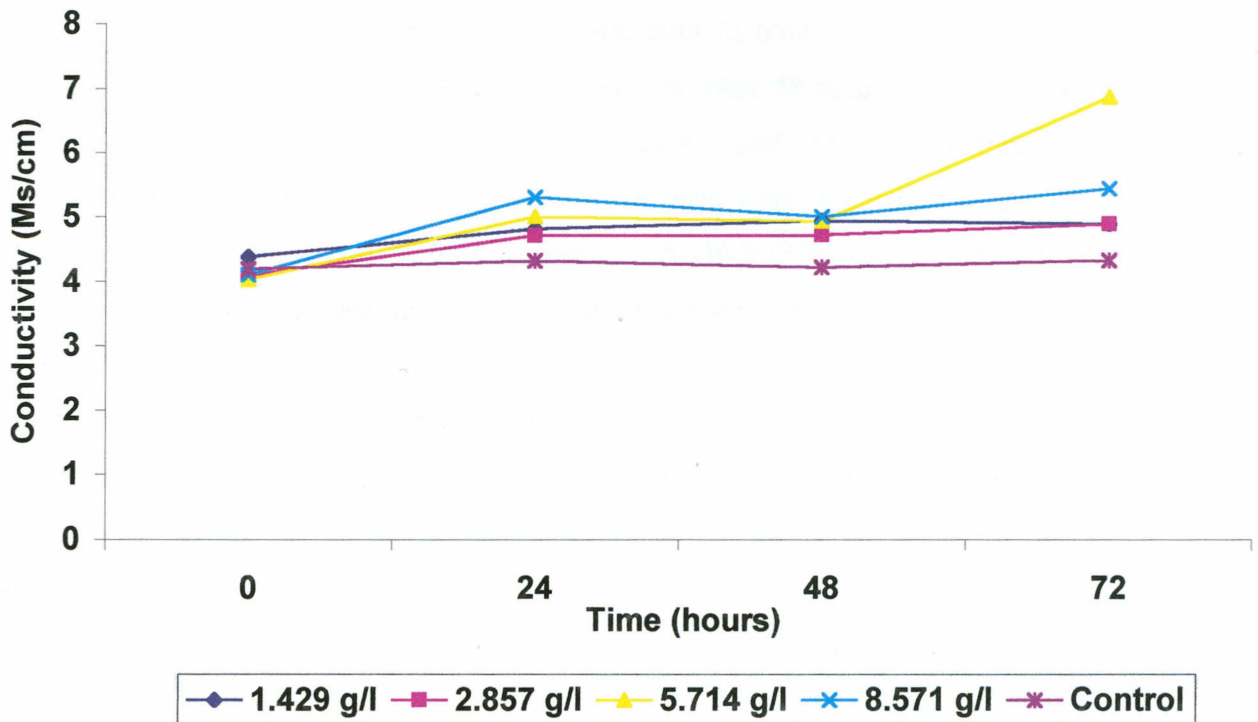


Figure 9b: Variation in water conductivity for *Spilanthes mauritiana*



4.7.2 Water temperature

4.7.2.1 Effect of *Piper guineense* on water temperature

Water temperature was recorded in each pool before and after introduction of the plant material. The mean water temperature recorded in the pools is shown in figure 10a. In both the control and treated pools, there was a general decrease in temperature after 24 hours for all treatments followed by an increase after 48 hours and then a further decrease after 72 hours. The minimum and maximum temperatures recorded were 28.47 and 33.94^0 C, respectively. There was no significant difference in the mean water temperature between the treated and the control pools ($F_{1, 29}$ = 0.925; p = 0.576). There was no significant difference ($F_{1, 10}$ = 4.748; p = 0.184) in mean temperature, 29.99^{0}C (range 29.10 - 32.94^{0}C) and 30.93^{0}C (range 29.78 – 33.04^{0}C) in the highest and lowest dose, respectively. In the control pools, a mean temperature of 30.93^{0}C (range 29.33 - 33.61^{0}C) was recorded.

4.7.2.2 Effect of *Spilanthes mauritiana* on water temperature

Water temperature was recorded on each pool after 0, 24, 48 and 72 hours, respectively. The variation in the water temperature between the doses is shown in figure 10b. Water temperature in all experiments including the controls decreased drastically from 0 to 24 hours, and then increased upto 72 hours except for the 5.714-g/l doses where there was a reduction in temperature after 48 hours. The minimum and maximum temperatures recorded were 28.93 and 31.77^{0}C, respectively. Significant difference ($F_{1, 64}$ = 1.667; p = 0.032) in mean water temperatures, 30.41^{0}C (range 28.99 - 31.77^{0}C) and 30.08^{0}C (range 29.25 -30.99^{0}C), was recorded in the pools treated with the highest and the lowest dose, respectively. In the control pools, mean water temperature of 30.9^{0}C range (29.76 - 31.73^{0}C) was recorded. There was a significant difference in the mean water temperatures between the hours of sampling in the controls ($F_{1,29}$ = 8.821; P = 0.019). There were no significant differences ($F_{1, 37}$ = 0.738; p = 0.782) in the mean water temperatures between the treated and control pools.

Figure 10a: Variation in water temperature for *Piper guineense*

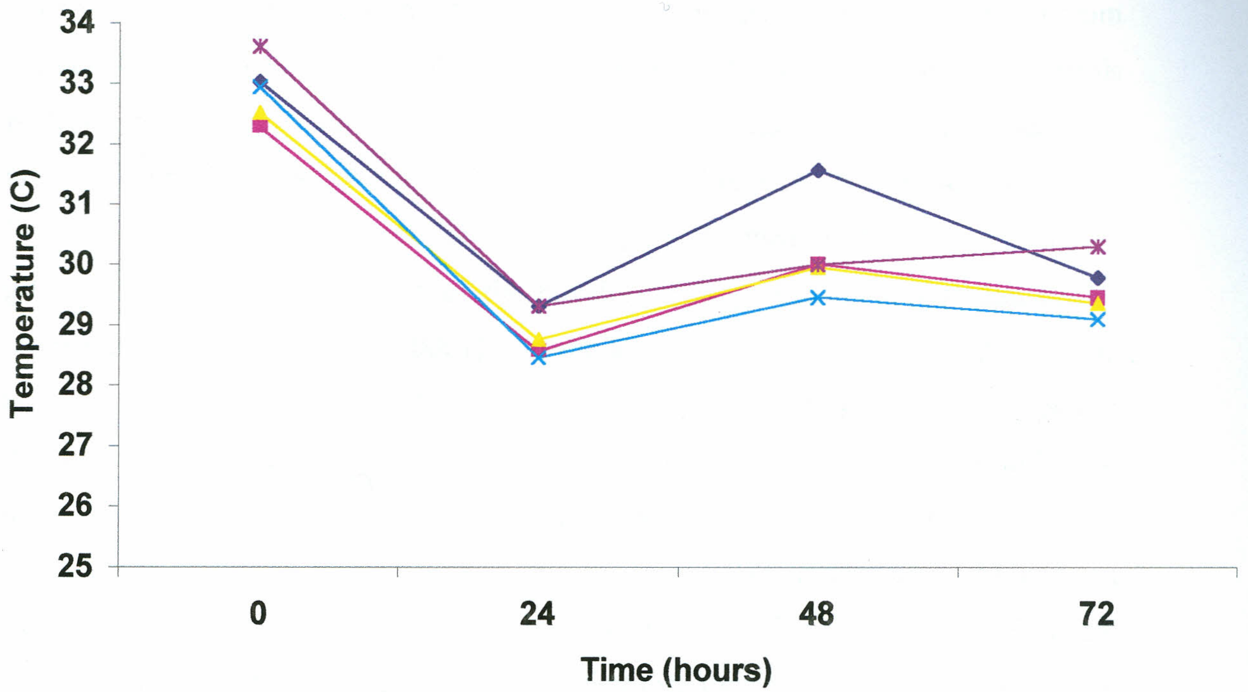
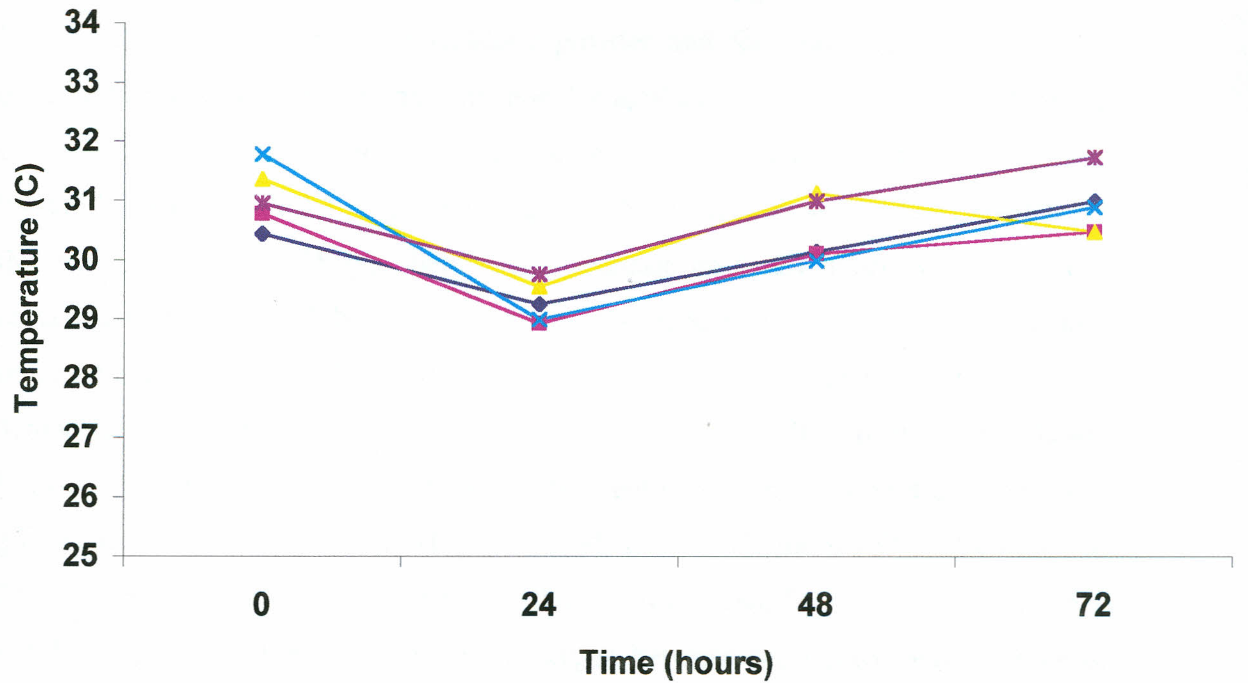


Figure 10b: Variation in water temperature for *Spilanthes mauritiana*



4.7.3 Concentration of dissolved oxygen

4.7.3.1 Effect of *Piper guineense* on dissolved oxygen

On each sampling day, concentration of dissolved oxygen (DO) was recorded from the pools. The variation in dissolved oxygen between the treatments and the controls is shown in figure 11a. In the treated pools, there was a drastic reduction in mean DO after 24 hours, followed by a slight reduction up to 72 hours. Slight reduction in mean DO was observed at the lowest dose of 1.429 g/l. The mean DO at a dose of 8.571 g/l, 72 hours after introduction of the powder was significantly lower than before treatment ($F_{1,\,9} = 8.847$; $p = 0.0001$). At the lowest dose, the mean DO at 72 hours post exposure was significantly ($F_{1,5} = 12.931$; $p = 0.004$) lower than before introduction. Non significant difference ($F_{1,\,19} = 1.411$; $p = 0.208$) in the mean DO of 4.50 and 3.19 mg/l was recorded in the control pools at 0 and 72 hours, respectively. There was a significant difference ($F_{1,\,29} = 5.225$; $p = 0.034$) in the mean DO between the treated and the control pools. In general, there was a significant ($F_{1,\,17} = 4.383$; $p = 0.0001$) reduction in the amount of water-dissolved oxygen in the pools treated with this plant material. There was a reduction of dissolved oxygen to 72 hours at doses 2.857, 5.714 and 8.571 g/l with minimum DO recorded at 5.714 g/l.

4.7.3.2 Effect of *Spilanthes mauritiana* on dissolved oxygen

In the pools treated with *S. mauritiana* powder and the controls, mean dissolved oxygen was recorded before and after powder application. The results are shown in figure 11b. In general, there was a drastic reduction in mean DO up to 72 hours in the pools treated with 2.857 g/l, 5.714 g/l and 8.571 g/l, respectively. However, at the lowest dose of 1.428 g/l, there was a decrease up to 24 hours after which DO remained constant to 72 hours. There was a significant ($F_{1,5} = 9.153$; $p = 0.001$) decrease in the mean DO from 2.77 to 0.38 mg/l, 72 hours after treatment demonstrated by the highest dose. A significantly ($F_{1,\,27} = 48.546$; $p = 0001$) lower DO of 1.73 mg/l was recorded 72 hours after treatment with the lowest dose of 1.428 g/l. Statistically non significant ($F_{1,\,14} = 2.284$; $p = 0.108$) mean DO of 3.84 mg/l at 72 hours post exposure was observed in the controls. Significant ($F_{1,\,29} = 6.512$; $p = 0.001$) reduction in the mean dissolved oxygen between the treated and the control pools was observed. In general, there was a significant ($F_{1,\,19} = 3.279$; $p = 0.0001$) reduction in the amount of water-dissolved oxygen in the pools treated with this plant material. The lowest DO was recorded at a dose of 8. 571 g/l.

Figure 11a: Variation in concentration of dissolved oxygen for *Piper guineense*

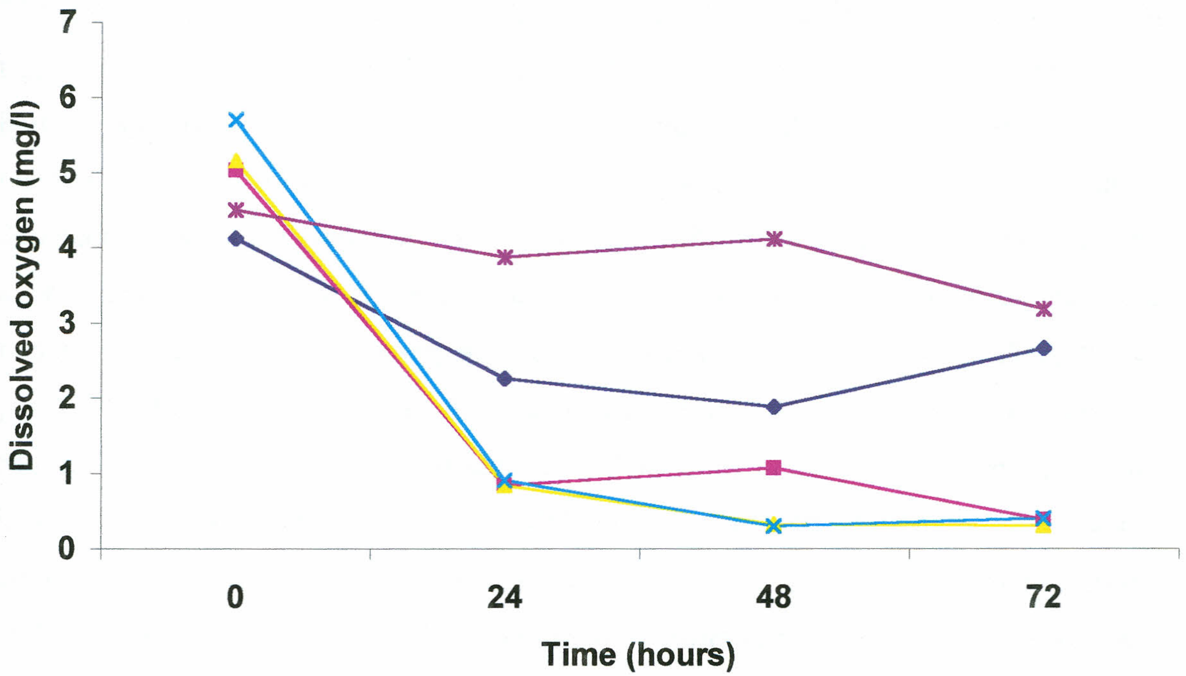
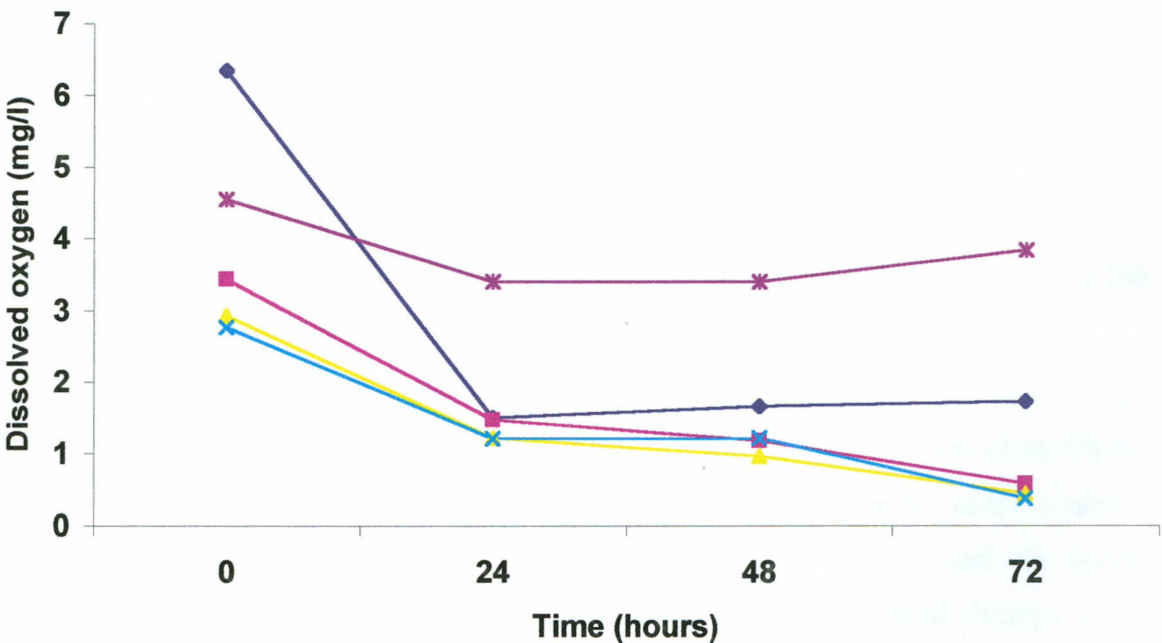


Figure 11b: Variation in concentration of dissolved oxygen for *Spilanthes mauritiana*



1.429 g/l 2.857 g/l 5.714 g/l 8.571 g/l Control

4.8 Effect of plant material on non-target aquatic organisms

4.8.1 Effect of *Piper guineense* on non-target aquatic organisms

The toxic effects of the powder from this plant were tested against eight species of non-target aquatic invertebrates and vertebrates. Mortalities were monitored after 24 and 48 hours, respectively. The results are as shown in table 6. Out of the eight tested species, microdytiscids and back swimmers showed the highest tolerance to the powder. Out of 160 microdytiscids exposed to a dose of 1.429 g/l, 158 (98.7%) and 153 (95.6%) survived after 24 and 48 hours of exposure, respectively. There was no significant difference in the number of microdytiscids surviving after 24 and 48 hours of exposure ($\chi^2 = 0.500$, df = 1, p = 0.779). Back swimmers were also relatively tolerant to the powder with mortalities of 5% (n=2) and 15% (n=6) recorded after 24 and 48 hours of exposure, respectively at the lowest dose. There was no significant difference in the mortalities of back swimmers after 24 and 48 hours of exposure ($\chi^2 = 1.000$, df = 1, p = 0.317). In the control pools, 100% (n=40) and 99.4% (n=159) of the backswimmers and microdytiscids, respectively were collected after 24 hours. There was no significant difference in the mortality of back swimmers in the control pools with time ($\chi^2 = 0.500$, df = 2, p = 0.779). Fish were the most susceptible to this powder. Out of the 60 fish exposed to the lowest dose, 48 (80%) and 39 (65%) were collected after 24 and 48 hours of exposure, respectively. In the control pools, fish mortalities of 20% and 26% were recorded after 24 and 48 hours of exposure. There was no significant difference in the mortality of fish in the control and treated pools after 24 and 48 hours of exposure ($\chi^2 = 0.500$, df = 2, p = 0.799). At the highest dose, 39 (65%) and 33 (55%) out of 60 exposed fish survived after 24 and 48 hours of exposure, respectively. There was no significant difference in the number of fish surviving after 24 and 48 hours at the highest dose ($\chi^2 = 0.500$, df = 2, p = 0.779).

When exposed to 8.571 g/l, mortality of 22% (n=13) and 18% (n=11) was observed for shrimps and damsel fly nymphs, respectively after 48 hours of exposure. In the controls, mortalities of shrimps were 5% (n=3) and 22% (n=13) after 24 and 48 hours, respectively. There was no significant difference in the mortalities of shrimps in the control and treated pools after 48 hours of exposure to the powder ($\chi^2 = 0.500$, df = 2, p = 0.779). Of the 60 introduced macrodytiscids, 53 (88%) and 48 (80%) were collected after

24 and 48 hours, respectively at the lowest dose. There was no significant difference in the number of macrodystiscids collected after 24 and 48 hours of exposure ($\chi^2 = 0.500$, df = 2, p = 0.779). In the controls, 55 (92%) and 48 (80%) were collected after 24 and 48 hours, respectively. This was not significant ($\chi^2 = 0.500$, df = 2, p = 0.779). At the lowest dose, 23 (92%) and 19 (76%) dragon fly nymphs were collected after 24 and 48 hours of exposure, respectively. There was no significant difference in the number of dragon fly nymphs collected after 24 and 48 hours of exposure ($\chi^2 = 1.000$, df = 1, p = 0.317). At 8.571 g/l, 15 (75%) and 10 (50%) tadpoles were collected after 24 and 48 hours, respectively. There was no significant difference in the number of tadpoles collected in the control and treated pools after 24 and 48 hours of exposure ($\chi^2 = 1.000$, df = 1, p = 0.317).

4.8.2 Effect of *Spilanthes mauritiana* on non-target organisms

The toxic effects of the powder from this plant were tested against six species of non-target aquatic invertebrates and two vertebrates. Mortalities were monitored after 24 and 48 hours, respectively. The results are as shown in table 7. Out of introduced 160 microdystiscids to the lowest dose (1.429 g/l), 160 (100%) and 154 (96%) were collected after 24 and 48 hours of exposure, respectively. Similarly, 40 backswimmers were introduced into the pools treated with the lowest dose with 37 (92.5%) and 34 (85%) of these being collected after 24 and 48 hours of exposure, respectively. This was not significant ($\chi^2 = 500$, df = 2, p = 0.779). While at the highest dose, 35 (87.5%) and 30 (83%) were also collected after the same duration, respectively. There was no significant difference in the number of backswimmers collected after 24 and 48 hours at this dose ($\chi^2 = 500$, df = 2, p= 0.779). In the controls, 39 (97.5%) and 37 (92.5%) of the introduced 40 backswimmers were collected after 24 and 48 hours, respectively. There was no significant difference in the mortality of backswimmers in the treated and control pools ($\chi^2 = 1.000$, df = 1, p = 0.779). Out of the introduced 60 fish at the lowest dose, 47 (78%) and 39 (65%) were collected after 24 and 48 hours of exposure, respectively. At the highest dose, 44 (73%) and 33 (55%) were collected after 24 and 48 hours of exposure, respectively. This was not significant ($\chi^2 = 0.500$, df = 2, p = 0.779). In the control pools, fish mortalities of 23% and 31.7% were recorded after 24 and 48 hours of

exposure, respectively. There was no significant difference in the mortalities of fish in the control and treated pools after 24 and 48 hours of exposure (χ^2 = 500, df = 2, p= 0.779).

Out of 60 shrimps introduced into the treated pools, 55 (92%) and 48 (80%) were collected after 24 and 48 hours of exposure, respectively at the lowest dose. There was no significant difference in the number of shrimps collected after 24 and 48 hours of exposure at this dose (χ^2 = 0.500, df = 2, p = 0.779). Mortalities of 8% (n=5) and 20% (n=12) among 60 shrimps in the controls were observed after 24 and 48 hours, respectively. There was no significant difference in the mortality of shrimps in the treated and control pools after 24 and 48 hours of exposure (χ^2 = 0.500, df = 2, p = 0.779). There were mortalities of 13% (n=8) and 23% (n=14) among the 60 damsel fly nymphs after 24 and 48 hours of exposure, respectively to the lowest dose, this was non significant (χ^2 = 0.500, df = 2, p = 0.779). At the highest dose, mortalities of 20% (n=12) and 37% (n=22) were recorded after 24 and 48 hours, respectively. There was no significant difference in the mortality of damsel fly nymphs after 24 and 48 hours of exposure (χ^2 = 0.500, df = 2, p = 0.779). At 1.429 g/l, 51 (85%) and 47 (78%) of the macrodystiscids were collected after 24 and 48 hours, respectively. There was no significant difference in the number of macrodystiscids collected after 24 and 48 hours of exposure (χ^2 = 1.000, df = 1, p = 0.317). In the control pools, 56 (93%) and 46 (77%) of the 60 introduced macrodystiscids were collected after 24 and 48 hours, respectively. There was no significant difference in the number of macrodystiscids collected in the treated and control pools after 24and 48 hours of exposure (χ^2 = 0.500, df = 2, p = 0.779). At 1.429 g/l, 15 (75%) and 14 (70%) of the tadpoles survived after 24 and 48 hours, respectively. There was no significant difference in the number of surviving tadpoles after 24 and 48 hours of exposure (χ^2 = 1.000, df = 1, p = 0.317). There was no significant difference in the mortalities of tadpoles in the treated and control pools after 24 and 48 hours of exposure to this powder (χ^2 = 0.500, df = 2, p = 0.779).

Table 6: Non-target organisms collected after the application of *Piper guineense* powder

Dose (g/l)			**1.429**			**8.571**			**Control**		
Time (hours)			**No. exp**	**24**	**48**	**No. exp**	**24**	**48**	**No. exp**	**24**	**48**
Common name	**Order**	**Family**									
Damsel fly nymphs	Odonata	Gomphilidae	60	52	45	60	45	39	60	53	49
Dragon fly nymphs	Odonata	Coenagrionidae	25	23	19	25	20	18	25	24	21
Macrodytiscids	Coleoptera	Dytiscidae	60	53	48	60	49	37	60	55	48
Microdytiscids	Coleoptera	Dytiscidae	160	158	153	160	159	146	160	159	153
Backswimmers	Hemiptera	Notonectidae	40	38	34	40	36	29	40	40	36
Fresh water shrimps	Decapoda	Palaemonidae	60	54	49	60	49	45	60	57	47
Tilapia fish	Perciformes	Cichlidae	60	48	39	60	39	33	60	48	44
Tadpoles	Anura	Ranidae	20	19	14	20	15	10	20	16	16

Legend:

No. exp = (n) - Total Number of non target organisms introduced into the pools before powder application

Table 7: Non-target organisms collected after the application of *Spilanthes mauritiana* powder

Dose (g/l)			**1.429**			**8.571**			**Control**		
Time (hours)			**No. exp**	**24**	**48**	**No. exp**	**24**	**48**	**No. exp**	**24**	**48**
Common name	**Order**	**Family**									
Damsel fly nymphs	Odonata	Gomphilidae	60	52	46	60	48	38	60	53	45
Dragon fly nymphs	Odonata	Coenagrionidae	25	23	20	25	22	17	25	23	20
Macrodytiscids	Coleoptera	Dytiscidae	60	51	47	60	50	43	60	56	46
Microdytiscids	Coleoptera	Dytiscidae	160	160	154	160	157	152	160	159	153
Backswimmers	Hemiptera	Notonectidae	40	37	34	40	35	30	40	39	37
Fresh water shrimps	Decapoda	Palaemonidae	60	55	48	60	51	43	60	55	48
Tilapia fish	Perciformes	Cichlidae	60	47	39	60	44	33	60	46	41
Tadpoles	Anura	Ranidae	20	15	14	20	14	12	20	17	15

Legend:

No. exp = (n) - Total Number of non target organisms introduced into the pools before powder application

CHAPTER FIVE

5.0 DISCUSSION

5.1 Larval mortality

Crude extracts of the plant *Piper guineense* have been studied previously and proved to be potential control agents against many destructive arthropods such as *Acrae eponina, Macrotermes nigeriensis* (Olaifa *et al.,* 1987), *Megalurothrips sjostedti* (Ivbjaro and Bolaji, 1990), *Callobruchus maculatus* (Ivbajaro 1990; Olaifa and Erhun, 1990) and *Zonocerus variegates* (Mbata *et al.,* 1995). Previous larvicidal activity on *Ae. aegypti* was reported and the bioactive principles isolated and identified (Addae-Mensah and Achieng, 1986). Methanol and ethyl acetate extracts of this plant have also shown high larvicidal activity against *An. gambiae s.l.* in the laboratory (Okinyo, 2002). *Spilanthes mauiritiana* extracts, as potential insecticide has not been extensively studied. However, Jondiko (1989) reported the mosquito larvicidal properties, isolated and identified the active principle. Results from this study show that, the powder of the two plants can be used as larvicides against anophelines and culicines, the principal vectors of malaria, filariasis and yellow fever among other diseases. Crude extracts from other plants have also shown high larvicidal activity against the same insects; *Melia volkensii* (Mwangi and Mukiama, 1988), *Azadirachtin indica* (Zebitz, 1984; 1986), *Swartzia madagascariensis* (Minjas and Sarda, 1986), *Tagetes minuta, T. patula, T. erecta* (Perich *et al.,* 1994; Green *et al.,* 1991). Other plants include: *Citrus sinensis, C. auratium, C. limon* (Mwaiko, 1992) and *Sorghum bicolor* (Jackson *et al.,* 1991). Xue *et al* (2001) reported that insect repellents from plants are also potential mosquito larvicides.

Coincidentally, certain stages of mosquitoes are more susceptible to phytochemicals at the same concentrations. In this study, the powder showed higher larvicidal activity against the early (1st & 2nd) than late instars (3rd & 4th) of anophelines especially with the *S. mauritiana* powder. Although this difference was not significant, high larval mortality was observed in early than in the late stages. The variation in toxicity to different stages has been reported in some other studies. Butanol extract of *Phytolacca dodecandra,* was more toxic to second and third instar larvae of *Ae. aegypti, Cx. pipiens* and *An. quadrimaculatus,* while the eggs and pupae of the same species were unaffected

(Spielman and Lemma, 1973). Similar results have been reported with *Cymbopogon citrus, Bursera delpechiana* and *Pelargonium roseum* (Osmani and Sighamony, 1980).

Rashed and Mulla (1989) demonstrated that species rate of ingestion is a function of age of larvae and water temperature; young larvae were more rapid feeders than older larvae. Based on these observations, the early instars, may have been feeding on the toxins more rapidly than the late instars. Younger instars have also been reported to be more susceptible to bacterial toxins (Ramoska *et al.*, 1977). The finding therefore, indicates that the feeding rate of early instars probably contributed to their greater susceptibility to the extracts. Besides, oxygen requirement for early instars is higher than that for late instars. The significant reduction in dissolved oxygen may have caused the higher mortality observed. The high mortalities of early instars compared to the late stages are advantageous for larvicide based control programmes since it prevents their development to other stages and eventually the less susceptible ones.

Between the two candidate larvicidal plants, *P. guineense* was more effective than *S. mauritiana.* Although this difference was not significant, similar studies have reported such differences between other larvicidal plants (Green *et al.*, 1991; Perich *et al.*, 1994). Green *et al.* (1991) observed that *Tagetes minuta* oil was highly larvicidal compared to *T. patula* and *T. erecta*. Similar results were observed by Perich *et al.* (1994). *Piper guineense* and *Spilanthes mauritiana* belong to different families; Piperaceae and Compositae, respectively. The observed difference in the larvicidal activity of the plant materials from these families may therefore be due to different larvicidal principles. The difference in family may have contributed to the variation in lethal effects.

The larvicidal activity of these plants was demonstrated against both anophelines and culicines. Although not significant, culicines were more susceptible to the *P. guineense* extracts than the anophelines. The high culicine larval mortality is advantageous in mosquito control since the biting nuisance by most culicine adults will be reduced. The lower mortality of anopheline larvae may have negative implication in malaria control since there will be more adults to act as vectors of malaria. This may however be

countered by more frequent application regimen of the larvicide. Unlike in this study, some studies have shown that anophelines are more susceptible to plant extracts than culicines. For instance, Mwangi and Rembold (1988) reported that *An. arabiensis* larvae were more susceptible to *M. volkensii* extracts than *Ae. aegypti*. Extracts of *Swartzia madagariensis* were more toxic to *An. gambiae s.l.* than *Ae. aegypti* and *Cx. quinquefasciatus* (Minjas and Sarda, 1986). The difference in susceptibility can be attributed to the difference in the mode of feeding and physiological characteristics between the two groups of mosquitoes. Anopheline larvae are filter feeders, mainly ingesting food particles floating at the air-water interface (Clements, 1992). Culicines feed below the water surface with their heads hanging down and the siphons anchored to the air water interface (Clements, 1992). The feeding behaviour of larvae has also been reported to affect the level of toxicity of bacterial larvicides in different mosquito species (Ramoska *et al.,* 1977) indicating that the mode of feeding may contribute to the higher susceptibility of culicines than anophelines.

In the larvae treated with *S. mauritiana* material, increasing exposure time resulted in asymptotic increase in larval mortality. The asymptotic increase of mortality with exposure to time suggests that the larvae were feeding on the toxins continuously over time without any inhibition. However, in the pools treated with *P. guineense* material, for the doses, 2.857, 5.714 and 8.571 g/l, there were high mortalities after 24 hours for 1st instars. The mortality reduced after 48 hours but increased after 72 hours of exposure. This suggests that during larval sampling, mosquito eggs may have been collected together with the larvae. These eggs successfully hatched in the pools to 1st instars thus increasing the number of larvae after 48 hours well above the initial sampled population at 24 hours. However, the larvae often died 24 hours after hatching suggesting that the plants are not ovicidal. These results support previous data by Mohsen *et al.* (1989) and Osmani and Sighamony (1980) who reported that ethanolic extracts of *Haplophylum tuberculatum* did not have any ovicidal effect but killed first instar larvae of *Cx. quinquefaciatus.*

From the results, there was a relatively high emergence rate of anophelines at the lowest doses compared to the higher doses, however, it is also worth noting that some of the larvae treated with the lowest doses of the extracts successfully pupated but often died before emergence of adults. This was observed in anophelines only. However, culicines that pupated successfully developed into mature adults. This is in agreement with studies of Mwangi and Mukiama (1988), who reported that, *Ae. aegypti* larvae treated with sub-lethal doses of the extracts pupated, survived and moulted to adult mosquitoes within the normal two days. The difference between the two groups may imply that the plants are slow acting larvicides or IGRs for anophelines eventually causing death. Although the culicines are more susceptible to the plant powder, the ones that survive are not affected. This is an advantage for malaria control programmes since anopheline adults being the vectors of malaria, their high non-emergence rates can reduce the entomological inoculation rates and parasite transmission.

The current study, therefore, demonstrates the potential of *P. guineense* and *S. mauritiana* as sources of mosquito larvicides. The lethal effects occur earlier (24 hours) than in *M. volkensii* extracts where lethal effects occurred after 48 hours (Mwangi and Mukiama, 1988) and after continuous exposure in neem seed kernel (Zebitz, 1984). Previous larvicidal experiments involved use of plant extracts and not powder. This is therefore the first instance where plant powders are being investigated for larval control under field conditions. The use of plant powder reduces the cost of extraction and thus would make the larvicides more accessible to the resource poor rural farming communities especially in irrigation schemes.

5.2 Influence of plant materials on dissolved oxygen

Several studies have reported the relationship between dissolved oxygen and larval abundance and occurrence (Rejmankova *et al.*, 1993; Grillet, 2000; Xue *et al.*, 2001). No previous studies have been done on the effects of plant material on DO and larval mortality. In this study, there was a significant reduction of DO in the treated pools compared to the control pools, indicating that the plant powders have significant effects on water DO. The DO range of 2.66-0.30 mg/l after treatment is much below the

minimum oxygen requirement for larval survival. Mittal *et al.* (1998) reported the, average DO requirement for *Culex* is 2.1 while *Aedes* and *Anopheles* is 6.2 and 6.6 ppm, respectively. Recent studies by Sunish and Reuben (2001) revealed the DO requirement for *Cx. vishnui* at 3-8 mg/l. Other studies show that most larvae occur in habitats with DO range of 3.0-7.0 mg/l (Laird, 1988). It has also been demonstrated that mosquito larvae can tolerate minimum DO levels of upto 4 ppm (Amerasinghe *et al.*, 1995). Kentucky water quality criteria show that oxygen levels that remain below 1- 2 mg/l for a few hours can result in large aquatic organism kills (Barbrara *et al.*, 1997). In line with these studies, the reduction in DO observed in the present study may have caused the larval mortality in the treated pools, since it was below the minimum requirement for mosquito larvae. Coincidentally, the mortality due to the reduction in DO is surprising and may not be the sole cause of death since most anophelines respire primarily at the water surfaces and oxygen is usually not considered as a relevant habitat factor in larval distribution (Grillet, 2000). However, it has also been observed that some anophelines are negatively affected in water that contains little DO in a yet unexplained way (Unti, 1943).

5.3 The influence of plant materials on water temperature

Many studies have reported positive correlation between larval growth and developmental rates of natural populations to water temperature (Sweeney, 1978; Mackay, 1979; Atkinson, 1994). No significant difference in temperature changes with time and between the treated and control pools was observed. The non significance changes of temperatures with time and between the treated and control pools can probably mean that the introduced powders had no effect on the temperatures. The mean minimum and maximum temperatures of 28.58^{0}C and 33.94^{0}C, respectively, recorded in this study are not different from the ones recorded in other studies. For example, *An. quadrimaculatus* can survive in pools of 21.2 – 28^{0}C (Wallace and Merrit, 1999) while *An. arabiensis* can survive in pools of temperatures between 18 and 32^{0}C (Awono-Ambene *et al.*, 1998). Pal (1945) and Sunish and Reuben (2001) have reported similar results. However, in the present study, there was a non significant increase in temperatures with time, this may have contributed to larval mortality indirectly. It is

likely that an increase in temperature increases the rate of movement of the larval mouthparts and consequently the rate of toxin ingestion. This is supported by previous studies (Dadd, 1971) in which the feeding rate of fourth instars of three species was influenced by water temperature. In studies on *B.t.i,* Wright *et al.* (1981) showed that increasing temperatures enhances the activity of bacterial control against mosquito larvae. Rate of larval development depends on water temperatures among other factors (Service, 1996). In this study, reduction in water temperature to a minimum of 28^0C after 24 hours was observed. The reduction could not have had effects on larval activity such as feeding since mosquito larvae such as *Ae. flavescens* have been shown to be active even at 25^0C (Service, 1996). The results in this study indicate that the higher temperatures may have increased the rate of ingestion of toxins per unit time and therefore resulted in increased susceptibility of larvae to the powder.

In this study, water temperature in all treatments and controls decreased drastically from 0 to 24 hours. This observation could have been due to the powders introduced into the pools. These powders may have been absorbing heat from the water surface, this heat absorption may have led to the reduction of temperatures with time as observed. However, after 24 hours of exposure to the powder, there was an increase in temperature, this could have been due to the fact that the powders may have been releasing the previously absorbed heat and thus increasing the temperatures. However, this study was still unable to explain for this observed abnormal decrease in temperatures with time. This may therefore warrant for further investigations. However, the observed trend in temperature changes between the exposure hours is more likely a reflection of time (between 0700 and 1200 hours) at which larvae were sampled on each particular day. During sampling, larvae were collected at different hours on different days. This difference in the hours of sampling could be responsible for the fluctuations in temperatures observed.

5.4 Influence of plant materials on water conductivity

Previous studies have demonstrated the relationship between larval occurrence and water conductivity (Rejmankova *et al.*, 1992; Grillet, 2000). The mechanism by which

conductivity affects the anopheline abundance and occurrence is unknown, although at the physiological level, it is likely to involve ionic balance in the larvae (Grillet, 2000). In this study, water conductivity in the treated pools increased with time and dose to a maximum of 5.44 Ms/cm in the highest dose. The observed value is not different from the water conductivity recorded in larval habitats in previous studies (Mittal *et al.*, 1998; Grillet, 2000). Most larvae occur in sites with conductivity below 5 Ms/cm (Mittal *et al.*, 1998). The preference of *An. stephensi* for highly saline habitats has been noted (Tyagi *et al.*, 1995). Therefore, the increase in conductivity may not have caused larval mortality in the treated pools. This is due to the fact that anophelines and culicines can tolerate a wide range of water salinity (Njogu and Kinoti, 1971). The reason of the adaptation to such a wide range of salinities is not known. Beadle (1939) suggested that impermeability to water and inorganic ions might enable *Ae. natronius* to breed in physiologically unfavourable waters. *An. gambiae s.l.* that is a common species along Kenyan coast is a complex of fresh water species *An. gambiae s.s.*, *An. arabiensis* and a salt-water species *An. merus* (Gillies and De Meillon, 1968). Two of the sibling species have been previously recorded in Jaribuni, the current study site (Kubasu, 1997). This study may have been dealing with similar species that can tolerate high salinities. The results of this study show that the plant material caused increased water conductivity. The increase, however, may not have had any influence on larval susceptibility to the powder.

5.5 Emergent mosquitoes

The significance of the threat of mosquitoes in disease transmission is determined by the numbers of adults emerging from the breeding site (Kettle, 1992). More adults emerged from the pools treated with the lowest dose compared to those treated with the highest dose. The difference in emergence rates could be due to the high mortality of fourth larval instars in the highest doses. In general, the overall results demonstrated that more anophelines than culicines emerged. The high mortality of fourth instar culicine larvae might have contributed to low number of emerging adults. More adults emerged from pools treated with *S. mauritiana* than *P. guineense* treated pools. The difference can be attributed to the high larval mortality in the *P. guineense*. Although pupal mortality was recorded, some successfully emerged as adults especially at the lower doses compared to

the higher doses. This is mainly because, there was higher pupal mortality at the highest dose compared to the lowest dose. The low pupal mortality at the lowest doses could explain for the high number of emergent mosquitoes at the lower doses compared to the high doses. In the control pools, relatively low numbers of emergent adults were collected than expected. During the study, high numbers of pupa and fourth instar larvae were introduced into the pools. However, all the introduced pupa and fourth instar larvae could not develop into adults because, the experimental time of 72 hours is relatively less than the average time needed for larvae and pupa to develop to adults. However, the observed emergence rate of 4.92 – 5.27% for anophelines in the present study are closer to those observed by Service, 1977.

Fewer *An. funestus* adults emerged than *An. gambiae s.l.* This suggests that *An. funestus* may be more vulnerable to the plant powder than *An. gambiae s.l.* In this study area, *An. funestus* is the predominant species (Kubasu, 1997) and their high mortality may be translated to low numbers of adults and hence reduction in malaria transmission. Although both species are primary malaria vectors in Kenya especially along the coast (Mbogo *et al.*, 1996), the higher emergence rate observed for *An. gambiae s.l.* has an implication in malaria control since they are also considered efficient vectors of malaria parasites (Kubasu, 1997). It is also worth noting that the majority of the larvae in the pools treated with the lowest doses successfully pupated, survived and moulted to adults within the two days as observed in the controls.

5.6 Influence of the plant materials on the body size

The wing length of adults emerging from treated and control pools did not show any significant difference between the pools. *An. gambiae s.l.* had a mean wing length of 2.69 ± 0.083 mm. The wings of the emerged mosquitoes from both the treated and control pools were normal and not malformed. According to the classification - based on wing length - mosquitoes can either be classified as large or small (Takken *et al.,* 1998). The classification is based on the normal distribution curve where mosquitoes with the wing length less than the overall mean are classified as "small" while mosquitoes with wing length greater than the overall mean are classified as "large". Based on this

classification, the emergent mosquitoes in this study could generally be classified as "large". The disadvantage of "large" mosquitoes in relation to parasite transmission has been reported in various studies (Lyimo and Takken, 1993; Lyimo and Koella, 1992). Briegel (1990) noted that, for *An. albimus* and *An. gambiae,* females that started egg maturation after one blood meal, were significantly larger than those that did not. The number of eggs produced by a female mosquito is related to its size (Bock and Milby, 1981; Steinwascher, 1982; Packer and Corbet, 1989). Hence the "large" mosquitoes recorded in this study may be very efficient vectors that are a disadvantage in terms of malaria parasite transmission, however, this may depend on the levels of transmission in specific areas. This may imply that the plant materials did not have any influence on the body size of emergent adults. The emerging adults could therefore be of high vector competence like the adults in the untreated pools. The results are in contrast to studies by Mwangi and Mukiama (1988), using *M. volkensii* extracts in which the emerged adults were usually smaller in size, incapable of flight, often having malformed or peculiarly folded wings and deformed appendages. The observations by Mwangi and Mukiama, 1988 and Zebitz, 1984 using mosquitoes treated with neem seed kernel also reported emerged adults of smaller size, incapable of flight, having malformed or peculiarly folded wings and deformed appendages.

From the results, the number of adults which had their wings measured was low compared to the total number of emerged adults. This was due to the difficulty experienced by most adults during emergence. The adults were unable to fly from the water surface where ecdysis took place and therefore died within 24 hours of emergence leaving the detached wings on the water surface. Mwangi and Mukiama (1988) also reported similar observations in *Ae. aegypti.* However, considering the adults that successfully emerged form the pupal exuviae, no morphological abnormalities were observed. This therefore indicates that these powders have no effect on body size of emerging adults.

5.7 Influence of the plant materials on larval and pupal morphology

In addition to the direct toxicity of the plant powder, several morphological abnormalities were observed in the larvae from the treated pools as compared to the controls. Larval-pupal intermediates, with a pupal head capsule and larval abdominal segments, were observed in anophelines. Similar observations were reported by Sujatha *et al.* (1988), using petroleum ether extracts of *Ageratum conyzoides*, in *Cx. quinquefasciatus, Ae. aegypti* and *An. stephensi.* They reported larvae with intermediary stages, discoloured and longer pupae. Mwangi and Mukiama (1988), Saxena and Saxena (1992) and Saxena *et al.* (1994) also reported similar results with *Ae. aegypti* larvae. This suggests that the plant material from *P. guineense* and *S. mauritiana* may be acting as IGRs against the mosquitoes.

The most obvious effect of the plant materials was observed when adults attempted to separate themselves from the pupal exuviae. Some adults extended only the head and thorax while others were able to remove their whole body but remained bound to the exuviae by their legs and tarsi. Similar abnormalities have been reported in mosquito larvae after treatment with insect growth regulators from Rose Bengal (Spielman and Skaff, 1967; Ittycheriah *et al.,* 1974; Arias and Mulla 1975; Pimprikir *et al.,* 1979). The results, therefore, indicate further that these plant powders may be acting as insect growth regulators against mosquitoes and suggest the presence of IGRs. However, this is a contrast to results reported by Olaifa *et al.* (1987) where *P. guineense* extracts induced symptoms of poisoning such as browning of treated area in *Acrae eponina,* with such parts usually turned blackish. These are symptoms common to many conventional chlorinated hydrocarbon and organophosphate insecticides. This suggests that *P. guineense* acts differently depending on the species of the target insect.

5.8 Effect of plant materials on larval predators

Predation influences the population dynamics of anophelines (Laird and Miles, 1985) and may be the most single most important factor determining vector abundance (Reisen *et al.,* 1989; Service, 1993). In Kenyan rice fields, larval mortality of *An. arabiensis* attributed to predators has been estimated at 93% (Service, 1977). During this study,

potential predators including fish, amphibians, coleopterans, notonectidae and odonates were recorded. Chesson (1984) observed that growth rates for larvae in the presence of notonectidae treatments were significantly reduced than the other treatments with other predators.

This study therefore shows that the plant materials, at the tested doses are relatively less toxic to some predatory non-target organisms. The non-interference of the tested powders with the predators accommodates supplementary contribution of the predators towards population regulation of mosquitoes. Miura *et al.* (1980), Gharib and Hilsenhoff (1988) and Sebastien and Brust (1981) demonstrated that *B.t.i* was not detrimental to non-target organisms. This suggests that the plant powders can be successfully integrated into mosquito control programmes where biological control is being used.

Since mosquito larvicides are applied to water for the control of the larval stages of mosquitoes, aquatic ecosystems are of the most immediate concern. Larvicides from *Tagetes* species were shown to be effective against mosquitoes but friendly to non-target organisms like the ostracod, caddis fly and *Physa* spp (Philogene *et al.*, 1985). The effect of neem on non-target organisms has been studied extensively. Neem is most selective against the lepidopteran pests and has less effect on parasitoids or predators (Mansour *et al.*, 1987). However, adverse effects of azadirachtin against beneficial organisms have been reported (Schumutterer and Holst, 1987; Price and Schuster, 1991). In this study, the powders of the two plants were relatively safe to macrodystiscids, microdytiscids and back swimmers of the tested invertebrates and vertebrates. In this study, fish was the least tolerant even in the controls with high mortality within 24 hours of exposure to the powder. The mortality of fish in the controls could be due to unfavourable environmental conditions in the artificial pools and the problems of delicate handling during sampling. Notonectids and microdystiscids were the least susceptible to the powders. The observed reduction in the number of macrodystiscids could be attributed to the fact that they can fly away once exposed to the powder. Indeed, the beetles were observed to fly away on the application of the powder. The plant powders may be repellent to the beetles.

The range of responses for the non-target organisms tested can be attributed to many factors. The individual test organisms used in this study were native populations and probably had a high degree of genetic, physiological, and behavioral variability within species. In general, the results indicate that these powders do not have a toxic effect on the predatory aquatic invertebrates and vertebrates studied. However, more information need to be obtained for firm conclusions to be drawn on consequences or benefits from the use of these powders as replacements/supplements to more commonly used larvicides in areas adjacent to streams and river systems. At the doses tested, the powder may not be harmful to non-target predatory organisms in aquatic environments.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

From this study, several conclusions and suggestions for future work can be made.

6.1 Conclusions

1. *Piper guineense* and *Spilanthes mauritiana* powder exhibit high larvicidal activity against immature anopheline and culicine mosquitoes under natural conditions.
2. Powders from both plants have no significant effect on the mean temperature and water conductivity of the treated pools.
3. The plant powder had no effects on the body size of adult mosquitoes emerging from the treated pools. No effect on phenotypic morphology was observed in the emerged adults.
4. Plant powder was also more effective against anopheline first and second instars than third and fourth instars, especially so for the *S. mauritiana* powder.
5. *Piper guineense* was found to exhibit higher larvicidal activity than *S. mauritiana.* Powder from the two plants was found to be more effective against the culicines than the anophelines. However, mortalities in anophelines would make the powder an important integral part of an integrated approach in malaria vector control.
6. The plant materials caused significant reduction in dissolved oxygen in the treated pools. The reduction in dissolved oxygen may have been responsible for the observed larval mortality.
7. The plant materials did not show any negative effect on non-target predatory organisms that also contribute in controlling mosquito larval population densities. The plant materials could therefore be used safely in mosquito control without causing negative effect to non-target organisms in mosquito breeding habitats.

In summary, it has been demonstrated in this study that *P. guineense* and *S. mauritiana* have larvicidal potential in mosquito control.

6.2 Recommendations

Plants may provide rural, community-based, affordable and accessible mosquito control tools that can impact on malaria transmission in endemic foci. It may be necessary to carry out further investigations before these plants are introduced to rural communities for mosquito control.

1. There is need to investigate the residual activity of the plants on the suitability of the treated pools as breeding sites. This would help to determine how frequent the breeding habitats need to be treated.
2. The vertebrate toxicology and its effects on a wide range of non-target aquatic organisms need further study before they can be seriously considered as an alternative to current mosquito larvicides. It is only after this that their use in mosquito control could be evaluated. It could also be necessary to investigate the effect of these plants on larger non-target organisms including mammals, to assess their suitability for mosquito control in habitats used as sources of drinking water.
3. Since the plants have shown high larvicidal activity in relatively small pools, it would be necessary to conduct more trials in larger water bodies such as ponds, rice fields, swamps, streams and drainages. This will help evaluate their effectiveness for mosquito control under a variety of expansive natural habitats.
4. There is need for field assessment of the active ingredients in the powders as this will help in accurate formulation and application.
5. It would be interesting to use sub-lethal doses of the two plants and investigate any insect growth regulation properties and the impact on mosquito populations in natural breeding habitats.
6. There is need to investigate the mode of action of these plants.
7. Assessment of the efficacy of these plants on a variety of mosquito species also need to be undertaken to define their range of action and consequently their geographical/ ecological boundaries in mosquito control measures.

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APPENDIX 1

LARVAL SAMLPLING SHEET 2002

Site code A B C Date Sample conc.

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	3rd(An.)																																									
	3rd(Cx.)																																									
	4th(An.)																																									
	4th(Cx.)																																									
	Pupae																																									

APPENDIX 2

RESULTS DATA SHEET

Habitat No.		Locality	
Control Yes/No			

Date		Sample concentration g/l	
Vol. (ml)		Plant spp.	

LARVAL RESULTS

ANOPHELINES PRESENT

Time (hours)	00	24		48		72	
Instar	Alive	Alive	Dead	Alive	Dead	Alive	Dead
1st							
2nd							
3rd							
4th							
TOTAL							

CULICINES PRESENT

Time (Hours)	00	24		48		72	
Instar	Alive	Alive	Dead	Alive	Dead	Alive	Dead
1st							
2nd							
3rd							
4th							
TOTAL							
PUPAE							

Emergent adults score sheet

Time (Hours)	24		48		72	
Sex	Males	Females	Males	Females	Males	Females
No. of Culicines						
No. of *An. gambiae s.l.*						
No. of *An. funestus*						
Other Anopheline spp						
Total						

Water parameters score sheet

Time (Hours)	00	24	48	72
Conductivity (MS/l)				
Temperature (^{0}C)				
Dissolved Oxygen (mg/l)				

Non-target aquatic organisms score sheet

Time (Hours)	00	24	48
Damsel fly nymphs			
Dragon fly nymphs			
Back swimmer			
Microdysticids			
Macrodystiscids			
Shrimps			
Tadpoles			
Fish			

APPENDIX 3

Table 8a: Larvae sampled in pools treated with 1.429 g/l of *Spilanthes mauritiana*

	Time (h)	00		24		48		72	
Species	Instar	T	C	T	C	T	C	T	C
Anopheles	1	1074	588	763	577	257	446	34	289
	11	715	404	549	394	270	380	49	388
	111	522	309	422	300	250	319	75	314
	1V	627	348	541	338	349	342	142	393
	Total	2938	1649	2275	1609	1126	1487	300	1384
Culicines	1	882	609	668	590	201	444	39	317
	11	674	605	531	600	313	569	85	520
	111	459	265	373	255	215	261	98	298
	1V	217	61	171	61	126	91	86	124
	Total	2232	1540	1743	1506	855	1365	308	1259

Table 8b: Larvae sampled in pools treated with 2.857 g/l of *Spilanthes mauritiana*

	Time (h)	00		24		48		72	
Species	Instar	T	C	T	C	T	C	T	C
Anopheles	1	1017	689	372	656	5	522	0	418
	11	728	377	422	381	31	354	2	321
	111	380	226	244	221	24	247	7	265
	1V	320	171	171	176	81	215	20	227
	Total	2440	1463	1209	1434	141	1338	29	1231
Culicines	1	751	277	214	239	5	101	3	71
	11	382	260	156	259	83	303	33	255
	111	267	143	105	145	90	180	73	196
	1V	73	24	46	34	40	54	35	56
	Total	1473	704	521	677	218	638	144	578

T- treated C- control

Table 8c: Larvae sampled in pools treated with 5.714 g/l of *Spilanthes mauritiana*

	Time (h)	00		24		48		72	
Species	Instar	T	C	T	C	T	C	T	C
	1	1059	438	11	433	0	302	0	197
	11	740	294	64	283	4	262	0	236
Anopheles	111	446	204	97	214	5	267	0	273
	1V	541	180	121	152	15	193	0	231
	Total	2786	1116	293	1282	24	1024	0	937
	1	438	325	0	299	0	171	0	133
	11	467	299	2	277	2	318	1	244
Culicines	111	378	99	38	149	27	156	18	178
	1V	277	114	8	113	11	116	7	136
	Total	1560	837	48	838	40	761	26	691

Table 8d: Larvae sampled in pools treated with 8.571 g/l of *Spilanthes mauritiana*

	Time (h)	00		24		48		72	
Species	Instar	T	C	T	C	T	C	T	C
	1	672	480	1	475	1	308	0	198
	11	523	268	3	262	1	302	0	305
Anopheles	111	490	190	13	187	3	192	0	238
	1V	248	116	16	105	5	164	0	168
	Total	1933	1054	33	1029	10	966	0	909
	1	380	141	0	138	0	72	0	31
	11	385	250	0	242	0	211	0	192
Culicines	111	159	121	0	122	0	155	0	148
	1V	74	30	0	31	0	53	0	82
	Total	998	542	0	533	0	491	0	453

T- treated C- control

APPENDIX 4

Table 9a: Larvae sampled in pools treated with 1.429 g/l of *Piper guineense*

	Time (h)	00		24		48		72	
Species	Instar	T	C	T	C	T	C	T	C
Anopheles	1	1374	541	1067	517	531	359	152	168
	11	572	266	462	246	255	275	152	278
	111	281	244	233	240	143	220	106	245
	1V	310	269	216	252	137	242	91	259
	Total	2537	1320	1978	1255	1066	1096	501	950
Culicines	1	863	493	639	473	256	276	68	142
	11	778	406	574	394	381	398	261	423
	111	386	223	298	208	241	279	258	297
	1V	174	98	95	84	81	65	87	133
	Total	2201	1220	1606	1159	959	1018	674	995

Table 9b: Larvae sampled in pools treated with 2.857 g/l of *Piper guineense*

	Time (h)	00		24		48		72	
Species	Instar	T	C	T	C	T	C	T	C
Anopheles	1	1413	626	25	607	228	547	167	471
	11	734	359	136	353	37	321	19	277
	111	404	151	130	141	36	132	10	115
	1V	202	165	74	154	25	120	5	96
	Total	2753	1301	365	1255	326	1120	201	959
Culicines	1	1029	530	81	515	273	455	99	387
	11	681	491	54	474	60	460	62	412
	111	262	283	11	263	8	244	3	215
	1V	143	129	8	100	2	88	3	71
	Total	2115	1433	154	1352	343	1247	167	1085

T-treated C- control

Table 9c: Larvae sampled in pools treated with 5.714 g/l of *Piper guineense*

	Time (h)	00		24		48		72	
Species	Instar	T	C	T	C	T	C	T	C
Anopheles	1	1145	581	8	563	53	500	13	420
	11	635	349	15	337	4	307	0	281
	111	395	224	26	218	2	203	1	172
	1V	289	182		175	6	141	0	126
	Total	2464	1336	49	1293	65	1151	14	999
Culicines	1	689	495	0	477	262	446	113	404
	11	780	574	5	549	5	505	2	473
	111	334	189	2	192	0	175	0	164
	1V	116	61	11	53	6	45	2	33
	Total	1919	1319	18	1271	273	1171	117	1074

Table 9d: Larvae sampled in pools treated 8.571 g/l of *Piper guineense*

	Time (h)	00		24		48		72	
Species	Instar	T	C	T	C	T	C	T	C
Anopheles	1	1246	719	8	700	17	649	4	571
	11	595	367	14	358	0	358	0	344
	111	434	243	23	235	4	221	0	212
	1V	289	174	20	163	9	132	0	109
	Total	2564	1503	65	1456	30	1360	4	1236
Culicines	1	825	377	0	362	48	325	45	276
	11	937	511	4	504	1	450	1	415
	111	263	269	1	262	0	264	0	237
	1V	102	107	2	85	2	80	0	67
	Total	2127	1264	7	1213	51	1119	46	995

T-treated C-control