EFFECTS OF EXTRACTS AND HYDRODISTILLATES OF *Clausena anisata* (Wild.) ex. Benth. AND *Hyptis spicigera* Lam. ON *Musca domestica* L. AND *Periplaneta americana* (L.)

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A Thesis presented in partial fulfilment of the requirements for the degree of M.Phil. Entomology of the University of Ghana.

Insect Science Programme*

University of Ghana

Legon

December 1997

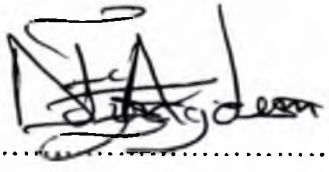
**Joint interfaculty international programme for the training of entomologists in West Africa. Collaborating Departments: Zoology (Faculty of Science) & Crop Science (Faculty of Agriculture).*

DEDICATION

Dedicated to my parents Mr.(Late) and Mrs. Dongdem, for their love and support, and to my brothers Anthony, Julius, and Achillis and to my sister, Mercy Anne.

DECLARATION

I, Mr. Ferdinand A. Dongdem, do hereby declare that this preliminary study was carried out entirely by me and that it has not been presented for any degree here or abroad.



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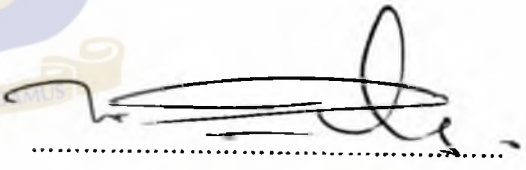

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ABSTRACT

Extracts of two indigenous Ghanaian medicinal plants *Clausena anisata* and *Hyptis spicigera* were bioassayed against housefly (*Musca domestica* L.) and cockroach (*Periplaneta americana* (L)) for various insect control activity. The extractives bioassayed were the petroleum ether and methanol extracts and essential oil of the plants. The lethal toxicity of the plant extractives were bioassayed using topical application and petri dish residual method against adult flies and cockroach nymphs respectively. The essential oils exhibited knockdown effect when they were bioassayed against housefly by topical application. A treated filter paper method was used to evaluate the repellency of the extractives against housefly. The crude extracts were also bioassayed for growth regulatory effect against the second larval instar of housefly using a treated larval breeding medium. A treated rearing medium method was also used to test the antifeedant effect of the crude extracts against cockroaches.

Per cent mortality, knockdown and index of repellency induced by the extractives against the insects were generally dose-dependent. The essential oil of the two plants were however more effective as toxicants and repellents than the crude extracts. The LD_{50} of the essential oil of *C. anisata* and *H spicigera* were 2.2101 and 0.8580 μg respectively, against flies and 0.0096 and 0.0310 μg cm^{-2} respectively against cockroach nymphs. The LD_{50} of the solvent extractives ranged between 0.4143 and 1746.2 mg for housefly and 0.6869 to 2.600 mg cm^{-2} for cockroach. The knockdown potency of essential oils from *H. spicigera* (KD_{50} = 0.2631) was better than for *C. anisata* (KD_{50} = 0.7445). The RD_{50} of *C. anisata* and *H. spicigera*

in 30 min were 0.0400 and 0.0509 μg cm^{-2} against housefly. The RD_{50} of the crude extracts ranged from 0.1288 to 1.3768 mg cm^{-2} against housefly. The crude extracts however demonstrated poor growth regulatory and antifeedant effects.

ACKNOWLEDGMENTS

My gratitude goes to the National Agricultural Research Project which provided funds toward the completion of this study.

Many individuals have also contributed toward the completion of this study and therefore deserve my gratitude. I gratefully acknowledge my supervisor, Dr. S. W. K. Gbewonyo, a Senior lecturer of the Department of Biochemistry, University of Ghana, Legon who responded with keen interest to the study. I am deeply indebted to him for his pieces of advice, criticisms, suggestions and reading through the work. In fact he personally taught and supervised every stage of the breeding of the insects to the extraction and application of the extracts. I also acknowledge with gratitude the constructive criticisms and corrections of Dr. D. Obeng-Ofori of the Crop Science Department and the time he spent in reading the manuscript.

I thank Prof. Marian Addy, Head of the Department of Biochemistry, for granting me permission to use facilities at their Department to carry out the study. I am also grateful to the laboratory technicians especially Mr. S. Feyi who helped me in diverse ways. I thank my colleagues, Mrs. Dorcas Osei-Sarfo who introduced me to the extraction procedure of the essential oils and Messrs. Sam Addo and George Collison who introduced me to the Ms Excel computer programme for the analysis of my data.

To God be the Glory.

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LIST OF ABBREVIATIONS

CO_2 - Carbon dioxide.

Conc. - Concentration.

DDT - 1,1,1 - trichloro - 2, 2 - bis (p - chlorophenyl) ethane.

GAFCO Ltd - Ghana Agro-Food Company Limited, Tema, Ghana.

GTV - Great Television Viewing.

HCH - 1, 2, 3, 4, 5, 6 - hexachlorocyclohexane.

IITA - International Institute of Tropical Agriculture.

KD_{50} - Dose required to knockdown 50% of the treated insects. An insect is considered knockdown if it is found lying on its back, no longer able to upright itself but is still moving.

LD_{50} - Dose required to kill 50% of the treated insects.

NCDC - National Communicable Disease Center.

N_2 - Nitrogen.

NPCA - National Pest Control Association.

RD_{50} - Dose required to repel 50% of the insects.

WHO - World Health Organization.

Symbols

% - Percentage

CHAPTER 1

1.0 GENERAL INTRODUCTION

1.1 Perspective and overview

The housefly (*Musca domestica* L.) and American cockroach (*Periplaneta americana* (L.)) are important household and public health pests. These insects, by virtue of their cohabitation with man, transmit many bacterial and viral diseases such as dysentery, leprosy, typhoid fever, cholera and poliomyelitis, through contact or food contamination. For almost half a century man has depended largely on synthetic insecticides to combat insect pests and vectors (Capstick, 1990) including the housefly and American cockroach. However, the misuse, overuse and abuse of these insecticides resulted in insecticide residues in the environment, development of insecticide resistance in insects, and the destruction of non-target organisms creating upsets in ecological balance (Carson, 1962; Newsom, 1967). Some of these insecticides are also neurotoxic and lethal to man (Murphy, 1986) and his domesticated animals. These problems have made the use of synthetic insecticides undesirable. The high cost of these insecticides has also made resource-deficient rural folks revert to the use of plant materials to control insect pests. These problems have led to the search for cheaper, safer and more biodegradable alternatives to synthetic pesticides.

Several studies have shown that natural products, particularly plant products, are a potential source of cheap and environmentally friendly bioactive substitutes for synthetic insecticides (Elliott *et al.*, 1978; Tierto Niber *et al.*, 1992; Wink, 1993). There is therefore an increased

interest among entomologists, phytochemists, plant protection agencies, etc in finding a role for natural plant products in orthodox insecticide practice. Among the plants noted for insecticidal activity are some medicinal plants. Most medicinal plants are less harmful to man and other organisms compared with other plants and therefore provide a more suitable alternative source for the search for botanical insecticides.

Clausena anisata (Wild.) ex. Benth. and *Hyptis spicigera* Lam. are two of the many tropical herbal plants (Ayensu, 1978) with insecticidal properties. *C. anisata* (Family: Rutaceae) is a shrub or small tree with odorous pinnate leaves (Plate 1), whilst *H. spicigera* (Family: Labiateae) is a tall erect, aromatic rather scabribe herb (Plate 2). Traditionally the leaves of these plants are burned to repel mosquitoes (Abbiw, 1990). The leafy branches are also used for grain protection (Parh *et al.*, 1990). However, claims over these medicinal plants showing insecticidal properties are based on only observations of farmers and herbalists (Abbiw, 1990; Parh *et al.*, 1990). It is also not certain whether the leaves of these plants act as repellants or toxicants or both because farmers and herbalists appear to have varying views. It is equally not certain if these are the only insect control properties they possess, for, Gebreyesus & Chapya (1983) isolated two antifeedants from the leaves of *C. anisata*. These considerations prompted the present study on the insect control properties of these traditional herbs with the view to providing some empirical data to establish the scientific basis for their continued use. The study is also an attempt to find affordable and readily available sources of botanical insecticides against household pests including the housefly and cockroach. It is along such lines of study that simple compositions of the leaf potions of these plants could be formulated

Plate 1. ***Clausena anisata*** **(Wild.) ex. Benth. (Family: Rutaceae).**

Plate 2. ***Hyptis spicigera*** **Lam. (Family: Labiatae).**

for household and agricultural application. Beyond this are also prospects of formulating new compositions for the insecticide industry and new prescriptions for biopesticidal administration. These together provide the rationale for screening the extracts and essential oils of the leaves of *C. anisata* and *H. spicigera* for their insect control properties against the housefly and cockroach.

1.2 Specific objectives of the present study:

To determine,

(i) The lethal toxicity of extracts and essential oils of *C. anisata* and *H. spicigera* on the housefly and cockroach,

(ii) The knockdown effect of the essential oils in the housefly,

(iii) The repellency of the extracts and essential oils of the plants to the housefly,

(iv) The growth regulatory effect of the extracts of the plants on the housefly,

(v) The antifeedant effect of the extract on the cockroach.

CHAPTER 2

2.0 REVIEW OF THE LITERATURE

2.1 Plant defence mechanisms

Plants as a consequence of their immobility, have evolved several different types of static defence against herbivores-majority of which are herbivorous insects and/or pathogens. These defence mechanisms have been reviewed by several authors including; Levin (1976), Swain (1977) and Wink (1988). The mechanisms may be distinguished as physical or chemical, but are not independent of each other and may interact cooperatively and synergistically.

Physical defence is provided by hooks, thorns, prickles and trichomes; glandular and stinging hairs on leaf surfaces; hard seed coats; thick bark in roots and stems; and latex or resin (Bell, 1987; Wink, 1993).

The production and storage of defence chemicals which are abundant is, however, the most important defence strategy (Bell, 1987; Wink, 1993). These defence chemicals are secondary plant compounds or metabolites. They were originally thought to play no part in the basic metabolic processes in the plant (Mothes, 1976; Bell, 1987) but are now generally assumed to be important for the survival and fitness of plants although the biological functions of many have not been studied experimentally (Wink, 1993). These secondary metabolites are believed to have resulted from the coevolution of plants with herbivorous insects, in particular (Bell, 1987; Wink, 1993). However, these secondary plant metabolites have long been and are still

being exploited for medicinal purposes (Evans-Anfom, 1984) and have until recently erroneously made traditional medicinal plants rather biopesticidal candidate plants.

2.2 Secondary plant metabolites

Natural plant compounds used in the control of insect pests are as varied as the plants from which they have been isolated. More than 30,000 secondary metabolites have been reported from plants (Swain, 1977; Wink, 1988). The major groups of compounds with insecticidal activity include: alkaloids (>10,000), amines (>100), non-protein amino acids (>400), cyanogenic glycosides (>50), glucosinolates (>100), lectins (>100), protease inhibitors (>50), all of which are nitrogen-containing allelochemicals (Wink, 1993). Other allelochemicals are monoterpenes (>1000), sesquiterpines (>1500), diterpenes (>1000), triterpenes/steroids (>800), tetraterpenes (>350), polyketides (>700), polyacetylenes (>750), flavonoids (>1,200), phenylpropanoids (>500). Since only 5-10 per cent of all higher plants comprising more than 300,000 species have been analysed phytochemically in some detail, the actual number of secondary products is certainly very large and exceeds 100,000 compounds (Wink, 1993).

2.3 Insect control activities

Many though these secondary plant metabolites are, they fall into two broad categories: the toxic preparations and the behavior controlling chemicals (Chaudhury, 1990). The toxic preparations may be ovicidal and/or larvicidal and/or adulticidal. Examples include nicotine, rotenone, ryania, some pyrethrins, quassin, etc. The protective effects of the behavior controlling chemicals range from antigonadal properties, oviposition deterrence, growth and

development inhibition, antifeedant effect, to repellency, and include; asarones, ajugarins, some pyrethroids, sparteine, cytisine, lupinine, angustifoline, etc (Chaudhury,1990; Wink, 1993).

2.4 Progress in the development of botanical insecticides

Only a small percentage (Grainge & Ahmed, 1988) of plants have been screened for insecticidal activity. In addition many of these studies are not complete and often the bioassay procedures used are inadequate or inappropriate (Khambay & O'Connor, 1993). Despite the problems associated with the identification, isolation, characterization and evaluation of botanical insecticides (Khambay & O' Connor, 1993) a number of new and established examples of plant insecticidal compounds are known. These are:

(a) Pyrethroids

Six pyrethroids have been extracted from *Chrysanthemum cinerariaefolium* Vis. with pyrethrin I (Fig. 1a) as the lead structure for optimization of insecticidal activity (Elliott *et al.*, 1975). Recent reviews have shown that the insecticidal activity of the synthetic pyrethroids can now be increased over 1000 fold relative to the lead compound (Elliott, 1989; Naumann, 1981).

(b) N-Alkylamides

Among the N-Alkylamides (Fig. 1b) are a number of natural isobutylamides from Compositae and Rutaceae (Jacobson, 1971) with insecticidal activity. These are more toxic to a pyrethroid

resistant (kdr) strain of housefly than to the susceptible strain (Elliott *et al.*, 1986) and may thereby reveal potential against economically important resistant insect strains (Khambay & O'Connor, 1993). Elliott *et al.*(1987) reviewed the literature on N-alkylamides.

(c) Anonaine and acetogenins

Annona species are known for several types of biological activity including medicinal properties. Anonaine (Fig. 1c) and the recently discovered insecticidal acetogenins (Rupprecht *et al.*, 1990) have been isolated from *Annona squamosa* (Annonaceae). The acetogenins occur in several *Annona* species. Though only a limited amount of synthesis has been reported, these compounds are believed to be generally biocidal (Khambay & O'Connor, 1993).

(d) Lignans

Lignans (Fig. 1d) (highly oxygenated phenylpropanoid dimers) are compounds not generally associated with insecticidal activity. However, Yamauchi & Taniguchi (1991) recently identified aedoxan A from *Phryma leptostachya* as a highly active lignan.

(e) Phototoxins

The phototoxins, polyacetylenes and thiophenes (e.g. α-terthienyl, Fig. 1e) isolated from Asteraceae have shown high activity (LD_{50} 40ppb against houseflies) but only when exposed to UV-radiation (e.g. Fields *et al.*, 1991) or in the presence of singlet oxygen (Khambay & O'Connor, 1993). They act by a novel mode of action and therefore have a great potential as a new family of insecticides.

(f) Naphthoquinones

Plumbagin (Fig. 1f), a naphthoquinone, present in many species of Plumbaginaceae (e.g. *Plumbago europea*) known for medicinal properties is claimed to have several types of activity including insecticidal and antifeedant activities (Gujar, 1990).

(g) Nicotinoids

Nicotine (Fig. 1g) (a nicotinoid), the main alkaloid of many *Nicotiana* species, such as *N. tabacum* has been used as an insecticide for at least 200 years. Its use in agriculture has declined since the advent of synthetic pyrethnoids mainly due to its high mammalian toxicity and low persistence. Although some variations retain insecticidal activity (Schmeltz, 1971) none is significantly more active than nicotine.

(h) Coumarins

Members of the genus *Mammea* are amongst the most insecticidal plants. The insecticidal activity of the leaves and seeds of the immature fruit of *M. americana* (Clusiaceae) has been attributed to a coumarin (Fig. 1h). Although much work on synthetic analogues has been done by Crombie (1989) no data on their activity is available.

(i) Rotenone

Rotenone (Fig. 1i) obtained from the roots of the genera *Derris* and *Lonchocarpus* (Leguminosae) have been used as commercial insecticides for almost 150 years. Structure-activity relationship studies on synthetic analogues have identified the key features necessary for activity (Crombie *et al.*, 1992). However, only one analogue has been found to be more

active than rotenone (Khambay & O'Connor, 1993). The use of rotenone as an insecticide is limited by its relatively high mammalian toxicity.

(j) Veratrum alkaloids

Veratrum alkaloids (e.g. veracevine, Fig. 1j) from *Schoenocaulon officinale* (Lilinaceae) (Crosby, 1971) are also known for their insecticidal and mammalian toxicity (Ujvary *et al.*, 1991).

(k) Quassins

Quassin (Fig. 1k), neoquassin, and 18-hydroxyquassin are the insecticides obtained from *Quassia amara* and *Picrasma excelsa* (Simaroubaceae). These plants also possess medicinal properties. Quassin seems to be a selective pesticide with no recorded toxicity to vertebrates (Wink, 1993).

(l) Ryanodine

Ryania speciosa (Flacourtiaceae) produces the insecticidal ryanodine (Fig. 1l). It shows relatively low vertebrate toxicity (Merck Index, 1989). Ryanodine was patented in 1946 by Merck and Co. ("Ryanex, Ryanicide"). Its use is limited by the shortage of raw material.

(m) Azadirachtins

Azadirachta indica (Neem), another medicinal plant and *Melia azederach* (Chinaberry) produce seeds rich in insect deterrent and insecticidal tetranortriterpeniods such as azadirachtin (Fig. 1m). This plant and its constituents have been developed and tested intensively during the last two decades for use as a natural insecticide (Schmutterer *et al.*, 1981). A commercial

product Margosan-O has been developed in the USA, but has been registered only for non-food plants and forestry (Wink, 1993).

(n) Quizolizidine alkaloids

Quizolizidine alkaloids constitute the main secondary products of many Leguminosae, especially in the genera *Lupinus, Genista, Cytisus, Baptisia, Thermopsis, Sophora,* and *Ormosia* (Wink, 1993). The main structural types belong to lupanine (Fig. 1n)/sparteine, multiflorine, 13α-hydroxylupanine, tetrahydrorhombifoline, 13α-tigloyloxylupanine, aphylline, anagyrine/cytisine, lupinine, natrine, and respective derivatives (Kinghorn & Balandrin, 1984; Wink, 1993). Quizolizidine alkaloids have insecticidal and insect deterrent properties.

2.5 Commercialization of botanical insecticides

Though crude extracts of plants or dried and powdered plant tissues producing toxic, repellent, antifeedant and growth regulatory effects have a long history of use in the Americas, South East Asia and Africa as insecticides, very few of these have attained commercial levels. Nicotinoids, rotenone, pyrethrins, ryanodine, quassin, and the newer product margosan-0 are the only substances to have found their way into agricultural application (Wink, 1993). A number of potential candidates have reached the preparatory stage e.g. the quizolizidine alkaloids (Jacobson, 1982).

2.6 The role of botanical insecticides

Interest in the search for botanical insecticides has recently increased for a number of reasons.

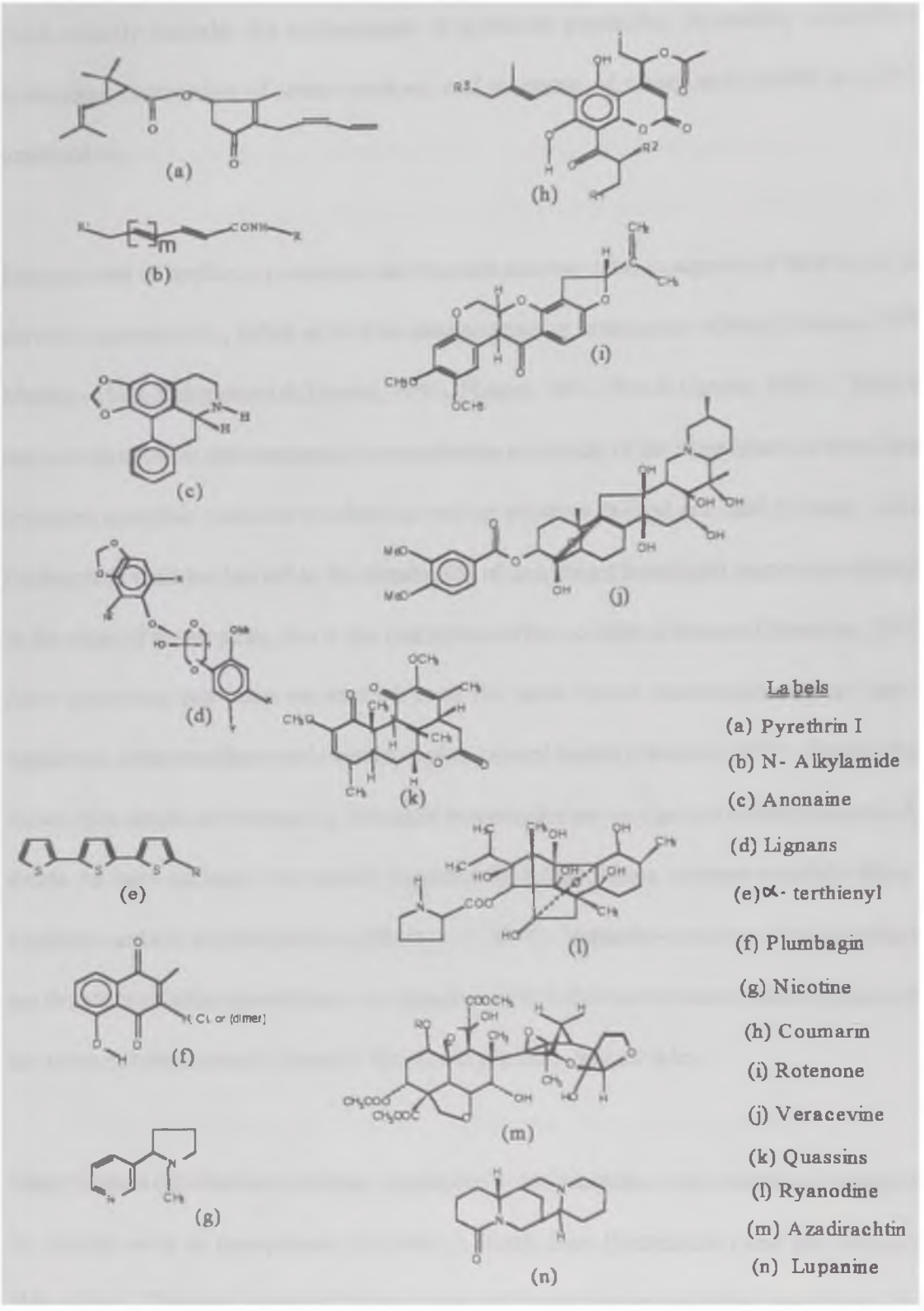


Fig. 1 Chemical structures of some botanical insecticides

These broadly include: the replacement of synthetic pesticides, increasing agricultural production, destruction of insect vectors, and a source of cheap and readily available insecticides.

Replacement of synthetic pesticides has become necesary due to reports of their acute and chronic neurotoxicity, lethal as well as carcinogenic or teratogenic effects (Hansen, 1983; Murphy, 1986; Kaloyanova & Batawi, 1991; Thayer, 1991; Wu & Casida, 1993). They are also associated with environmental contamination as a result of the persistence of these broad spectrum synthetic pesticide residues as well as residues in food and man (Carson, 1962). Furthermore their use has led to the elimination of non-target beneficial organisms resulting in the surge of newer pests, due to the distruption of the ecological balance (Newsom, 1967). Also resistance has been reported in even the most recent insecticides, insect growth regulators, chemosterilants and even biological control agents (Sawicki, 1979). Studies have shown that natural substances e.g. botanical insecticides are very potent contact insecticides, stable on inert surfaces, but readily degraded by metabolizing systems, notabily those of mammals and soil microorganisms (Elliott *et al.*, 1978). Naturally-occurring plant constituents are therefore suitable alternatives to developing stable and selective bioactive compounds that are more environmentally friendly than most synthetic insecticides.

Many tropical diseases like: malaria, elephantiasis, onchocerciasis and cholera are transmitted by insects such as mosquitoes (Culicidae), black flies (Simuliidae) and the houseflies (Muscidae). There are reports of the resurgence of insect vectors including mosquitoes, black

flies and houseflies as a result of insect resistance to insecticides (Busvine & Pal, 1969; WHO, 1970; Chapman *et al.*, 1993) and reinfestation from uncontrolled areas. According to WHO (1976), resistance is probably the biggest single obstacle in the struggle against vector borne diseases. There is therefore a growing interest in the use of new plant-based insecticides e.g. the pyrethroids as a control measure.

With the current economic situation in Sub-saharan African countries in general and Ghana in particular, state subsidies for agricultural inputs have been withdrawn and cost of safe and efficient pesticides has escalated. In addition, the use of synthetic insecticides in subsistence farming - the system practised by majority of the rural folks - is uneconomical. For such resource-deficient farmers, attention must be focused on indigenous plants as a source of cheap and locally available insecticides (Wink, 1993; Tierto Niber, 1994). Tierto Niber (1994) published simplified steps for making water extracts of powdered neem seeds (*A. indica*) for application on crops. A practical demonstration of this is currently going on in Niger, Benin and Burkina Faso where farmers are being taught by researchers and extension officers under the IITA subregional cowpea project, the preparation and method of application of water extracts of powdered neem seeds to cowpea (GTV, 1997).

2.7 Indigenous Ghanaian medicinal plants with insecticidal properties

Some indigenous Ghanaian plants are selectively used by farmers and other rural folks as insecticides and repellents against stored product insects especially termites; crop pests e.g. grasshoppers and beetles; medical insects especially mosquitoes and lice and other arthropods

of veterinary importance e.g. ticks and fleas. Abbiw (1990) recorded different potions of various parts of 58 plants with insecticidal properties. Fifty two of these, including *Clausena anisata* and *Hyptis spicigera*, are being used by herbalists and fetish priests to cure various illnesses (Ayensu, 1978; Abbiw, 1990).

Many though these plants are, very few have received empirical attention to authenticate claims about their insecticidal properties. More attention is drawn towards their medicinal properties. Nonetheless, Tierto Niber *et al.* (1992) screened eleven of these plants against three storage beetles for their toxicity. *Ricinus communis* (seed), *Solanum nigrum* (leaf), *Cissampelos owariensis* (leaf and root) and *Erythrophyleum suaveolens* (leaf) gave very promising results. Cobbinah & Osei-Owusu (1988) also studied the effects of neem seed extracts on insect pests of eggplant, okra and cowpea.

2.8 Experimental plants

2.8.1 *Clausena anisata* (Wild.) J.D. Hook f. ex Benth., (Family: Rutaceae)

2.8.1.1 Description and distribution

Clausena anisata is a shrub or small tree, up to 6 m high. It has odorous pinnate leaves, with leaflets numbering 17-32, with alternate, obliquely ovate or ovate-lanceolate leaf arrangement. The leaves are cuneate to rounded at the base, acuminate, acute, obtuse or rounded at the apex; up to about 11 cm long and 5 cm broad; with entire or crenulate leaf margins, and nearly glabrous to densely pubescent beneath (Hutchinson & Dalziel, 1958). It produces cream-white flowers in lax narrow panicles at least half as long as the leaf. The fruits are ellipsoid,

shinning black drupes, about 9 mm long and 7 cm broad. *C. anisata* is widespread in tropical Africa. In Ghana, it is found in Cape Coast, Kintampo, Volta River basin and the Accra plains (Hutchison & Dalziel, 1958).

2.8.1.2 Medicinal and insecticidal properties

The medicinal properties of *C. anisata* are many and varied and include the traditional treatment of eye infections, headache, sinusitis. It is antihelminitic and antiseptic (Abbiw, 1990). The root decotions are used for treatment of heart diseases and palpitations (Ayensu, 1978). Crushed leaves are stuffed into wounds to drive away maggots (Biegel & Mavi, 1972) and the bruised leaves repel mosquitoes (Abbiw, 1990). The leafy branches are also used in Cameroon to protect stored products such as maize ears, beans and cowpea (Parh *et al.*, 1990).

Despite this wide utilization, the insecticidal properties of the extracts and essential oil of *C. anisata* have received little attention. Most works have centred on the phytoconstituents and antimicrobial activity of the essential oil (Gundidza *et al.*, 1994). Mester (1983), however, isolated mupanine an insecticidal compound, and Gebreyesus & Chapya (1983) isolated two antifeedants, imperatorin and xanthoxyletin from the leaves.

2.8.1.3 Other phytoconstituents

Gundidza *et al.* (1994) isolated about thirty compounds from the essential oils of the fresh leaves of the plant. The major components were sabinene (23.0%), germacrene-D (17.0%),

(*Z*)-ß-ocimene (6.0%), germacrene-ß(5.5%), (*E*)-ß-ocimene (4.9%) and terpinen-4-ol (4.7%). Clausanitine and ananisatine (Okorie, 1975) and anisocoumarins A, B, C, and D (Ngadjui *et al.*, 1989) were isolated from the stem and roots. Two new geranyl coumarins were also isolated from the leaves and identified as anisocoumarins I and J (Ngadjui *et al.*, 1991). Addae-Mensah *et al.* (1996) studied the chemovarieties of *C. anisata* in 3 West African countries including Ghana, Togo and Benin. Three chemotypes were classified based on the constituents of their essential oils. Two of these which had methyl chavicol (80 - 100%) as the major constituent were observed in collections from the 3 countries. The third chemotype which yields between 85 - 100% (*E*) - anethole as the major constituent was found only in collections from Ghana.

2.8.2 *Hyptis spicigera* Lam. (Family: Labiateae)

2.8.2.1 Description and distribution

Hyptis spicigera is a tall erect, aromatic rather scabribe herb, probably a native of Brazil (Hutchinson & Dalziel, 1963). The leaves are lanceolate, acute, up to 8 cm long and 3 cm broad and closely gland-dotted beneath. The inflorescence is terminal and forms a dense cylindrical or ovoid cycli spike up to 9 cm long. The flowers are very small with linear-filiform ciliate bracts. The mature calyx is 5 cm long, strongly 10-ribbed, pubescent, but lacking conspicuous tufts of white hairs between the subulate teeth (Hutchinson & Dalziel, 1963). *H. spicigera* is widespread in tropical Africa and Asia, and in Ghana is common in the Gonja land and Yendi (Hutchinson & Dalziel, 1963) of the Northern region.

2.8.2.2 Medicinal and insecticidal properties

Crushed leaves of *H. spicigera* are applied to the head as a relief from headache (Abbiw, 1990). Traditionally, the leafy branches are put in layers below bundles of millet to keep away termites. They are also burned to get rid of mosquitoes (Abbiw, 1990).

Very little is known about the phytoconstituents of the leaves of *H. spicigera*. Ellis (1990) suggested that like *H. suaveolens* and *H. mutabilis*, *H. spicigera* may contain a combination of terpenoid compounds as active ingredients (Rogelio & Mariano, 1988). The only preliminary insecticide bioassay done on *H. spicigera* appears to be that against *Callosobruchus maculata* Fab. (Ellis, 1990). The indications were that it had the potential as a pesticide and further detailed studies were recommended.

2.9 Insects used for bioassays

2.9.1 The housefly (*Musca domestica* L.; Diptera: Muscidae)

The housefly is one of the most widely studied insects. West & Peters (1973) published an annotated bibliography containing 5720 references on the housefly. It is widely used in studies in the development of new insecticides because of its short life-cycle, ready availability and ease of handling.

2.9.1.1 Description, biology and life-cycle

The adult housefly measures 6-7 mm long and is recognized by the presence of 4 black-and-

grey striped thorax, a sharp angle in the fourth longitudinal vein of the wings, and a protruded but retractable sponging mouthpart. It can live for 2 - 3 weeks (Keiding, 1986). Copulation is possible 24 - 30 h after emergence and oviposition occurs 4 - 8 days after copulation (West, 1951). The white to cream coloured banana-shaped eggs, each measuring 1 - 1.2 mm long are deposited in clusters on moist decaying, fermenting or putrefying organic matter and hatch between 8 - 12 h later. The maggot (larva) assumes a creamy or yellowish appearance and is blunt at the posterior, tapering to a point anteriorly and is transparent except towards the end of the larval period. There are 3 larval instars; the first instar (1 - 3 mm) last for 20 h - 4 days; the second (3 - 5 mm) 24 h - several days, and the third (12-13 mm), 3 - 9 days (Keiding, 1986). A non-feeding prepuparium matures into a dark brown puparium (about 6 mm long) from which the adult emerges in about 5 days. The duration of the life-cycle is dependent on temperature and humidity.

2.9.1.2. Taxonomy, distribution and ecology

The genus *Musca*, consists of 26 species (Keiding, 1986). Though the taxonomy of the domestic forms are still unclarified four different species have been distinguished. *Musca domestica domestica* L. is found in temperate zones; *M. d. vicina* Macq., in subtropical and tropical zones in the Mediterranean, Asia, Africa, South and Central America, the Pacific, and Australia; *M. d. nebulo* Fab. in tropical Asia; and *M. d. curviforceps* Sacca & Rivosechi, is restricted to Africa, where it is the common housefly south of the Sahara (Sacca, 1964). Adult flies are diurnal feeding on human food and garbage, and use human buildings for shelter. They breed in decaying, fermenting or rotting organic matter including: dung (Haines,

1953, 1955), garbage and wastes (Schoof *et al.*, 1954), organic manure other than dung, and sewage and compost heaps (Silverly & Schoof, 1955). They survive on water plus sugar or other assimilable carbohydrates, though females require protein for development of eggs (Spiller, 1964).

2.9.1.3 Public health importance

The housefly is regarded an important agent for transmission of diseases. Bacterial infections transmitted include: Shigellosis (bacillary dysentery) (Bidawid *et al.*, 1978), Salmonellosis (typhoid, paratyphoid, enteritis, food poisoning, etc.), Cholera (Echeverria *et al.*, 1983) and Campylobacteriosis (*Campylotacter fetus jojuni*) (Rosef & Kapperud, 1983). They are capable of transmitting protozoan (e.g. Amoebic dysentery) and helminthic infections (e.g. eggs and cysts of *Enterdous*, *Ascaris*, *Trichiuris*, *Ancylostoma*, *Necator*, *Taenia*, *Dipylidium*, etc.) (Dipeolu, 1977).

Houseflies have the ability to transmit poliomyelitis and related viral (e.g. Coxsackie viruses) diseases to human through contaminated food and may also contribute to the spread of infectious hepatitis (Keiding, 1986). Other diseases include: rickettsial infections (e.g. *Coxiella burnettii*) (Hucko, 1984); eye diseases e.g. trachoma (Viral) and epidemic conjunctivitis (bacillary) (Keiding, 1986); and skin and wound infections such as cutaneous diphtheria, mycoses, yaws and leprosy (Geater, 1975).

2.9.1.4 Control of houseflies

Long term control of flies involves improved sanitation, chemical control only being a supplement (Busvine, 1980; Keiding, 1974). Environmental sanitation and hygiene eliminates or reduces fly breeding sources, excludes flies from contact with matter that contains pathogenic germs and protects food, utensils and man from contact with flies. These could only be achieved through education and public co-operation. Other non-chemical control methods include the use of baited or light traps, sticky fly paper (Davidson, 1962; Thimijan *et al.*, 1970), fly swatters, electric grids combined with attractants, and screening buildings against fly invasion.

Chemical control of preadult stages involves treating breeding places with larvicides and growth regulatory inhibitors (e.g. Diflubenzuron and Cyromazine). Residual treatments, mainly sprays to resting-sites and other surfaces, introducing toxic resting-sites e.g. impregnated strips, cords, etc (Chow & Thevasagayam, 1953) and toxic baits; space sprays and direct spraying of fly aggregations indoors and outdoors and fumigation (WHO, 1984) are effective against adult flies. Ascher & Levinson (1953), Sampson (1956) and Keiding (1986) have provided reviews of the chemicals used in fly control. Chemical control is, however, only effective provided resistance is not a problem.

There are also potentials in the fields of biocontrol (e.g. Axtell, 1969; Pimentel & Uhler, 1969), sterility and genetic control (e.g. LaBrecque & Weidhaas, 1970) of houseflies.

2.9.1.5 Resistance to insecticides

The housefly is an insect species that has shown the greatest ability to develop resistance to insecticides (Brown & Pal, 1971; Keiding, 1977). WHO (1986) gave the global situation of housefly resistance to insecticide in 1985 though it was incomplete in many areas. There are indications of increased resistance to more insecticides following the 1984-85 housefly resistance survey (Chapman *et al.*, 1993). Very high resistance to organochlorine compounds in particular, (e.g. DDT, HCH and Cyclodienes) has been reported globally. On the global scale organophosphorus-resistance increased greatly in distribution, levels, and number of compounds involved (Keiding, 1986). Information on the present occurrence of carbamate resistance in housefly populations is more limited than for the organophosphates. Resistance to the pyrethroids - including natural pyrethrum - was only found locally in Denmark and Sweden, but has rapidly developed in Switzerland, the United Kingdom and Germany. Their resistance to insecticides has been attributed to genetic factors including kdr - factor (Keiding, 1977); cross-resistance (WHO, 1976); and multiple resistance (Keiding, 1975).

2.9.2 The American cockroach (*Periplaneta americana* (L.); Dictyoptera: Blattidae)

Periplaneta americana is a common domiciliary species of cockroaches commonly called the American cockroach. Mckittrick (1964) provided a classification and taxonomic key for *P. americana.*

2.9.2.1 Description, biology and life-cycle

P. americana is a large cockroach with adults measuring about 35 - 40 mm in length. They

may live about 100 days under adverse conditions whereas at the extreme an adult life span of 2 - 3 years is known (Griffiths & Tauber, 1942). Gould & Deay (1938) gave an average life-span of 1 year and probably longer. The sexes are separated by the presence of styli in the males and stouter abdomens in females. The wings in the fully developed male extend slightly beyond the abdomen but are approximately as long as the abdomen in females. When both sexes are together mating takes place within a few days after emergence but parthenogenesis is known to occur in this species (Roth & Willis, 1956). A dark brown ootheca measuring about 8mm in length is produced every 4 - 10 days and is carried for about 24 h after which it is deposited in a carefully selected location. A normal ootheca has 16 eggs in two parallel rows. The incubation of eggs takes 30 - 45 days with extremes of 24 - 100 days (Cornwell, 1968). The nymphs moult 7 - 13 times over the course of 5 - 15 months (Cochran *et al.*, 1975). All stages are shiny-red to chocolate brown.

2.9.2.2 Distribution and ecology

P. americana is virtually a cosmopolitan species. It is believed to have originated in tropical Africa from whence it dispersed through commerce to South America, the West Indies and southern North America (Rehn, 1945), Great Britain, southern Japan (Asahina, 1961) and many other parts of the northern and southern temperate zone (Cochran *et al.*, 1975). They are omnivorous insects and feed on a wide variety of food stuffs, in addition to biological wastes such as garbage and sewage (Roth & Willis, 1957; James & Harwood, 1969). They may be found in restaurants and "chop bars", food processing plants, grocery stores, bakeries, latrines, outhouses, sewers, kitchens and other places where food occurs. Historically, they

are of importance as ship galley and cargo hold pests (Cochran *et al.*, 1975). There are reports of their occurrence in garbage dumps, unoccupied buildings, trees, mines and under decaying matter (Cornwell, 1968). *P. americana* prefers warm humid environments.

2.9.2.3 Economic importance of cockroaches

Sixteen species of cockroaches including *P. americana* are considered vectors of pathogenic organisms affecting man and have been found to be contaminated with 40 different bacterial species that are pathogenic (Roth & Willis, 1957). These cause diseases including; leprosy, bubonic plague, dysentery, diarrhoea, urinary tract infections, boils and abscesses, pus formation, urogenital tract and intestinal infections, enteric fevers and gastroenteritis, food poisoning and typhoid fever (Cornwell, 1968). Pathogenic helminths are also transmitted by cockroaches (Roth & Willis, 1957, 1960). The eggs of 7 species of helminths occur naturally in cockroaches and are also natural intermediate hosts for 12 species of helminths. *P. americana* was also found to be a natural intermediate host of *Moniliformis moniliformis* in Puerto Rico (Acholonu & Finn, 1974). In addition about 45 non-pathogenic species of helminths are known to be primary parasites of cockroaches.

Roth & Willis (1957) showed conclusively that cockroaches may acquire, maintain and excrete various viruses e.g. Coxsackie virus and several strains of poliomyelitis. Tarshis (1962) suspected cockroaches as vectors of infectious hepatitis. Cockroaches also transmit four pathogenic protozoans (Roth & Willis, 1957, 1960). *Aspergillus fumigitus and A. niger* have also been reported as occurring naturally in cockroaches. Blister-raising properties (Roth &

Willis, 1960), dermatitis of the skin and oedema of the eyelids have been attributed to cockroaches. *P. americana* has been found to excrete compounds which are either mutagenic or carcinogenic (Mullins & Cochran, 1973).

2.9.2.4 Control of cockroaches

Preventive measures involve minimizing entry of cockroaches into buildings and eliminating breeding and harbourage sites through environmental sanitation (Cochran *et al.*, 1975).

Organophosphates, organochlorides and carbamates have been the principal types of compounds used in cockroach control, especially for residual application (Grayson, 1966). Attempts are being made to extend the residual effectiveness of certain chemicals (e.g. organophosphates and synthetic pyrethroids) or to maximize their safe use by slow release of the toxicant, through encapsulated formulation or impregnation of plastic strips (Cochran *et al.*, 1975). These approaches appear promising. Several chemicals are commercially available as baits for control of cockroaches. Some of these baits include, 1.9% dichlorvos paste, 2% propoxur pelleted baits, and 0.125% kepone paste, pellets and paraffin baits. Chemicals with knockdown and flushing action e.g. pyrethrum and repellents e.g. R - 874 and R - 11 plus the synergist MGK - 264 (Mallis *et al.*, 1961) have also been useful in cockroach control by preventing them from invading new areas.

2.9.2.5 Resistance to insecticides

Insecticide resistance in cockroaches occurs primarily in the German cockroach (*Blattella*

germanica L). The first appearance of resistance in a field population was to chlordane in 1951-52 in Texas USA (Fisk & Isert, 1953; Heal *et al.*, 1953). Resistance has been reported in other species of cockroaches including *P. americana*. Populations of *P. americana* in New Orleans, Louisiana, USA in late summer e.g. 1972 survived applications of chlordane and dieldrin at rates 30 times the normal concentration, though details of occurrence apparently have not been published. Undoubtedly the populations had resistance to organochlorine compounds, although they were susceptible to organophosphates (Cochran *et al.*, 1975).

CHAPTER 3

3.0 MATERIALS AND METHODS

Details of the materials and methods that are common to several experiments are presented below, whereas specific details relating to individual experiments are described in the appropriate sections.

3.1 Rearing and breeding of experimental insects

3.1.1 The housefly (*Musca domestica* L.)

Stock of adult houseflies were obtained from Madina Estates, a suburb of Accra by setting traps baited with sliced pieces of sugar cane that were sprinkled with fresh "pito" (a local northern Ghanaian drink) and yeast. The flies were reared in 30 cm × 30 cm × 30 cm metal frame cages covered completely on all sides with muslin (Plate 3a). A plywood floor was fixed at the base of each cage. The muslin ended in sleeves at both the rear and front sides of each cage. The front sleeve served as entrance for the introduction or harvesting of flies, and changing of food, water and oviposition containers. The front and rear sleeves of the cages were closed with elastic bands. The cages rested on raised stands on petri dishes which contained a layer of engine oil to provide a barrier against cross infestation from crawling insects and mites. Adult flies were fed on sucrose (granulated sugar) provided in shallow petri dishes in the cages. Water was given in soaked cotton-wool pads pressed into the petri dishes. Food and water were changed daily. Each cage could accommodate up to 700 flies.

Plate 3 a. Houseflies in breeding cage.

Plate 3 b. Cockroaches in breeding cage.

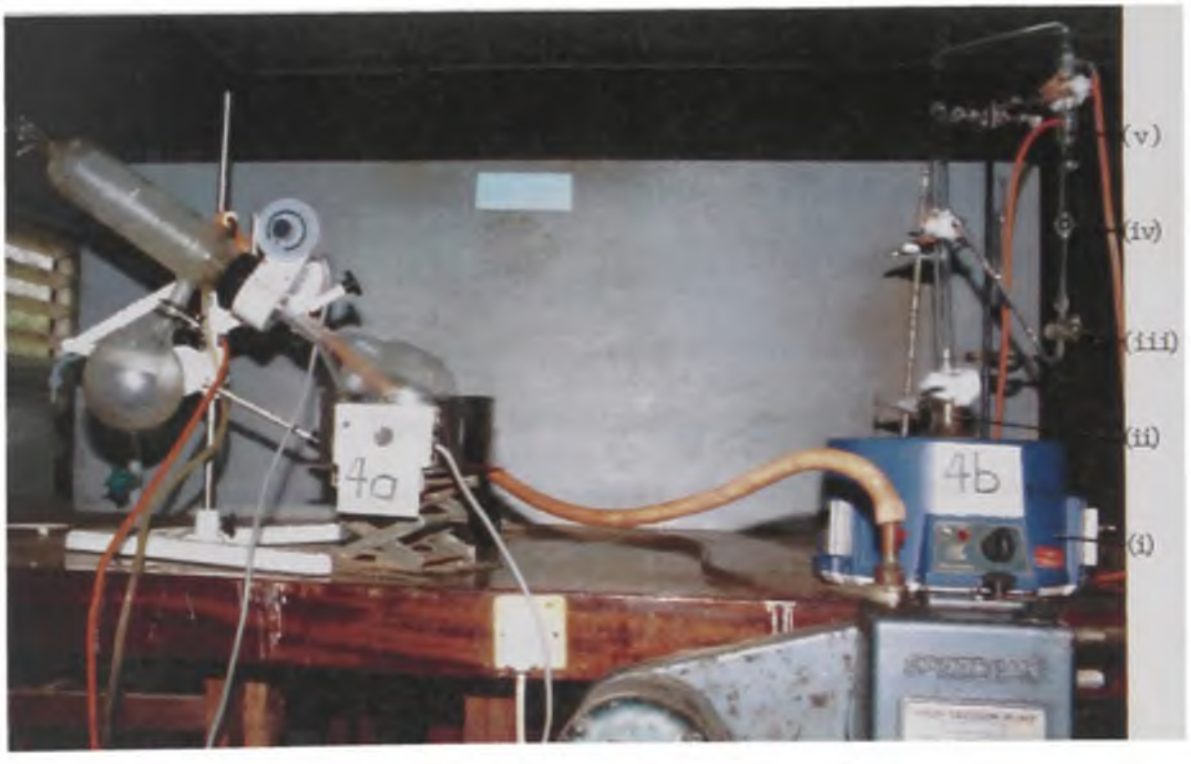


Plate 4 a. Eyela rotary vacuum evaporator used to concentrate crude extracts.

Plate 4 b. Clevenger-type apparatus used for hydrodistillation of essential oil.

(i) Heating mantle (ii) Round botton flask

(iii) Three-way tap (iv) Oil Collection point (v) Condenser

Mating occurred a few hours after emergence from the pupal cases. Pieces of liver were provided in petri dishes for eggs to be laid on. Eggs laid on the liver were transferred into the larva rearing medium. The larvae were reared in 17 cm × 11 cm × 4 cm transparent plastic containers with lids. Each lid had a circular opening (6.5 cm diameter) covered with muslin. A synthetic food medium consisting of a mixture of wheat bran, grass (elephant grass) meal, bakers yeast, malt extract and water was provided for the larvae. This was prepared by adding 3.5 g of dried brewers yeast and a spatula full of marmite malt to 200 ml of warm water, and stirring to dissolve. This was then poured into a bowl and 400 ml of previously boiled warm water added and stirred. To this was added 61 g of dry grass meal, then 122 g of wheat bran and stirred to mix thoroughly to give a loose texture.

Each rearing container was filled with the prepared medium to about a third of its volume and seeded with 100 - 200 eggs and/or larvae. The observed average period for larval development was six days. The mature larvae migrate to the upper layer of the medium to pupate. The pupae were carefully removed from the rearing containers with a pair of forceps and transferred to petri dishes. They were kept in the petri dishes till they began to emerge approximately five days after pupation. The petri dishes were put in the oviposition cages and opened for the emerging adults to fly into the cages. Four-day old flies were used for the bioassays involving the housefly because of their superior response to handling, selection and insecticides (Dahm *et al.*, 1961). The age of the flies used for each experiment was counted from the day of maximum adult fly emergence.

3.1.2 The American cockroach (*Periplaneta americana* (*L.*))

Stock of cockroaches were obtained from the insectary of the Biochemistry Department, University of Ghana, Legon. A slight modification of the breeding method of cockroaches used at the Infestation Control Laboratory of the Ministry of Agriculture, Fisheries and Food at Tolworth, Surbiton, Surrey, England was adopted. The colonies were kept in transparent plastic rearing cages 45 cm × 35 cm × 30 cm with perforated metal lids (Plate 3b). The cages rested on raised stands on petri dishes which contained a layer of engine oil to provide a barrier to cross infestation from crawling insects and mites. Each cage was occupied by five platforms of hardboard measuring 25 cm × 15 cm separated by wooden balls (2.5 cm diameter) and held together by nails.

Water and food were provided on the top platform of the Tolworth system (cage). Water was given in soaked cotton-wool stacked to the bottom of petri dishes. A pig grower, a product of GAFCO Ltd., Ghana, composed of protein (16.0%), energy (3000 k cal/kg), calcium (0.5%), phosphorus (0.7%), lysine (0.7%), and methionine (0.5%) was provided in petri dishes as food. Ootheca which were produced after seven days were deposited and glued to the corners of the cages and within the food pellets (pig grower) in the petri dishes. These were removed regularly with a pair of forceps and transferred to petri dishes where they remained until they hatched four to five weeks later. The nymphs were returned to other breeding cages to reduce crowding and cannibalism.

3.2 Collection and processing of plant materials

3.2.1 Extraction of crude extracts

Leaves of *Clausena anisata* (Wild) ex. Benth. were obtained from Ofankor, a town 2 km north of Accra (lat. 5°58′ N along the coast of Ghana) whilst *Hyptis spicigera* Lam. was obtained from a stretch of land between the Department of Biochemistry and the Botanical Gardens, University of Ghana, Legon, Accra. The leaves were bagged in envelopes and oven-dried at a temperature of 29 - 31°C for 2 weeks. The dry leaves were milled with a Size 8 inch (20.3 cm) laboratory mill (Christy & Norris Ltd. Chelmsford, England) and kept in black polythene bags in an air conditioned room.

A one-in-ten (weight of powdered plant material to volume of extracting solvent) extracts were prepared. The extraction was done at room temperature. Methanol (Fisons, England) and petroleum ether (BDH Chemicals Ltd.,England) which were used for extraction were obtained from Fregesco Chemical Co., Ltd., Accra, Ghana. The mixture of solvent and leaf powder was stirred on a magnetic stirrer for about eight hours and left over night. The extracts were filtered with a buckner filtration system using a vacuum pump (Edwards High Vacuum Ltd., Crawley, England). The filtrates were collected in labelled flat bottom flasks and concentrated to 100 ml using an Eyela rotary vacuum evaporator (Tokyo Rikakikai Co., Ltd., Tokyo, Japan) (Plate 4a). The concentrations of the crude extracts were determined. In determining the concentrations, 5 ml vials were labelled and weighed and 1 ml of each extract measured into the appropriate vial. The extracts were dried with N_2 gas (obtained from Equip Liquified-gas Com., Ltd., Tema) and the vials reweighed. The difference in weight per millilitre represented

the concentrations of the corresponding extracts.

3.2.2 Hydrodistillation of essential oils

Fresh leaves of *C. anisata* and *H. spicigera* were collected and subjected to hydrodistillation using a Clevenger-type apparatus. The set up consisted of a heating mantle (i), a two litre round bottom flask (ii) in which the fresh leaves were boiled with tap water, and a Clevenger-type apparatus (Plate 4b). The Clevenger-type apparatus consisted of a condenser (iii), an oil collection point (iv) and a three-way tap (v) via which the oil collected over water during the distillation could be tapped. Hydrodistillation of the leaves was done in batches of 150 g for 2 h each. A total of 3 and 4.33 kg of fresh leaves of *C. anisata* and *H. spicigera* respectively, was boiled. The oils were separated from water with a Pasteur pipette and dried by filtration over anhydrous sodium sulphate.

3.3 Bioassay techniques of crude extractives

Standard approaches were adopted in these studies. Completely randomized experimental design method was used for the experiments. At least 30 insects were used for each tested dose. Four-days old flies were used for bioassays involving the housefly. On the other hand, cockroach nymphs or adults of similar weight were used for the assays involving the cockroach. Flies were collected from the cages by scooping with a plastic boiling tube whilst cockroaches were immobilized with CO_2 (Equip Liquified-gas Com., Ltd., Tema) directly in the cages before collection.

Topical application and residual film methods (Dahm *et al.*, 1961) were used to evaluate the toxicity of the plant extractives against the housefly and cockroach, respectively. An inverted cone trap method (LaBreque & Wilson, 1959) was used to test the repellency of the extracts on the housefly. A treated rearing medium bioassay was used to test the growth inhibition effect of the crude extracts against the second larval instars of housefly. A no-choice-dietary bioassay was used to determine the antifeedant effect of the crude extracts on the cockroach. Finney's Probit-log statistical analysis for quantal data (Finney, 1971) was used to determine the LD_{50}s, KD_{50}s and RD_{50}s.

3.3.1 Toxicity and knockdown effects

Standard direct-contact tests for preliminary evaluation of a new candidate material as a toxicant were used to determine the toxicity of the crude extracts.

3.3.1.1 Toxicity of crude extracts to houseflies by topical application

A Burkards hand microapplicator (Burkards Manufacturing Co. Ltd, Richmansworth, England) (Plate 5) was used to topically apply acetone solutions of the crude extracts to well-fed flies. One microlitre of the acetone solution of the extract was applied on the thorax of four day old adult flies of mixed sexes selected randomly. The experiments were designed as 4 × 4 experiments, and the method consisted of four 10-fly replicates for each of four graded doses of each crude extract. In general the doses ranged from 0.02 - 0.8 mg μl^{-1} and the interval between doses varied geometrically. For *C. anisata* the doses included: 0.02, 0.04, 0.08, 0.16 (Pet. ether), and 0.1, 0.2, 0.4, 0.8 µg / fly (Methanol); whilst 0.03, 0.06, 0.12, 0.24

Plate 5. Burkard hand microapplicator used for topical application of extractives.

Plate 6. Set up of treated and control traps for repellency experiments.

(Pet. ether), and 0.08, 0.16, 0.32, 0.64 µg / fly (Methanol) were the graded doses for *H. spicigera*. To prepare the concentrations, the crude extracts were further concentrated by measuring 10 ml of each extract into a separate vial, drying with N_2 gas and redissolving in 1 ml of the appropriate solvent. A Hamilton microsyringe (50 µl capacity) was used to measure out the appropriate volumes ranging from 100 - 800 µl, corresponding to the graded doses. These were dried again and picked in 200 µl of acetone for application. The treatment series included four groups of flies treated with acetone only to serve as control.

The flies were lightly anaesthetized with CO_2 and treated at the rate of 10-flies per minute. Each group of flies was held in a petri dish (8.5 cm diameter) for 24 h after treatment and observed for the number of deaths (a fly was considered dead when it remained motionless when pricked with a needle) including moribund flies (flies that responded with unco-ordinated movements to probes with a needle). The LD_{50}, Fiducial limits, X^2, and Slope, were determined from probit-log analysis (Finney, 1971). Abbotts formula (Abbott, 1925) was used to correct for deaths in control experiments:

$$AM = \frac{\%\,T - \%\,C}{100 - \%\,C} \times 100.$$

AM = the adjusted mortality, % T = % test effect (mortality) and % C = % control mortality.

3.3.1.2 Toxicity of crude extracts to cockroach nymphs by petri dish residual film method

A residual film method of continuously exposing insects to a contact insecticide was used. 10 randomly selected unsexed nymphal instars of average weight 0.16 (±0.1)g were exposed to graded concentrations of the crude extracts in pyrex petri dishes (9 cm diameter; area, 63.6 cm^2). Four graded concentrations which generally ranged between 0.2 and 5 mg cm^{-2} with geometrically increasing dosage intervals constituted the treatments. For *C. anisata* the doses include: 0.18, 0.36, 0.72, 1.45 (Pet. ether), and 0.65, 1.29, 2.58, 5.16 mg cm^{-2} (Methanol); whilst 0.21, 0.4, 0.82, 1.64 (Pet. ether), and 0.65, 1.29, 2.58, 5.16 mg cm^{-2} (Methanol) were the graded doses for *H. spicigera*. They were prepared by measuring the appropriate volumes (0.5 - 4 ml) and either making up, or drying with N_2 gas and redissolving in 1 ml of the appropriate solvent for application.

The graded concentrations of crude extracts were introduced into labelled pyrex petri dishes and dispersed over the interior surface by rotating the dishes horizontally. Control experiments were set up with petri dishes treated with only the extraction solvents. Treated petri dishes were allowed to dry for 30 - 60 min. The nymphs were anaesthetized with CO_2 gas, transferred to treated petri dishes which were covered. Each group of nymphs was held for 24 h and the number of dead nymphs (nymphs that did not respond to, including those that responded with unco-ordinated movements to probes with a needle) recorded after this period. Each treatment and control was replicated six times. The LD_{50}, Fiducial limits, X^2, and Slope, were determined from probit-log analysis (Finney, 1971). Abbotts formula (see section

3.3.1.1) was used to correct for deaths in control experiments.

3.3.1.3 Toxicity and knockdown effects of essential oils

The toxicity of the essential oil of *C. anisata* and *H. spicigera* were tested against the housefly (adults) and cockroach (nymphs) as described in sections 3.3.1.1 and 3.3.1.2, respectively. The tests were designed as 4 × 5 experiments, and the method consisted of four 10-insect replicates for each of five graded doses of essential oil including 0.1875, 0.375, 0.75, 1.5, 3, 6 μg / fly for the housefly and 0.003, 0.006, 0.012, 0.024, 0.048, 0.096 μg cm^{-2} for the cockroach nymphs. Dose intervals varied geometrically. The essential oils were diluted in acetone to make 10% stock solutions from which further dilutions were made in order to acquire the desired doses. Samples were topically applied to the thorax of the housefly (Section 3.3.1.1) whilst cockroach nymphs where exposed to residual films of the samples in pyrex petri dishes as described previously (Section 3.3.1.2). Samples in petri dishes were allowed 10 - 15 min to dry. The number of insects found dead (flies that did not respond to, including those that responded with unco-ordinated movements to probes with a needle) 24 h after treatment were recorded. The knockdown effect on houseflies 15 min after treatment were also recorded. The LD_{50} for housefly and cockroach nymphs after 24 h were calculated. The KD_{50} was also determined for the first 15 min by probit analysis (Finney, 1971).

3.3.2 Repellency to housefly

3.3.2.1 Repellency of crude extracts

An inverted cone trap method (LaBreque &Wilson, 1959) was used to evaluate the repellency

of the crude extracts. The crude extracts were applied uniformly via Pasteur pipette to semi-circular Whatmans No. 4 filter paper (diameter, 12.5 cm; area, 61.4 cm^2). One millilitre of four different doses of crude extract, prepared as described in section 3.3.1.2, were tested. Actual concentrations varied geometrically and generally ranged from 0.05 to 1.6 mg cm^{-2}. For *C. anisata* the doses include: 0.05, 0.10, 0.20, 0.40 (Pet. ether), and 0.20, 0.40, 0.8, 1.60 mg cm^{-2} (Methanol); whilst 0.20, 0.40, 0.8, 1.60 (Pet. ether), and 0.1625, 0.325, 0.65, 1.3 mg cm^{-2} (Methanol) were the graded doses for *H. spicigera*. All the treated filter papers were allowed 5 - 10 min to dry. Each was then rolled into a cone and sellotaped. An aperture, large enough to allow a fly to pass through was made at the apex of each cone. The cones were inverted over 350 ml glass bottles (diameter, 5.5 cm; height, 15.5 cm) to form cone traps (Plate 6). The filter paper for control experiments were treated with 1 ml of the corresponding solvent of extraction.

Casein hydrolysate (10 g) moistened with 10 ml of water was placed at the bottom of each bottle and covered with wire gauze to serve as an attractant. In each experiment, a control and treated cone trap were exposed for 30 min in a cage (18 cm × 25 cm × 25 cm) containing 100 houseflies of mixed sexes. The flies were starved overnight before use. Counts were made of the number of flies trapped in the treated and untreated (control) traps after this period of time. Each experiment was replicated four times. The sum of the four counts represented a replicate. After each count the cage was turned through 90° so that at the end of the fourth count 360° was covered. This was done to minimise any bias for a particular side or bottle.

The index of reaction (IR) was determined in accordance with the method of Bar-Zeev (1962). The percentage repellency (R%) (Campbell, 1983) for each dose was also calculated.

$$IR = \frac{100\,(T - C)}{T + C},$$

$$R\% = \frac{100\,(C - T)}{C},$$

where C = the number of flies trapped in control trap and T = the number trapped in the treated trap. The IR is a measure of the intensity of repellency of the crude extract as compared with the control. An IR of zero indicates no preference for either the treated or control. A positive value (max., +100) indicates that the control repels more strongly than the treated and a negative value (max., -100) indicates the contrary. A value double the standard error is considered to indicate a significant difference. Probit analysis (Finney, 1971), was used to determine the RD_{50} s.

3.3.2.2 Repellency of essential oils

An inverted semicircular-filter-paper cone trap method described in section 3.3.2.1 was used to evaluate the repellency of the essential oils against the housefly. One millilitre of four graded doses, prepared as described in section 3.3.1.3 were tested. Petroleum ether was used as the solvent. The actual doses varied geometrically and included 0.012, 0.024, 0.048, and

0.096 μg cm^{-2}. The index of repellency (IR), percentage repellency (R%) and the RD_{50}s were determined as shown in section 3.3.2.1.

3.3.3 Growth rergulatory and antifeedant effects of crude extracts

3.3.3.1 Growth regulatory effect of crude extracts

A treated rearing medium bioassay method was used and only housefly larvae were assayed. Four dietary doses were tested along with a solvent treated control for each extract. Actual doses used varied geometrically but generally ranged from 0.9 to 32 mg of extract g^{-1} of larval rearing medium. For *C. anisata* the doses include: 0.9, 1.8, 3.6, 7.2 (Pet. ether), and 4.0, 8.0, 16.0, 32.0 mg cm^{-2} (Methanol); whilst 1.0, 2.0, 8.0, 8.0 (Pet. ether), and 3.0, 6.0, 12.0, 24.0 mg cm^{-2} (Methanol) were the graded doses for *H. spicigera*. The concentrations were prepared as described in section 3.3.1.2. One millilitre of each extract concentration was added to 1 g of milled grass in 250 ml glass bottles and 15 - 30 min allowed for the solvent to evaporate. Synthetic larva rearing medium (see section 3.1.1 for preparation) was added to make up to 25 g and the diet was thoroughly stirred. Ten 2nd instar larvae were introduced into each bottle and sealed-off with muslin. Each treatment was replicated three times.

The morphological features of larvae, pupae and adult flies emerging from treated larval media were studied with a hand lens. Individual larvae, pupae and adult flies emerging were scored on a scale of 0-3 as follows:

0 - normal adults,

1 - abnormal adults,

2 - deformities and incomplete emergence from pupal case,

3 - deformities on larvae.

Larval and pupal mortalities were also recorded.

The potency (P) of the extracts in imparting deformities was determined as follows:

$$P = \frac{\sum (n_i \times s_i)}{n_T} \times 100.$$

n_i = the number of individuals, s_i their numerical scores and n_T the number of test flies. The P values were then coded and described as follows: 100-90 (++++) very good activity; 89-80 (+++) good activity; 79-60 (++) moderate activity; 59-50 (+) poor activity and 49-0 (-) no activity. An index of more than 80 indicates a significant potential of crude extract in imparting deformities to the developmental stages of housefly.

3.3.3.2 Antifeedant effect of crude extracts

A no-choice-diet bioassay was used to investigate the antifeedant effects of the crude extracts against adult cockroaches of average weight 0.9 (±0.2)g. Two adult cockroaches, a male and a female, constituted the sample size per test. Each cockroach was, however, confined in a separate transparent plastic container (diameter, 8 cm; height, 4.5 cm) covered with a perforated lid to avoid crowding. They were starved overnight before use. Three graded of each crude extract, prepared as described in section 3.3.1.2 were tested for their antifeedant effect. The doses ranged from 23 to 385 mg of extract. For *C. anisata* the doses include: 23, 46, 92, (Pet. ether), and 96, 192, 384 mg of extract (Methanol); whilst 26, 52, 104 (Pet.

ether), and 82, 164, 328 mg of extract (Methanol) were the graded doses for *H. spicigera*.

About 1 g of food pellets was treated by dipping the pellets in 1 ml of extract, ensuring that they were uniformly coated with the residue of the extract. The treated pellets were then oven dried at 28 | ±1 | °C for five minutes and weighed. These were presented in petri dishes (diameter 3 cm) to test insects to feed on for 48 h. After this duration the treated pellets were oven-dried again, reweighed and the weight differences calculated. Control experiments in which food pellets were treated with only 1 ml of the appropriate solvent were also set up. Test food-pellets were usually kept in a desiccator after they were oven-dried to avoid moisture absorption. Each experiment was replicated 10 times.

The antifeedant index (AI) for each graded extract concentration was calculated according to the formula suggested by Escoubas *et al.* (1992);

$$AI = 100 \times \frac{\%\ T}{\%\ T + \%\ C}$$

% T = % of treated food pellets consumed and % C = % of control food pellets consumed. The index varies from 0 (total feeding-inhibition) to 100 (total feeding-stimulation). A value of 50 indicates treated and untreated pellets have been consumed in equal amounts. An index of less than 20 indicates significant antifeedant activity (Alkofahi *et al.*, 1989; Escoubas *et al.*, 1992). The AI values were then coded and described as follows: 0-10 (++++) very good activity; 11-20 (+++) good activity; 21-40 (++) moderate activity; 41-50 (+) poor activity and 51-100 (-) no activity.

CHAPTER 4

4.0 RESULTS AND DISCUSSION

4.1. Extractives from plant materials

The percentage yield (%) of extractives and essential oil of *C. anisata* and *H. spicigera* are shown in Table 4.1. Among the crude extracts, the methanol extractives from *C. anisata* and *H. spicigera* were four and three times respectively, more than petroleum extractives. The methanol extractive of *C. anisata* was higher in content compared with that of *H. spicigera* though the yield of petroleum ether extractives was the same in both plants. Percentage yield of oil in *C. anisata* was about 70 times that of *H. spicigera*. Unlike the oil of *H. spicigera*, that of *C. anisata* characteristically solidified on storage in a refrigerator.

4.2 Lethal toxicity of extractives

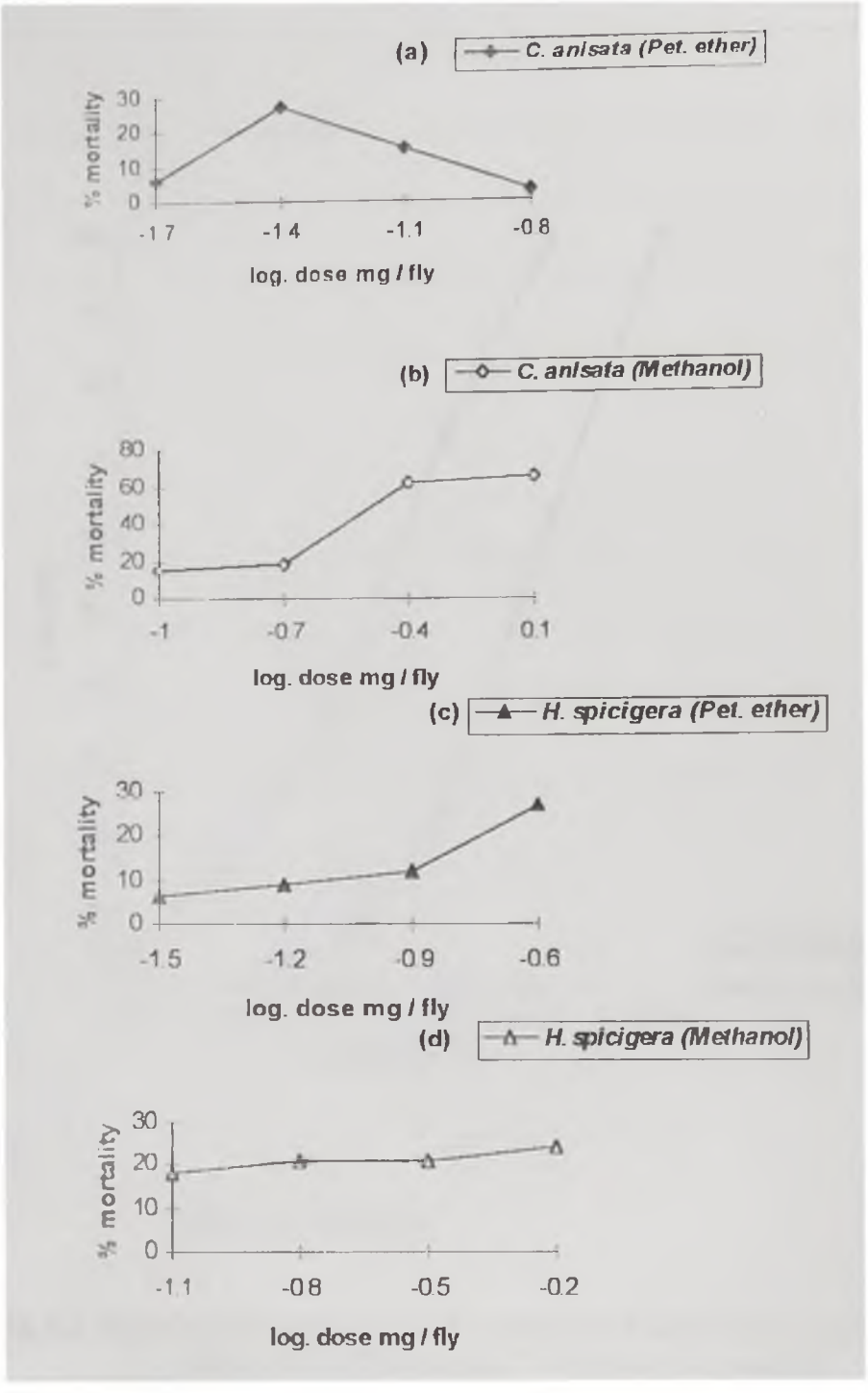
4.2.1 Contact toxicity by topical application.

Per cent mortality of houseflies obtained by topical application of increasing doses of petroleum ether extracts, methanol extracts and essential oils of *C. anisata* and *H. spicigera* are shown in Fig. 4.1 and 4.2, respectively. Among the crude extracts tested, dose-dependent fly mortality was observed for methanol extract of *C. anisata* (Fig. 4.1.b) and petroleum ether extract of *H. spicigera* (Fig. 4.1.c). Per cent mortality of flies treated with petroleum ether extract of *C. anisata* declined after a dosage of 0.04 mg which produced only 27% kill (Fig. 4.1.a). The lethal toxicity of petroleum ether extract of *C. anisata* against housefly did not

Table 4.1. Extractives obtained from solvent extraction and hydrodistillation of the leaves of *C. anisata* and *H. spicigera*.

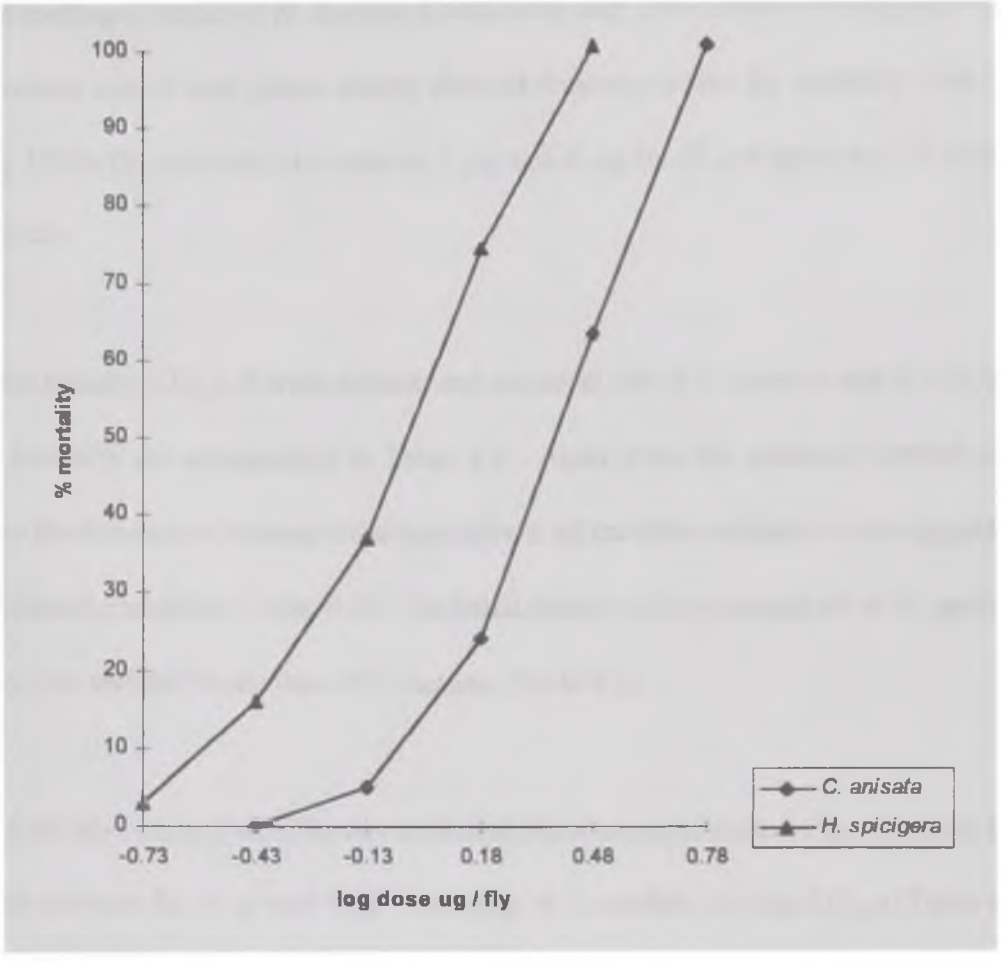
Plant species	Extraction solvent	Yield of extractive (%)
C. anisata		
	Petroleum ether	2.65
	Methanol	10.72
	Hydrodistillate	1.43
H. spicigera		
	Petroleum ether	2.63
	Methanol	8.21
	Hydrodistillate	0.02

The yield of crude extract (concentrated solvent extract) are expressed as a percentage of the weight of dried and powdered leaves weighed for extraction whilst the yield of essential oils are expressed as a percentage of the weight of fresh leaves boiled.



40 flies were used per dose

Fig. 4.1. Relationship between per cent mortality of housefly and graded doses of petroleum ether and methanol extracts of *C. anisata* and *H.spicigera*.



40 flies were used per dose

Fig. 4.2. Relationship between per cent mortality of housefly and log. graded doses of essential oils of *C. anisata* and *H. spicigera*.

show dose-dependency. Per cent mortality of flies only increased slightly with increasing doses of methanol extract of *H. spicigera*, achieving only 24% kill at 0.64 mg (Fig. 4.1.d). The essential oils of both plants clearly showed dose-dependent fly mortality (Fig. 4.2), causing 100% fly mortality at a dose of 3 μg and 6 μg for *H. spicigera* and *C. anisata*, respectively.

The lethal toxicity (LD_{50}) of crude extracts and essential oils of *C. anisata* and *H. spicigera* on the housefly are summarized in Table 4.2. Apart from the methanol extract of *H. spicigera* the differences between lethal toxicities of all the other extractives were significant at 0.95 fiducial probability (Table 4.2). The lethal toxicity of the essential oil of *H. spicigera* was over two and half times that of *C. anisata* (Table 4.2).

Although all other extractives of the two medicinal plants caused significant mortalities in flies, the crude extracts did so at very high doses (Fig. 4.1) resulting in high LD_{50}s (Table 4.2). Comparing the performance of these extractives to the typical procedure at Rothamsted used by Khambay & O'Connor (1993) for the screening and selection of extracts for further examination, only the essential oils of the two medicinal plants satisfy the standards for further study. At Rothamsted were topical application of extracts are done at 20 μg / insect, when the overall activity is low, selection of extracts for further examination is based on variation in activity between the extracts. Typically when the variation is greater than 30 %, the most active fraction is subjected to short column chromatography. Only when a significant increase in activity hs been realized is there further purification to the pure compound(s), often using

Table 4.2 Lethal toxicity (LD_{50}) of crude extracts and essential oil of the leaves of *C. anisata* and *H. spicigera* to 4-day old adult housefly.

Plant species	Extract	LD_{50} (mg / μg^a)	95% Fiducial limits upper - lower	X^2	Slope
C. anisata					
	Methanol	0.4143*	0.5055 - 0.3501	13.5029	0.0513
	Essential oil	2.2101[a]*	2.4361 - 2.0034	9.8178	0.0230
H. spicigera					
	Pet. ether	1.0860*	9.2698 - 0.4915	1.5953	0.2282
	Methanol	1746.2 [n]	0.0000 - 4.3400	0.1105	3.9444
	Essential oil	0.8580[a]*	0.9548 - 0.7708	5.6382	0.0197

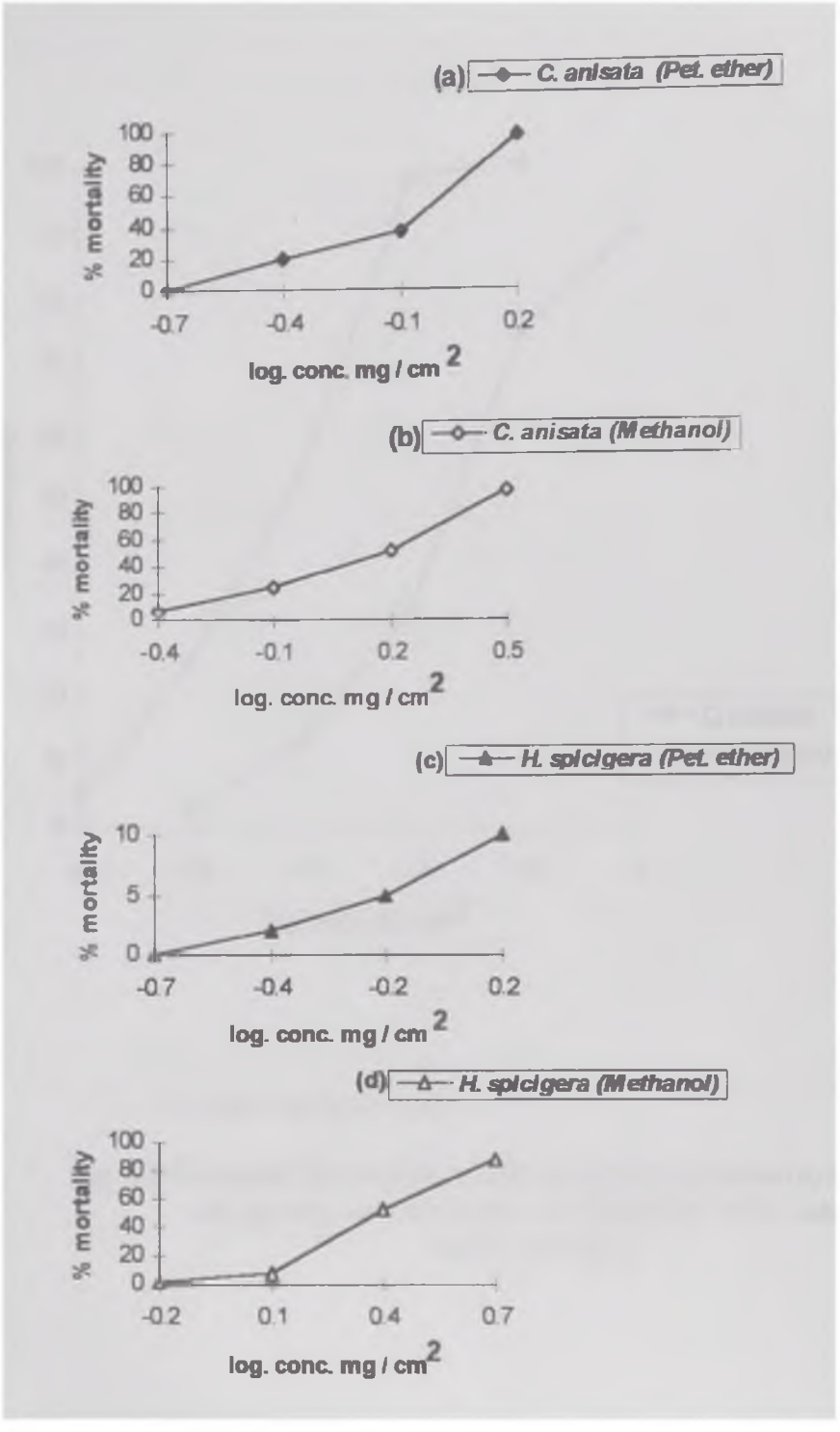
X^2 - Chi square value.
Pet. ether - Petroleum ether.
Pet. ether extract of *C. anisata* mortality did not show any lethal toxicity.
[a] - LD_{50} is quoted in μg for essential oils, but in mg for other extracts.
* - Significant at 0.95 fiducial probability.
[n] - Not significant at 0.95 fiducial probability.

a simpler and a quicker bench top bioassay (e.g mosquito larvae).

4.2.2 Contact toxicity in petri dish

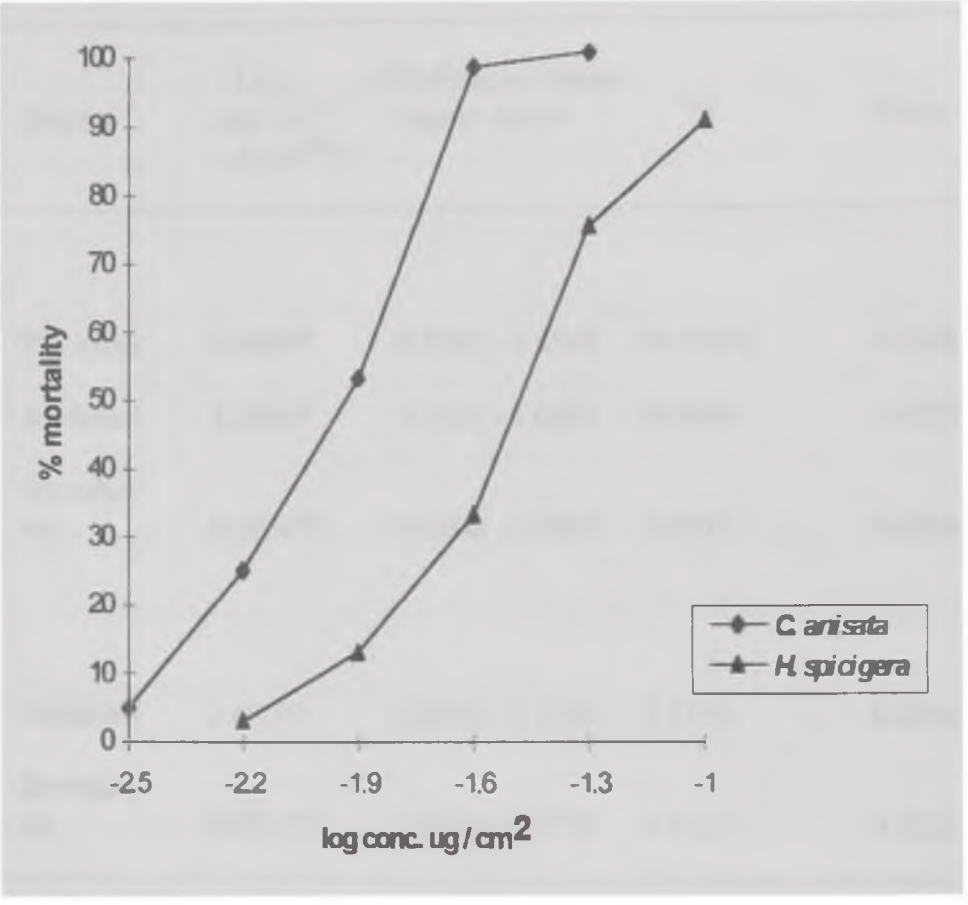
Per cent mortality of cockroach nymphs after 24 h exposure to increasing doses of petroleum ether extracts, methanol extracts, and essential oils of *C. anisata* and *H. spicigera* are shown in Fig. 4.3 and 4.4, respectively. Mortality was dose-dependent for both crude extracts and essential oil of the two plants. Except for the petroleum ether extract of *H. spicigera* of which nymphal mortality was low (10% at the highest tested dosage of 1.64 mg cm^{-2}) (Fig. 4.3.c) all the other extracts caused over 80% nymphal mortality at the highest tested dosage (Fig. 4.3.a, b, d). At a dose of 0.048 μg cm^{-2}, 100% nymph mortality was achieved with essential oil from *C. anisata* (Fig. 4.4). Ninety percent nymph mortality was, however, observed at a dose of 0.096 μg cm^{-2} of essential oil from *H. spicigera* (Fig. 4.4).

The lethal toxicity (LD_{50}) of crude extracts and essential oil of the leaves of the two plants on cockroach nymphs are given in Table 4.3. The LD_{50} of the methanol extract of *C. anisata* was twice as effective as that of *H. spicigera*. However, the essential oil of *C. anisata* was three times as effective as the essential oil of *H. spicigera*. Petroleum ether extracts of *H. spicigera* showed extremely low lethal toxicity. At 95% Fiducial limits the extractives bioassayed against the cockroach were significant. Heterogeneity ($h = 9.8374$) (at $P < 0.001$) was shown only in the bioassay of petroleum ether extract of *C. anisata* on cockroach nymphs. The LD_{50} of the essential oil from *C. anisata* and *H. spicigera* against cockroach nymphs were several thousand fold better than the methanol and petroleum ether extracts (Fig.



60 nymphs were used per dose

Fig. 4.3. Relationship between per cent mortality of cockroach nymphs and graded concentrations of petroleum ether and methanol extracts of *C. anisata* and *H. spicigera*.



60 nymphs were used per dose

Fig. 4.4. Relationship between per cent mortality of cockroach nymphs and graded concentrations of essential oil of *C. anisata* and *H. spicigera*.

Table 4.3 Lethal toxicity (LD_{50}) of crude extracts and essential oils of the leaves of *C. anisata* and *H. spicigera* to cockroach (nymphs).

Plant species	Extract	LD_{50} (mg cm^{-2}/ $\mu g\ cm^{-2a}$)	95% Fiducial limits upper - lower	X^2	Slope
C. anisata					
	Pet. ether	0.6869*	0.7562 - 0.6248	19.6747ϕ	0.0266
	Methanol	1.2090*	1.3421 - 1.0890	10.0890	0.0257
	Essential oil	0.0096[a]*	0.0106 - 0.0087	9.6917	0.0204
H. spicigera					
	Methanol	2.6000*	2.8756 - 2.3590	3.5138	0.0293
	Essential oil	0.0310[a]*	0.0350 - 0.0276	2.6121	0.0221

X^2 - Chi square value.
[a] - LD_{50} for essential oil is in $\mu g\ cm^{-2}$ and mg cm^{-2} for other extracts.
ϕ - Heterogenous (heterogeneity factor, $h = X^2 / (k - 2) = 9.8374$, where k is the number of doses) at $P < 0.001$.
For *H. spicigera*, pet. ether showed no lethal toxicity.
Pet. ether - Petroleum ether.
* - Significant at 0.95 fiducial probability.

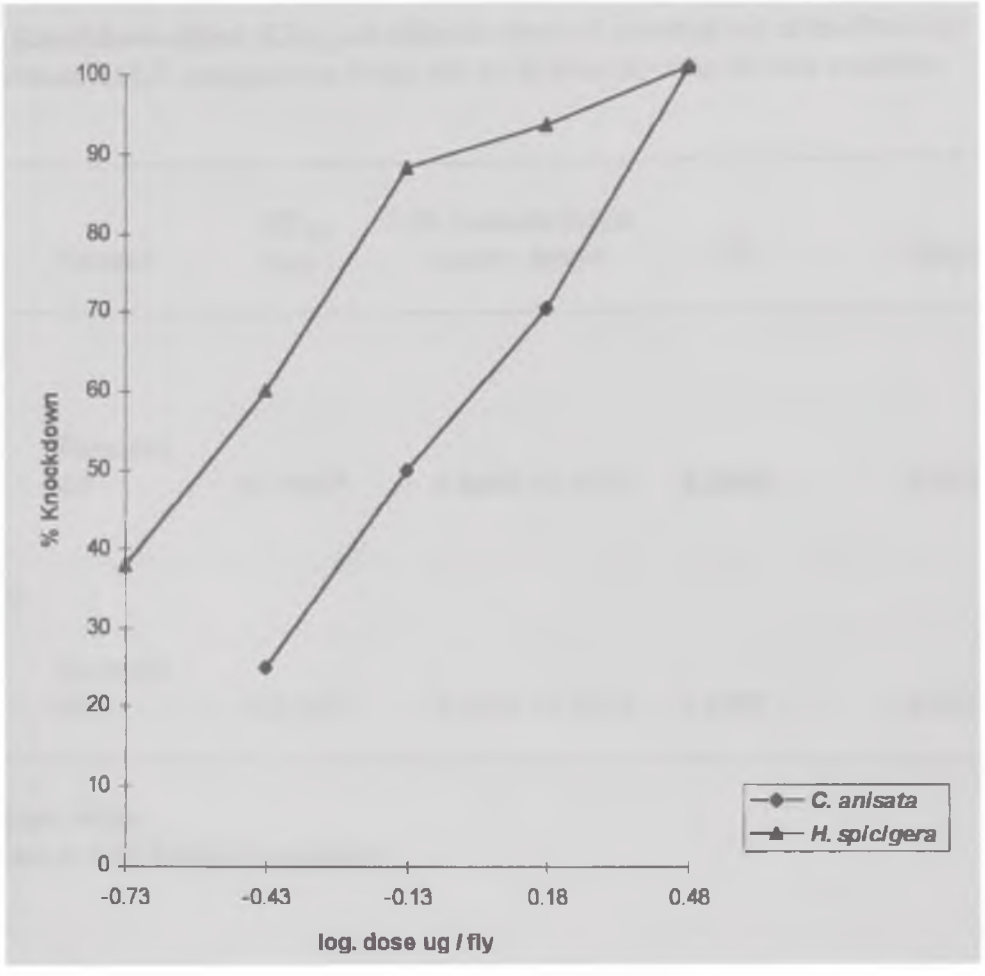
4.2; Table 4.3).

The results obtained show that the essential oil of *C. anisata* and *H. spicigera* contained the biologically active principles. The main active principle in the essential oil of *C. anisata* could be its major constituent (*E*)-anethole (85 - 100%) (Addae-Mensah *et al.*, 1996). Mupamine (Mester, 1983) and geranyl coumarins (Ngadjui *et al.*, 1991) isolated from the leaves of *C. anisata* may be responsible for mortality in insects treated with crude extracts of *C. anisata*. The active ingredient(s) in *H. spicigera* responsible for lethal action may be terpenoids similar to those isolated from related species, *H. sauveolens* and *H. mutabilis* (Rogelio & Mariano, 1988). Mortality recorded for the insects treated with crude extracts of *H. spicigera* may also be attributed to the presence of these terpenoid compounds.

4.2.3 Knockdown effect of extractives on housefly

Crude extracts of *C. anisata* and *H. spicigera* did not show any knockdown effect on housefly. The essential oil of both plants, however, showed knockdown on housefly following the topical application of graded doses of the oils. Figure 4.5 shows the knockdown effect on housefly of the essential oil of the plants 15 min after treatment with the oils. For both plants, the percentage knockdown of flies was dose-dependent. The knockdown of 50% of flies (KD_{50}) treated with essential oil of *C. anisata* and *H. spicigera* are summarized in Table 4.4. The essential oil of *H. spicigera* was three times as potent as essential oil of *C. anisata*.

In practical usage in the home, on industrial premises or outdoor treatments throughout the



40 flies were used per dose

Fig. 4.5. Relationship between per cent knockdown of housefly and graded doses of essential oil of *C. anisata* and *H. spicigera*.

Table 4.4. Knockdown effect (KD_{50}) of different doses of essential oil of the fresh leaves of *C. anisata* and *H. spicigera* on 4-day old adult housefly after 15 min exposure.

Plant species	Extract	KD_{50} (μg)	95% Fiducial limits upper - lower	X^2	Slope
C. anisata					
	Essential oil	0.7445*	0.8445 - 0.6472	8.3005	0.0325
H. spicigera					
	Essential oil	0.2631*	0.3104 - 0.2141	2.5988	0.0378

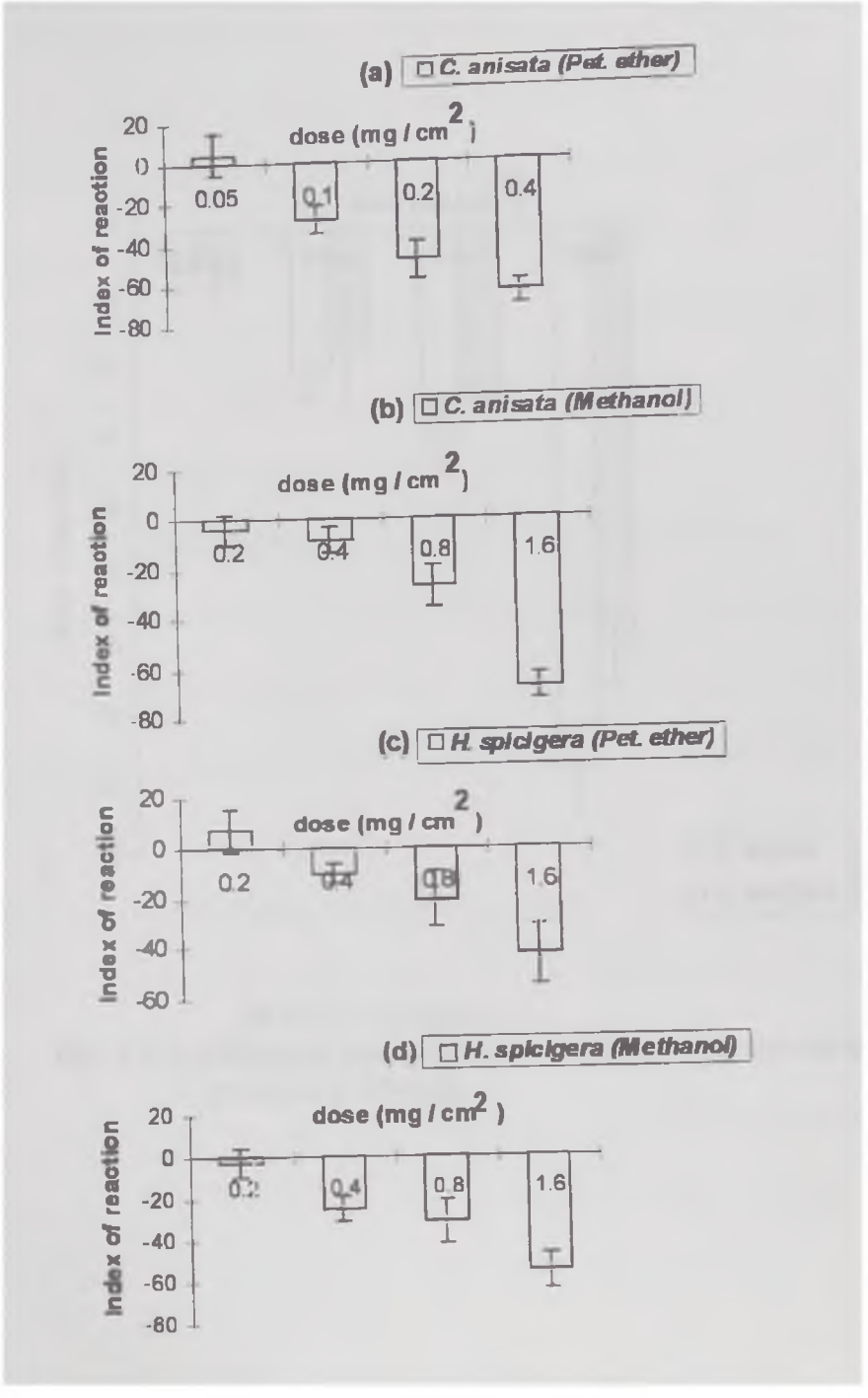
X^2 - Chi square value.
* - Significant at 0.95 fiducial probability.

world, there are varying interpretations of knockdown (Wickham *et al.*, 1974). From the entomologist's point of view, a knockdown in 5 - 15 min followed by death is satisfactory (Wickham *et al.*, 1974). Results obtained from knockdown bioassays have shown 100% knockdown of flies treated with 3 μg of essential oil from both plants (Fig. 4.5). *H. spicigera*, however, gave a better knockdown activity (KD_{50}) compared with *C. anisata* (Table 4.4). This probably indicates that the active ingredient(s) in essential oil of *H. spicigera* have a rather rapid and efficient penetration into the haemolymph of the flies and subsequently to the site of action in the central nervous system than that of *C. anisata* (Briggs *et al.*, 1974). According to Briggs *et al.* (1974) an important property determining a good knockdown and kill of a molecule is the overall polarity of the molecule since this has a marked influence on the rate at which the molecule penetrates to the site of action. This suggests that the active ingredient(s) in the essential oil of *H. spicigera* may be more polar than that of *C. anisata*.

The chemical constituent(s) in *H. spicigera* responsible for the knockdown activity is likely to be the same or similar to the terpenoid compound(s) that caused mortality of the insects (Rogelio & Mariano, 1988). Similar arguments could be advanced for *C. anisata* of which the major component is (*E*)-anethole (Addae-Mensah *et al.*, 1996).

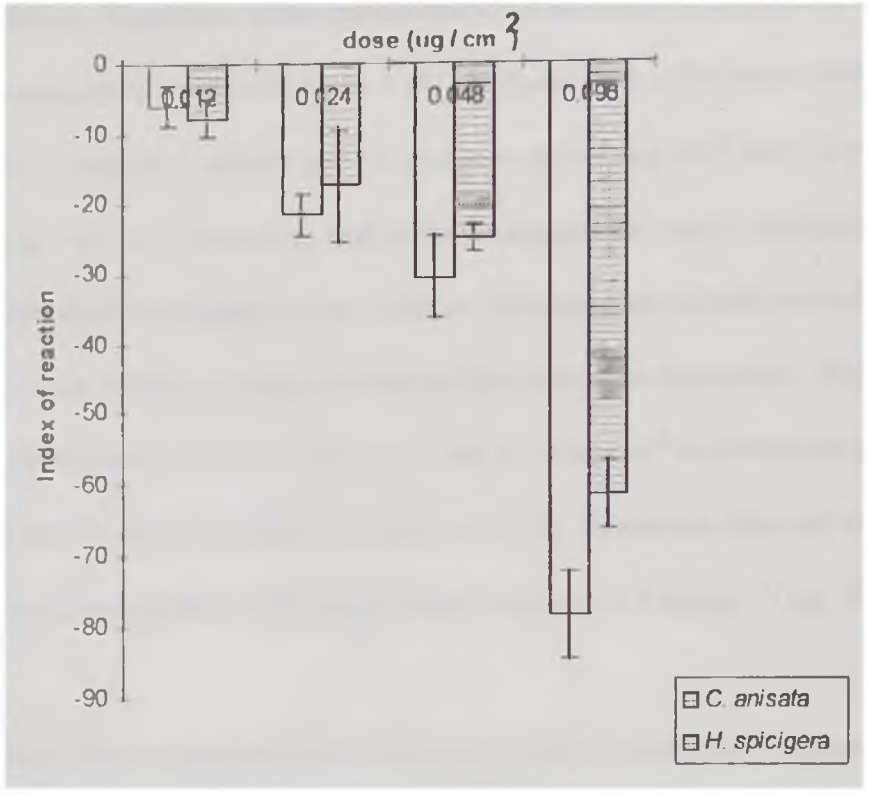
4.3 Repellency of plant extractives to housefly

Repellency of petroleum ether and methanol extracts of *C. anisata* and *H. spicigera* against housefly are shown in Fig. 4.6. Repellency of essential oils of *C. anisata* and *H. spicigera* on housefly are shown in Fig. 4.7. In each figure a negative index of reaction indicates that



100 flies were used per dose

Fig. 4.6. Repellency of graded doses of petroleum ether and methanol extracts of *C. anisata* and *H. spicigera* to housefly.



100 flies were used per dose

Fig. 4.7. Repellency of graded doses of essential oil of *C. anisata* and *H. spicigera* to housefly.

the houseflies were repelled by the extract or essential oil more than the control (treatment with solvent of application) used for comparison. A value double the standard error is considered significant. Repellency of the crude extracts and essential oils appeared to be dose-dependent. Mean index of reaction values of 4.35 (±10.2) and 6.75 (±8.9) were obtained for petroleum ether extract of *C. anisata* and *H. spicigera* at 0.05 mg cm^{-2} and 0.2 mg cm^{-2}, respectively (Fig. 4.6 a, c) indicating that at these dosages the control treatments gave significantly better repellency than the crude extracts. All other doses of both petroleum ether and methanol extracts of the two plants repelled the flies better than the control. Repellency was, however, significant for doses ≥ 0.1 mg cm^{-2} and ≥ 0.8 mg cm^{-2} for petroleum ether and methanol extracts of *C. anisata*, respectively (Fig. 4.6.a, b). Petroleum ether and methanol extracts of *H. spicigera* repelled the flies significantly at doses ≥ 0.4 mg cm^{-2} (Fig. 4.6. c, d).

The essential oil of the two plants repelled the flies significantly better than the control at all tested dose levels (Fig. 4.7). Essential oil of *C. anisata* repelled the flies better than *H. spicigera* at all tested doses except at 0.012 μg cm^{-2} where *H. spicigera* gave 10% more repellency than *C. anisata*. The highest dose (0.096 μg cm^{-2}) bioassayed achieved a mean index of reaction of -77.86 (±6.0) and -60.81 (±4.7) for *C. anisata* and *H. spicigera*, respectively.

The repellent action (RD_{50}) of crude extracts and essential oils of the leaves of *C. anisata* and *H. spicigera* on the housefly are given in Table 4.5. The RD_{50} values (Table 4.5) indicate that the essential oils repelled the flies better than the crude extracts. Essential oil of *C. anisata*

Table 4.5 Repellent action (RD_{50}) of crude extracts and essential oils of the leaves of *C. anisata* and *Hyptis spicigera* to 4-day old adult housefly.

Plant species	Extract	RD_{50} (mg cm^{-2}/ $\mu g\ cm^{-2}$ [a])	95% Fiducial limits upper - lower	X^2	Slope
C. anisata					
	Pet. ether	0.1288*	0.1604 - 0.0908	0.2113	0.1427
	Methanol	0.8944*	1.0223 - 0.7929	3.1251	0.0339
	Essential oil	0.0400[a]*	0.0460 - 0.0350	8.4463	0.0351
H. spicigera					
	Pet. ether	1.3768*	1.9470 - 1.1117	0.4877	0.1245
	Methanol	0.6821*	0.8148 - 0.5855	15.2778ϕ	0.0427
	Essential oil	0.0509[a]*	0.0620 - 0.0431	4.3547	0.0504

X^2 - Chi square value.
[a] - RD_{50} for extract is in mg cm^{-2} and for essential oils, $\mu g\ cm^{-2}$.
Pet. ether - Petroleum ether.
ϕ - Heterogenous (heterogeneity factor, $h = X^2 / (k - 2) = 7.6389$ where k is the number of dose levels) at $P < 0.001$.
* - Significant at 0.95 fiducial probability.

showed better repellency than *H. spicigera*. The petroleum ether extract of *C. anisata* also repelled better than all the other crude extracts. The methanol extracts of *H. spicigera* gave two times better repellency than the petroleum ether extract.

The RD_{50} values of all the extractives of *C. anisata* and *H. spicigera* bioassayed were significant at 0.95 fiducial probability (Table 4.5). Heterogeneity (h = 7.6389) between counts in bioassays was encountered (at $P < 0.001$) for the methanol extract of *H. spicigera*. The essential oil of the two medicinal plants demonstrated more repellency than the crude extracts. The essential oil of *C. anisata* was over 3000 times more a potent repellent against the housefly than the petroleum ether extract. That of *H. spicigera* was approximately 13,500 times as potent as the methanol extract. This suggests that the active principle for the repellent action could be the essential oils. Whilst (*E*)-anethole (85 - 100%) (Addae-Mensah *et al.*, 1996) may be responsible for the repellent action in *C. anisata* against the housefly, repellency in *H. spicigera* may be attributed to the presence of some terpenoid compounds (Rogelio & Mariano, 1988). Traces of these compounds may be responsible for the repellent action observed in the crude extracts.

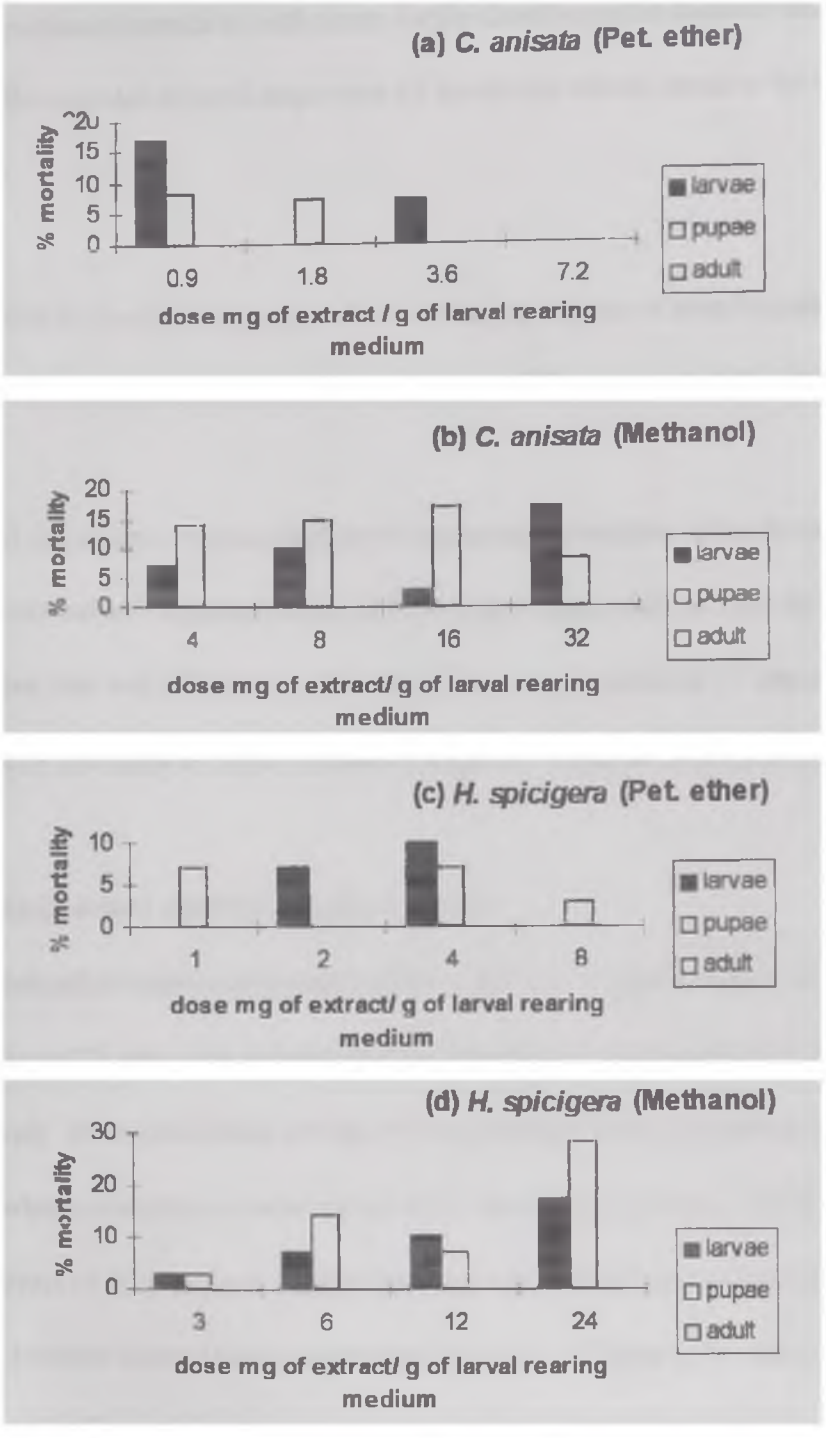
The bioassay method (section 3.3.2.1) used in determining the repellency of extractives against the housefly was satisfactory although few flies were often trapped after each test period (30 min). This may be attributed to the type and quality of the attractant (Casein hydrolysate; a housefly feeding stimulant) used for the bioassay. Houseflies are however, known to be less well equipped with organs of smell than related Diptera (Busvine, 1971).

4.4 Growth regulatory and antifeedant effects of crude extracts

4.4.1 Mortality and morphogenetic abnormalities in flies

The percentage mortalities observed at different stages of development induced by graded doses of petroleum ether and methanol extracts of *C. anisata* and *H. spicigera* after treatment of second instar larvae of housefly are shown in Fig. 4.8. The percentage death at each stage included those that died as a result of morphogenetic aberrations at various stages of fly development. No deaths were recorded in adult flies that emerged after crude extract treatment which were monitored for 24 h. Deaths obtained at larval (larvae that did not pupate) and pupal (pupae that did not emerge in to adults) stages did not show any dose-dependent response with the crude extracts of the two plants except the methanol extract of *H. spicigera* against the larval stage of the housefly (Fig. 4.8.d).

The highest per cent mortality of larvae (17%) and pupae (8%) were obtained at 0.9 mg of extract g^{-1} of larval rearing medium (lowest dose tested) for petroleum ether extract of *C. anisata* (Fig. 4.8. a). The highest larval and pupal per cent mortality induced by methanol extract of *C. anisata* was also 17% but at 32 and 16 mg of extract g^{-1} of larval rearing medium, respectively (Fig. 4.8. b). At 1.0 and 4.0 mg of extract g^{-1} of larval rearing medium of petroleum ether extract of *H. spicigera* 7% pupal mortality was recorded whilst the highest larval mortality (10%) was obtained at 4 mg of extract g^{-1} of larval rearing medium (Fig. 4.8. c). The highest larval and pupal mortality after treatment with methanol extract of *H. spicigera* were 17% and 28% respectively, and this was achieved at a dose of 24 mg of extract g^{-1} of larval rearing medium. Mortality was less than 30% at all doses tested for the petroleum



30 larvae were used per dose

Fig. 4.8. Per cent mortality in larvae, pupae and adults induced by different doses of petroleum ether and methanol extracs of *C. anisata* and *H. spicigera*.

ether and methanol extracts of both plants for the developmental stages of the housefly. Most of the deaths recorded at larval stage were for larvae that did not moult to the third instar stage (Fig. 4.8).

With regards to the deformities, very few but varying degrees of morphogenetic abnormalities were recorded at the doses of the crude extracts of *C. anisata* and *H. spicigera* used.

In general the potency of crude extracts in imparting deformities to the fly were very low and not dose-dependent. A potency value of 17 was the highest achieved among the crude extracts bioassayed and was obtained for the petroleum ether extract of *C. anisata* and methanol extract of *H. spicigera* at 3.6 and 12 mg of extract g^{-1} of larval rearing medium, respectively.

4.4.2 Antifeedant activity of crude extracts

Antifeedant effect (expressed as antifeedant index, AI) of graded doses of crude extracts of *C. anisata* and *H. spicigera* against adult cockroaches are summarized in Table 4.6 and 4.7, respectively. Poor antifeedant activity (+) was obtained for the petroleum ether extract of *C. anisata* whilst no activity (-) was recorded for the methanol extract (Table 4.6). Petroleum ether extract of *H. spicigera* did not show any antifeedant activity (-) but two doses of the methanol extract showed poor antifeedant activity (+) (Table 4.7). Absorption of moisture before reweighing of food pellets eaten probably accounted for no antifeedant activity registered at 328 mg (highest dose tested) of methanol extract of *H. spicigera*. Generally the antifeedant activity decreased with increasing dosage of extract except at 46 mg of the

Table 4.6 Antifeedant effect of crude extracts of the leaves of *C. anisata* on cockroach.

Extraction Solvent	dose (mg)	no. of Replicates	Mean % C eaten	Mean % T eaten	AI
Petroleum ether					
	23	10	4.80 ± 0.37	3.98 ± 0.58	42.76 ± 2.31 (+)
	46	10	4.80 ± 0.37	3.73 ± 0.55	40.84 ± 2.80 (+)
	92	10	4.80 ± 0.37	4.43 ± 0.54	45.10 ± 2.65 (+)
Methanol					
	96	10	4.70 ± 0.32	5.02 ± 0.32	51.86 ± 1.37 (-)
	192	10	4.70 ± 0.32	6.74 ± 0.25	59.59 ± 1.38 (-)
	384	10	4.70 ± 0.32	9.64 ± 0.26	67.86 ± 1.28 (-)

0-10 very good activity (++++);11-20 good activity (+++); 21-40 moderated activity (++) ; 41-50 poor activity (+);51-100 no activity (-).
% C - Percentage of control pellets eaten expressed as mean ± standard error.
% T - Percentage of treated pellets eaten expressed as mean ± standard error.
AI - Antifeedant index.
Twenty cockroaches (adults) were bioassayed at each dosage.

Table 4.7 Antifeedant effect of crude extracts of the leaves of *H. spicigera* on cockroach.

Extraction Solvent	dose (mg)	no. of Replicates	Mean % C eaten	Mean % T eaten	AI
Petroleum ether					
	26	10	6.55 ± 0.69	8.00 ± 0.67	55.85 ± 2.18 (-)
	52	10	6.55 ± 0.69	8.38 ± 0.66	56.51 ± 2.53 (-)
	104	10	6.55 ± 0.69	10.78 ± 0.78	62.77 ± 3.31 (-)
Methanol					
	82	10	7.04 ± 0.50	5.88 ± 0.49	45.09 ± 2.09 (+)
	164	10	7.04 ± 0.50	5.50 ± 0.35	44.36 ± 1.68 (+)
	328	10	7.04 ± 0.50	12.76 ± 0.70	64.40 ± 2.20 (-)

0-10 very good activity (++++);11-20 good activity (+++); 21-40 moderated activity (++) ; 41-50 poor activity (+);51-100 no activity (-).
% C - Percentage of control pellets eaten expressed as mean ± standard error.
% T - Percentage of treated pellets eaten expressed as mean ± standard error.
AI - Antifeedant index.
Twenty cockroaches (adults) were bioassayed at each dosage.

petroleum extract of *C. anisata* and 164 mg of the methanol extract of *H. spicigera* where the AI increased slightly by 1.92 and 0.73, respectively. No significant antifeedant activity (Table 4.6 and 4.7) was achieved for the crude extracts bioassayed against adult cockroaches. However, the poor activity obtained for petroleum ether extract of *C. anisata* may be attributed to the presence of imperatorin and xanthoxyletin which were previously reported to occcur in *C. anisata*, and showed this property (Gebreyesus & Chapya, 1983).

CHAPTER 5

5.0 GENERAL DISCUSSION AND CONCLUSION

The results from the study of the insect control activity of *Clausena anisata* and *Hyptis spicigera* on *Musca domestica* and *Periplaneta americana* have shown clearly that the two medicinal plants have both toxic and repellent properties. The results have also revealed a good knockdown effect of the essential oils of the plants on housefly followed by death, a property previously unknown to users of these plants. The study has also indicated that the insect control activities may be more closely associated with the essential oils and hence may be ascribed to the major components of the oils rather than the solvent extractives. Whilst (*E*)-anethole the major component of *C. anisata* (Addae-Mensah *et al.*, 1996), may be responsible for these activities, terpenoid compounds are likely to be the active principles which account for the activities in *H. spicigera* (Rogelio & Mariano, 1988). The essential oils of the plants may therefore serve as a potential source of botanical insecticides against the housefly, and cockroach and other related household pests. The toxic effect coupled with the repellent and knockdown action of these plants increases the protection potential of these plant materials against insect bites and crop and grain damage.

Although it is more likely that the repellent property of *C. anisata* and *H. spicigera* play a role when leafy branches of these plants are layered with grain to protect them from stored products insects, it is possible that contact with fresh leaves could be toxic to these insects. A

combination of the toxic, repellent and knockdown properties of the two plants are, however, probably evoked when the fresh leaves of the plants are burned in homes. Burning activates the release of the active principles with the smoke and thus repel mosquitoes from gaining entrance to the room. Mosquitoes resting indoors are knocked-down and eventually killed with increasing concentration of smoke containing the active ingredients from the plant oil.

Using these plants as insecticides may involve using aqueous formulations of the essential oils directly as sprays for crop and grain protection instead of dried leaf powders or leafy branches. This would eliminate the bulk of leafy branches required for storage, creating space for more grain. In addition this would increase the protective potential of the plants since the grains would be treated with known concentrations of plant materials that are more closely associated with toxic and repellent properties. Other aqueous formulations of the oils could be used as washing liquids for cleaning restaurant and "chop" bar floors and tables to repel flies and related dipterans. The fine aroma of these oils makes them appropriate for this purpose. The oils may also be incorporated in coils, paper, ropes in shear butter or wax that could be burned to liberate the active ingredients to repel or knockdown and kill insects confined indoors. It is equally possible to incorporate these oils in skin lotions including shear butter to act as repellents. These are achievable if simple and affordable extraction and application technologies are developed for adoption by resource-deficient farmers and other rural folks.

Further studies on all toxicological aspects including the level of persistence on agricultural products, the spectrum of activity, environmental contamination etc, of these oils are required.

Modes of insect control actions of the oils should also be studied. In addition, a means of replacing these plants in times of over-exploitation needs to be considered for their use as botanical insecticides to be a reality.

REFERENCES

Abbiw, D.K. (1990) Useful plants of Ghana. Kew, Intermediate Technology Pub. & the Roy. Botanic Gardens, 337 pp.

Abbott, W.S. (1925) A method of computing the effectiveness of insecticides. *J. Econ. Ento.* **18 :** 265-267.

Acholonu, A.D. & Finn, O.J. (1974) *Moniliformis moniliformis* (Acanthocephala: Moniliformidae) in the cockroach *Periplaneta americana* in Puerto Rico. *Trans. Amer. microsc. Soc.*, **93 :** 141-142.

Addae-Mensah, I., Asomaning, W.A., Oteng-Yeboah, A., Garneau, F.X., Gagnon, H., Jean, F.I.,Moudachirou, M. & Koumaglo, K.H. (1996) (E)-Anethole as a major constituent of *Clausena anisata*. *J. Essent. Oil Res.* **8** : 513-516

Alkofahi, A., Rupprech, J.K., Anderson, J.E., McLaughlin, J.L., Mikolajczak, K.L. & Scott, B.A. 1989) Search for new pesticides from higher plants. In, *Insecticides of plant origin*, J.T., Arnason, B.J.R., Philogene, & P., Morand (eds.), Washington, D.C., *ACS Symposium series*, pp. 25-43.

Asahina, S. (1961) A revised list of the Japanese cockroaches of sanitary importance (Insecta, Blattaria), *Jap. J. Med. Sci. Biol.*, **14** : 147-156.

Ascher, K.R.S. & Levinson, Z.H. (1953) Chemicals affecting the preimaginal stages of the housefly. I. Review of the literature, *Revista di Parassitologia*, **14** : 235-259.

Ayensu, E.S. (1978) Medicinal Plants of West Africa. Michigan, Reference Pub. Inc., 233 pp.

Axtell, R.C. (1969) Macrocheklidae (Acarina : Mesostigmata) as biological control agents for synanthropic flies, WHO/VBC/69.119, 17 pp.

Bar-Zeev, M. (1962) A rapid method for screening and evaluating mosquito repellents. *Bull. Ento. Res.*, **53 (3)** : 521-528.

Bell, E.A. (1987) Secondary compounds and insect herbivores. In, Insects - Plants, V. Labeyrie, G. Fabres, & D. Lachaise (eds.), Netherlands, Dr. W. Junk Pub., pp. 19-23.

Bidawid, S.P., Edeson, J.F.B., Ibrahim, J. & Matossin, R.H (1978) The role of non-biting flies in the transmission of enteric pathogens (*Salmonella* spp. and *Shigella* spp.) in Beirut, Lebanon. *Annals of Trop. Med. & Parasito.*, **72** : 117-121.

Biegel, H.M. & Mavi, S. (1972) Rhodesian Botanical Dictionary of African and English Plant Names. 1st edn., Rhodesia, Salisbury, Government Printers, p. 123.

Briggs, G.G, Elliott, M., Farnham, A.W. & Janes, N.F. (1974) Structural aspects of the knockdown of pyrethroids. *Pestic. Sci.*, **5** : 643-649.

Brown, A.W.A. & Pal, R. (1971) Insecticide resistance in arthropods. 2nd edn., Geneva, WHO, Monograph, **38** : 225-387.

Busvine, J.R. (1971) Techniques for testing insecticides. 2nd edn. England, CAB, 345 pp.

Busvine, J.R. (1980) Insects and hygiene: the biology and control of insect pests of medical and domestic importance. London, Chapman & Hall, pp. 190-211.

Busvine, J.R. & Pal, R. (1969) The impact of insecticide resistance on control of vectors and vector-borne diseases. *Bull. WHO*, 40 : 731 - 744.

Campbell, M.M (1983) A test for repellency to non-biting flies and a comparison of repellents using *Musca domestica* L. *Pestic. Sci.* **14** : 199-212.

Capstick, P.B. (1990) Biopesticides and pest management in the future. In, *Proc. Biospesti.pest mgt. in the Dev. world*, M.F.B. Chaudhury (ed), Nairobi, Kenya, ICIPE Sci. Press, pp. 1-6.

Carson, R. (1962) Silent Spring. Houghton - Mifflin, Boston.

Chapman, P.A., Learmount, J., Morris, A.W. & McGreevy, P.B. (1993) The current status of insecticide resistance in *Musca domestica* in England and Wales and the implications for housefly control in intensive animal units. *Pestic. Sci.* **39 (3)** : 225-235.

Chaudhury, M.F.B. (1990) Proc. Biopesti. Pest Mgt. in the Dev. world, M.F.B. Chaudhury (ed),Nairobi, Kenya, ICIPE Sci. Press, 170 pp.

Chow, C.Y. & Thevasagayam, E.S. (1953) A simple and effective device for housefly control with insecticides. *Bulletin of the WHO*, **8** : 491-495.

Cobbinah, J.R. & Osei-Owusu, K. (1988) Effects of neem seed extracts on insect pests of eggplant, okra and cowpea. *Insect Sci. Appl.* **9 (5)** : 601-607.

Cochran, D.G., Grayson, J.M. & Gurney, A.B.(1975) Cockroaches-Biology and Control WHO/VBC/75.576. 48 pp.

Cornwell, P.B. (1968) The Cockroach. Vol. I, Hutchinson, London, 391 pp.

Crombie, L. (1989) Natural models for the design of insect control compounds : the mammicins. In, *Recent advances in the chemistry of insect control II*, L. Crombie (ed.), London, Roy. Soc. of Chem. pp. 23-51.

Crombie, L., Joseph, J.L., Cayley, J., Larkin, J. & Weston, J.B. (1992). The rotenoid core structure: modifications to define the requirements of the toxophore. *Bio-org. Med. Chem. Lett.*, **2** : 13-16.

Crosby, D.G. (1971) Minor insecticides of plant origin. In, *Naturally occurring insecticides*, M. Jacobson & D.C. Crosby (ed.), Dekker, N.Y., pp. 177-239.

Dahm, P.A., Gurland, J., Lee, I. & Berlin, J. (1961) A comparison of some housefly bioassay methods. *J. Econ. Ento.* **54 (2)** : 343 - 347.

Davidson, A. (1962) Trapping houseflies in the rural areas of Israel. *Rivista da Parasitologia*, **23** : 61-70.

Dipeolu, O.O. (1977) Field and laboratory investigations into the role of the *Musca* species in the transmission of intestinal parasitic cysts and eggs in Nigeria. *J. of hyg. epidemio. microbio. and immuno.* **21** : 209-214.

Echeverria, P., Harrison, B.A., Tirapat, C. & McFarland, A. (1983) Flies as a source of entericpathogens in a rural village in Thailand. *Appl. & environ. microbio.*, **64** : 32-36.

El-Gammal, A.A.M (1990) Anti-feedants and insect growth regulators as a new means of control of *Schistocerca gregaria* in Egypt. In, *Biopesti. and Pest Mgt. in the Dev. World*, M.F.B. Chaudhury (ed), Nairobi, Kenya, ICIPE Sci. Press, pp. 23-30.

Elliott, M. (1989) The pyrethroids: early discovery, recent advances and the future. *Pestic. Sci.* **27** : 337-351.

Elliott, M., Farnham, A.W., Janes, N.F., Johnson, D.M. & Pulman,D.A. (1987) Synthesis and insecticidal activity of lipophilic amides. Parts 1-6. *Pestic Sci.* **18** : 191-244.

Elliott, M., Farnham, A.W., Janes, N.F., Johnson, D.M. & Pulman, D.A. & Sawicki, R.M. (1986) Insecticidal amides with selective potency against a resistant (Super-*kdr*) strain of houseflies (*Musca domestica* L.). *Agric. Biol. Chem.* **50** : 1347-1349.

Elliott, M., Janes, N.F. & Potter, C. (1978) The future of pyrethroids in insect control. *Ann. Rev. Ento.* **23** : 443-469.

Elliott, M., Janes, N.F., & Graham-Bryce, I.J. (1975) Retrospect on the discovery of a new

insecticide. In, *Proc. 8th Brit. Insect. Fung. Conf.*, Croydon, U.K., BCPC Pub. pp. 373-379.

Ellis, P.J.A. (1990) Control of bruchids and weevils using traditional insecticidal plants from Sierra Leone. In, *Biopesti. and Pest Mgt. in the Dev. World*, M.F.B. Chaudhury (ed), Nairobi, Kenya, ICIPE Sci. Press, pp. 61-68.

Evans-Anfom, E. (1984) Traditional medicine in Ghana, practice, problems and prospects. The J.B. Danquah memorial lectures 17th series, *Aca. Arts & Sci.* Accra (1990).

Escoubas, P., Fukushi, Y., Lajide, L ., & Mizutani, J. (1992) A new method for fast isolation of insect antifedant compounds from complex mixtures. *J. Chem. Eco.* **18 (10)** : 1819-1832.

Fields, P.G.,Arnason, J.T., Philogene, B.J.R., Aucoin, R.R., Morand, P. & Soucy-Breau, C. (1991) Phototoxins as insecticides and natural plant defences. *Mem. of the Ento. Soc. of Canada*, **159** : 29-38.

Finney, D.J. (1971) Probit analysis. S. Chand. & Com. Ltd., New Dehli, 328 pp.

Fisk, F.W. & Isert, J.A. (1953) Comparative toxicities of certain organic insecticides to resistant and non-resistant strains of the German cockroach, *Blattella germanica* (L.). *J. Econ. Ento.* **46** : 1059-1062.

Geater, J.G. (1975) The fly as potential vector in the transmission of leprosy. *Leprosy review*, **46** : 279-286.

Gebreyesus, T. & Chapya, A. (1983) Antifeedants from *Clausena anisata* (Wild) Hook F. Ex. Benth. (Rutaceae). In, *Natural Products for Innovative Pest Management*, D.L. Whitehead & W.S. Bowers (eds), Oxford, Pergamon Press, pp. 237-242.

Gould, G.E. & Deay, H.O. (1938) The biology of six species of cockroaches which inhabit buildings. Purdue Univ. *Agri. Exp. Sta. Bull.*, **451** : 31 pp.

Grainge, M. & Ahmed, S. (1988) Handbook of plants with pest control properties. Wiley, New York. 137 pp.

Grayson, J.M. (1966) Recent developments in the control of some arthropods of public health and veterinary importance-cockroaches. *Bull. Ento. Soc. Amer.*, **12** : 333-338.

Great Television Viewing (GTV) (1997) IITA Cowpea project: Zoom TV Documentary. Shown on GTV in Ghana, 13th March, 1997.

Griffiths, J.T. & Tauber, O.E. (1942) The nymphal development of the roach, *Periplaneta americana* L. *J.N.Y. Ento. Soc.*, **50** : 263-272.

Gundidza, M., Chinyanganya, F. & Chagonda, L. (1994) Phytoconstituents and antimicrobial activity of the leaf essential oil of *Clausena anisata* (Willd.) J.B. Hook ex. Benth. *Flavour & Fragrance J.*, **9** : 299-303.

Gujar, G.T. (1990) Plumbagin, a naturally occurring naphthoquinane: its pharmacological and pesticidal activity. *Fitoterapia*, **61** : 387-394.

Haines, T.W. (1953) Breeding media of common flies. I. In urban areas. *Amer. J. of Trop. Med. & hyg.* **2** : 933-940.

Haines, T.W. (1955) Breeding media of common flies. II. In rural areas. *Amer. J. of Trop. Med. & hyg.*, **4** : 1125-1130.

Hansen, L.G. (1983) Biotransformation of organophosphorous compounds relative to delayed neurotoxicity. *Neurotoxicology*, **4** : 97-112.

Heal, R.E., Nash, K.B. & Williams, M. (1953) An insecticide-resistant strain of the German cockroach from Corpus Christi, Texas. *J. Econ. Ento. Soc.*, **68** : 239-311.

Hucko, M. (1984) The role of housefly (*Musca domestica* L.) in the the transmission of *Coxiella burnettii*. *Folia Parasitologica* (Prata), **31** : 177-181.

Hutchinson, J. & Dalziel, J.M. (1958) Flora of West Tropical Africa. Vol. 1. Part 2, 2nd edn., Rev. by R.W.J. Keay, London, Crown Agents for Oversea Governments and Adm. p. 686.

Hutchinson, J. & Dalziel, J.M. (1963) Flora of West tropical Africa. Vol.II, 2nd edn., F.N. Hepper (ed.), London, Crown Agents for Oversea Governments and Adm. p. 466.

Jacobson, M. (1971) The unsaturated isobutylamides. In, *Naturally occurring insecticides*, M. Jacobson & D.G. Crosby (eds),Dekker, N.Y. pp. 137-176.

Jacobson, M. (1982) Plant, insects & man: Their interrelationships. *Econ. Bot.* **36** : 346-354.

Jacobson, M.T. & Harwood, R.F. (1969) Herm's medical entomology. London, Macmillan, p. 117.

James, M. T. & Harwood, R.F. (1969) Herm's medical entomology. London, Macmillan, 117 pp.

Kaloyanova, F.P. & Batawi, M.A. (1991) Human toxicology of pesticides. Boca Raten, Florid, CRC Press. 196 pp.

Keiding, J. (1974) Houseflies *(Musca domestica)*. In, *Control of arthropods of med. and vet. importance*, R. Pal, & R.H. Wharton (eds), N.Y., London, pp. 5-30.

Keiding, J. (1975) Problems of housefly (*Musca domestica*) control due to multiresistance to insecticides. *J. of hyg., epidemio., microbio. and immuno.* (Prugue), **17** : 340-355.

Keiding, J. (1977) Resistance in the housefly in Denmark and elsewhere. In, *Pesticide mgt. and insecticide resistance*, D.L. Watson & A.W.A. Brown (eds.). N.Y., Aca. Press, pp. 61-302.

Keiding, J. (1986) The housefly - Biology & Control. WHO/VBC/86.937, 63 pp.

Khambay, B.P.S. & O'Connor, N. (1993) Progress in developing insecticides from natural compounds. In, *Phytochem and Agric. Proc. Phytochem. Soc. Europe,* T.A. van Beek & H, Breteler, (eds.), Oxford, Sci. Pub., **31 (1)** : 41-61.

Kinghorn, A.D. & Balandrin, M.F. (1984) Quinolizidine alkaloids of the Leguminosae : Structural types, analysis, chemotaxonomy, and biological activities. In, *Alkaloids : chemical and biological perspectives*, W.S. Pelletier (ed.), N.Y., John Wiley and Sons Press, **2** : 105-148.

LaBreque, G.C. & Weidhaas, D.E. (1970) Advantages of integrating sterile-male releases with other methods of control against houseflies. *J. Econ. Ento.* **63** : 379-382.

LaBreque, G.C. & Wilson, H.G. (1959) Laboratory test for housefly repellents. *Florida Ento.* **42** : 175.

Levin, D.A. (1976) The chemical defences of plants to pathogens and herbivores. *Annu. Rev. Ecol. Syst.* **7** : 121-159.

Mallis, A., Esterlin, W.E. & Miller, A.C. (1961) Keeping German cockroaches out of beer. *Pest Control*, **29 (6)** : 32-35.

McKittrick, F.A. (1964) Evolutionary studies of cockroaches. Cornell Univ. Agric. Exper. Sta. Mem., **389** : 1-197.

Merck Index (1989) "Ryanex, Rynicide". 11th edn. Merck & Co., Rathway.

Mester, I. (1983) Structural diversity and distribution of alkaloids in Rutales. In, *Annu Proc. of the Phytochem. Soc. of Europe No. 22: Chemistry & Chemical Taxonomy of the*

Rutales, P.G. Waterman & M.F. Grundon (eds.), London, Aca. Press, pp. 31-96.

Mothes, K. (1976) Secondary plant substances as materials for chemical high quality breeding in higher plants. In, *Biochem. interaction between plants & insects*, J.W. Wallance & P.R.L. Monsell (eds), London, New York, Plenum Press, pp. 385-405.

Mullins, D.E. & Cochran, D.G. (1973) Tryptophan metabolite excretion by the American cockroach. *Comp. Biochem. Physiol.* **44B** : 549-555.

Murphy, S.D. (1986) Toxic effects of pesticides. In, *Casarett & Douli's Toxicology*, N.Y.,MacMillan, pp. 519-581.

Naumann, K. (1981) The chemistry of plant protection and pest-control products. Vol.7. Chemistry of synthetic pyrethroid insecticides: structures and properties, and patents. Springer-Verlag, Berlin, 217 pp.

Newsom, L.D. (1967) Consequences of insecticide use on non-target organisms. *Annu. Rev. Ento.* **12** : 257-286.

Ngadjui, B.T. Ayafor, J.F., Sondengam, B.L. & Connolly, J.D. (1989) Coumarins from *Clausena anisata*. *Phytochem.*, **28** : 585-589.

Ngadjui, B.T., Mouncherou, S.M, Ayafor, J.F., Sondengam, B.L. & Tillequin, F. (1991) Geranyl coumarins from *Clausena anisata*. *Phytochem.*, **30 (8)** : 2809-2811.

Okorie, D.A. (1975) A new carbazole alkaloid and coumarin from the roots of *Clausena anisata*. *Phytochem.*, **28** : 585-589.

Pimentel, D. & Uhler, L. (1969) Ants and the control of houseflies in the Philippines. *J. Econ. Ento.*, **62** : 248.

Parh, I.A., Forbuzo, B.C., Matheney, E.L. & Ayafor, J.F. (1990) Plants used for control of insect pests on stored grains in Cameroon. In, *Biopesti. & Pest Mgt. in the Dev. World*, M.F.B. Chaudhury (ed), Nairobi, Kenya, ICIPE Sci. Press. pp. 83-90.

Rehn, J.A.G. (1945) Man's uninvited fellow traveller-the cockroach. *Sci.* Monthly, **61** : 265-276.

Rogelio, P.M. & Mariano, G.F. (1988) Chemistry of *Hyptis mutabilis*: new pentacyclic triterpenoids. *J. Natur. Prod.*, **51** : 996-998.

Rosef, D. & Kapperud, G. (1983) Houseflies (*Musca domestica*) as possible vectors of *Campylobacter fetus* subsp. *jejuni*. *Applied & environ. microbio.*, **45** : 381-383.

Roth, L.M. & Willis, E.R. (1956) Parthenogenesis in cockroaches. *Annals. Ento. Soc. Amer.*, **49** : 195-204.

Roth, L.M. & Willis, E.R. (1957) The medical and veterinary importance of cockroaches. *Smithsonian Misc. Coll.*, **134** : 1-147.

Roth, L.M. & Willis, E.R. (1960) The biotic association of cockroaches. *Smithsonian Misc. Coll.*, **141** : 1-470.

Rupprecht, J.K., Hui, Y.H., & McLaughlin, J.L. (1990) Annonaceous acetogenins : a review. *J. Natur. Prod.*, **53** : 237-278.

Sacca, G. (1964) Comparative bionomics in the genus *Musca*. *Annu. Rev. Ento.* (1975), **9** : 341-358

Sampson, W.W. (1956) Insecticides used to control housefly larvae. *J. Econ Ento.*, **49** : 74-77.

Sawicki, R.M. (1979) Resistance to pesticides 1. Resistance of insects to insecticides. *SPAN*, **22 (2)** : 50-52, 87.

Schmutterer, H. (1990) Properties and potential of natural pesticides from the neem tree. *Annu. Rev. Ento.* **35** : 271-291.

Schmutterer, H. Ascher, K.R.S. & Rembold, H. (1981) *Proc. Ist Int. Neem Conf., Rottach-Egern, 1980.* GTZ, Eschborn, 290 pp.

Schmeltz, I. (1971) Nicotine and other tobacco alkaloids. In, *Naturally occurring insecticides*, M. Jacobson & D.G. Crosby (eds.), Dekker, N.Y., pp. 99-136.

Schoof, H.F., Mail, G.A. & Savage, E.P. (1954) Fly production sources in urban communities. *J. Econ. Ento.*, **47** : 245-253.

Silverly, R.E. & Schoof, H.F. (1955) Utilization of various production media by muscoid flies in a metropolitan area. I. Adaptability of different flies for infestation of prevalent media. *Annals Ento. Soc. Amer.* **48** : 258-262.

Singh, G & Sidhu, H.S. (1992) Morphologenetic abnormalities of alatiform mustard aphid, *Lipaphhis erysimi* following topical application of juvenile hormone analogues. *Insect Sci. Appl.* **13 (1)** : 27-33.

Spiller, D. (1964) Nutrition and diet of Muscoid flies. *Bull. of the WHO*, **31** : 551-554.

Swain, T. (1977) Secondary compounds as protective agents. *Annu. Rev. Plant Physiol.*, **28** : 479-501.

Tarshis, I.B. (1962) The cockroach - a new suspect in the spread of infectious hepatitis. *Amer. J. trop. Med. Hyg.* **11** : 705-711.

Tierto Niber, B. (1994) Potential for using indigenous plant-derived products as protectants against pests of stored grain on small farms in Africa: Appendix 1. Helsinki, Julkaisuja/Reports **20** : 28-31.

Tierto Niber, B., Helenius, J. & Varis, A.L. (1992) Toxicity of plant extracts to three storage beetles (Coleoptera). *J. Appl. Ento.*, **113** : 202-208.

Thayer, A.M. (1991) Most uses of FMC pesticide to end by 1995. Chem. Eng. News, May, 5 pp.

Thimijan, R.W., Pickens, L.G. & Morgan, N.O. (1970) A trap for houseflies. *J. Econ. Ento.*, **63** : 1030-1031.

Ujvary, I., Eya, B. k., Grendell, R.L. & Casida, J.E. (1991) Insecticidal activity of various 3-acyl and other derivatives of veracevine relative to the veratrum alkaloids veratridine and cevadine. *J. Agric Food Chem.*, **39** : 1875-1881.

West, L.S. (1951) The housefly, its natural history, medical importance and control. Comstock, Ithaca, N.Y., 584 pp.

West, L.S. & Peters, O.B. (1973) An annotated bibliography of *Musca domestica* L. Folkestone & London, Dawsons, 741 pp.

WHO (1970) Insecticide resistance and vector control. Geneva 5-6 WHO Tech. Rep Ser. No.443, 279 pp.

WHO (1976) Resistance of vectors and reservoirs of disease to pesticides. *Twenty-second report of the WHO Expert Committee on Vector Biology and Control*, Geneva, Tech. Rep. Ser. **585**, : 49-53.

WHO (1984) Chemical methods for the control of arthropod vectors and pests of public health importance, Geneva. *WHO/VBC/84.* 108 pp.

WHO (1986) Resistance of vectors and reservoirs of disease to pesticides. *Tenth report of the WHO Expert Committee on Vector Biology and Control*, Geneva, 84 pp.

Wickham, J.C., Chadwick, P.R. & Stewart, D.C. (1974) Factors which influence the

knockdown effect of insecticide products. *Pestic. Sci.* **5** : 657-664.

Wink, M. (1988) Plant breeding: Importance of plant secondary metabolites for protection against pathogens and herbivores. *Theor. Appl. Genet.*, **75** : 225-233.

Wink, M. (1993) Production and application of phytochemicals from an agricultural perspective. In, *Phytochem. & Agric. Proc. Phytochem. Soc. of Europe*, T.A. van Beek & H. Breteler, Oxford Sci. Pub. **34** : 171-213.

Wu, S.Y. & Casida, J.E. (1993) Neuropathy target esterase inhibitors: high potency and stereospecificity of 4*H*-1,3,2 -benzodioxaphosphorin 2-oxides. *Abstr. 205th Natl. Meeting* Am. Chem. Soc., Denver, Co, March 1993, Abstr. **98**.

Yamauchi, S. & Taniguchi, E. (1991) Synthesis and insecticidal activity of lignan analogs. *Agric. Bid. Chem.*, **55** : 3075-3085.