EFFECTS AND USE OF SOME *OCIMUM* PLANT SPECIES AND THEIR ESSENTIAL OILS ON SOME STORAGE INSECT PESTS

BEKELE JEMBERE ADGEH
B.Sc. Addis Ababa Uni. (Ethiopia)
M.Sc. UP Losbanos (Philippines)

THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN ENTOMOLOGY, AT UNIVERSTY OF NAIROBI, DEPARTMENT OF ZOOLOGY

AUGUST, 1994

DECLARATION

I HEREBY DECLARE THAT THE MATERIAL CONTAINED IN THIS THESIS IS MY OWN ORIGINAL WORK AND HAS NOT BEEN PRESENTED FOR A DEGREE IN ANY OTHER UNIVERSITY

Signed..... Date.30th Aug-1994
Bekele Jembere Adgeh

WE HEREBY CERTIFY THAT THIS THESIS WORK HAS BEEN SUBMITTED FOR EXAMINATION WITH OUR APPROVAL AS UNIVERSITY OF NAIROBI AND ICIPE SUPERVISORS

Signed ..... Date 30th Aug. 1994
Dr. G. H. N. Nyamasyo, Lecturer, University of Nairobi, P. O. Box 30197, Nairobi, Kenya

Signed ..... Date 30th Aug 94
Prof. A. Hassanali, Senior Principal Research Scientist and Head, Behavioral and Chemical Ecology Research Unit, ICIPE, P. O. Box 30072, Nairobi, Kenya

TABLE OF CONTENTS

Pages

LIST OF FIGURES

LIST OF PLATES

LIST OF TABLES

ACKNOWLEDGMENT

I express my deepest gratitude and sincere appreciation to Dr. G. H. N. Nyamasyo and Prof. A. Hassanali, my University of Nairobi and ICIPE supervisors, respectively, for sharing their precious talent and time to guide me in conducting the experiments and preparing this manuscript. I am also very grateful to a member of my supervisory committee, Dr. Obeng Ofori for his constructive criticisms and suggestions in the course of the study.

I extend my special appreciation to Mr. H. Amiani for his help in collection of plant materials and extraction of essential oils. Mr. K. Wanyama is gratefully acknowledged for conducting GC-MS analysis of the essential oils. I also wish to thank Dr. W. Lwande and Dr. Hassan Mahamat for their advice and suggestions in conducting the experiments.

I express my special thanks to BCERU technical staff Mr. Edward Nyandat, Mr. L. Moreka, Mr. N. K. Gikonyo, Mr. D. Omondi, Mr. H. Odongo, Mr. V. G. Acheng, Mr. W. Bundi and Mr. D. Nyrago for their cooperation and help in laboratory work.

I am greatly indepted to German Academic Exchange Programme (DAAD) and ARPPIS programme for the scholarship grant and support, without which this work should have not been possible.

My gratitude goes to Prof. Z. T. Dabrowski, who was a member of supervisory committee at the beginning of my study. Finally, I wish to thank my 1991- ARPPIS colleagues for their

friendly cooperation and moral support through out my study period in Kenya.

ABSTRACT

The bioactivity of *O. suave, O. kenyense* and *O. kilimandscharicum* against three storage insects (*Sitophilus zeamais, Rhizoperta dominica* and *Sitotroga cerealella*) was evaluated in the laboratory. Fresh, dry, ground plant materials, and essential oils of the three *Ocimum* plant species at three levels of applications were mixed with 250 g of disinfested maize and sorghum seeds in one liter volume glass jars. The effects of treatments on insect mortality, survival, reproduction and feeding were assessed. The repellent action of the materials and their effect on seed germination were also evaluated.

Chemical analysis of the essential oils of the three plants was done using GC and GC-MS. The identity of a total of 28 constituent compounds of the oils of the three plants were confirmed and their relative proportion determined.

Toxicity tests of the essential oils and their constituent compounds (at their natural relative amounts) was conducted on filter paper discs. Similarly, source of toxicity of the ground plant materials of *O. kilimandscharicum* and *O. suave* was studied.

The toxic effect of the three *Ocimum* plant species was observed only in the treatments involving ground plant materials and essential oils. All other treatments showed

no significant level of toxicity to the insects. Of the three *Ocimum* plant species tested, *O. kilimandscharicum* was the most toxic (inducing 100% mortality) and *O. kenyense* the least. Toxicity of *O. suave* essential oil was attributable mainly to eugenol, that of *O. kilimandscharicum* to camphor and that of *O. kenyense* to 1,8-cineole and *iso*-eugenol. Toxicity of the major compounds in the essential oils were dose-dependent. Toxicity of the ground plant materials was attributed to the essential oils of the plants as no mortality was observed in treatments involving ground plant materials free of essential oils.

The highest dose of fresh plant materials of the succulent *O. kenyense* and *O. kilimadscharicum* increased reproduction and feeding of the insects, which could be due to the high humidity maintained in the glass jars. No significant effect of the other treatments on reproduction and feeding was observed.

O. suave repelled *S. zeamais* in all forms, while fresh plant materials at all levels of application were not repellent to *R. dominica* and *S. cerealella*. *S. cerealella* was significantly repelled only by the highest doses (level 3) of dry and ground plant materials and essential oil of the plant. Similarly, all materials of *O. kilimandscharicum* repelled *S. zeamais* and *R. dominica*, with the exception of fresh plant materials (levels 2 and 3). Treatments involving fresh, ground plant materials and essential oil of *O. kilimandscharicum* at level 3 were significantly repellent

to S. cerealella. *O. kenyense* was repellent to *S. zeamais* in all forms of treatment. However, the highest repellent activity of the plant was obtained with the highest doses of fresh, dry and ground plant materials and essential oil treatments. All plant materials and essential oil of *O. kenyense* with the exception of dry plant materials levels 1 and 2 were also repellent to *R. dominica*. Significant repellent activity of *O. kenyense* against *S. cerealella* was obtained with only fresh plant materials level 3 treatment.

Essential oil of *O. suave* reduced the germination of maize seeds while dry plant materials of the same plant enhanced the germination of the seeds. None of the other treatments had any significant effect on the germination of maize or sorghum seeds.

Large scale evaluation of the most effective treatment (ground plant material of *O. kilimandscharicum)* was carried out by mixing the powder with 25 kg of disinfested maize and sorghum seeds. The effectiveness of the treatment was evaluated by sieve sampling, grain probe and pheromone traps. 100% mortality of *R. dominica* and *S. zeamais* at 2% and 10% levels of treatments respectively, was observed 48 h after treatment. Pheromone baited grain probes were the most effective in sampling live adults of *R. dominica*. Grain probe was more effective compared to sieve sampling in sampling adults of *S. zeamais* . Under small scale farmers conditions the most effective and practical treatment could be one based on the use of ground plant materials.

CHAPTER 1

INTRODUCTION

The Third World food production is inadequate for the ever-growing human population and an increase in agricultural production and preservation will continue to be of vital importance. In this context, great demands will be made on effective and affordable pre- and post-harvest protection. Considerable quantities of foods are destroyed by insects, rodents and micro-organisms between harvest and use in tropical countries. Dichter (1976) estimated that in the sub-sahara regions of Africa losses of food grains during storage at farm or village level can amount to 25-40%. On average, it has been estimated that at least 10% of food is lost in storage (FAO, 1985).

A large number of insects including many species of beetles and moths attack stored crop products in farmers bins, mills, ware houses, retail stores and homes. Insect infestation of stored grain causes weight and quality losses that lead to a reduction of commercial value and seed germination (Seck et al., 1993). Effective control of storage pests in these places can result in immediate increase in world food supplies without any increase in agricultural productivity.

The initial feeling of optimism in pest control by the use of synthetic insecticides since the discovery of

DDT in 1942 soon received a jolt when several reports on manifold adverse effects of the synthetic pesticides became apparent from different parts of the world (Georghiou,1986). Among these adverse effects, the most frequently reported ones included the development of resistant strains in pests, the destruction of non-target species including useful pollinators and natural enemies of the insect pests, and toxic residues left in the environment due to indiscriminate use of these pesticides. At least 447 insects and mite species, including storage insects, have become resistant to one or more classes of insecticides (Georghiou, 1986). At present, insect control in stored food products relies heavily upon the use of gaseous fumigants and residual insecticides which are toxic to the consumer (Shaaya et al., 1991). The poor storage facilities of traditional farmers in the developing countries are also unsuitable for effective conventional chemical control as most storage types are open to reinfestation by insect pests. Great attention, therefore, began to be paid to measures aimed at reducing the adverse effects of pesticides, finding safer methods of pest control and identifying pesticidal materials of natural origin (Jain, 1983).

Naturally occurring anti-pest secondary metabolites appear to have a role in the development of future crop protection strategies, because they are environmentally non-polluting, biodegradable, often times species-specific and

safe to humans. Some are consumed by humans and livestock and yet appear to have no detrimental effect (Mandava, 1985). Some insects fail to damage resistant plants because of the presence of a complex array of inherent defense chemicals. Many of these plant species are potential sources of botanical pesticides (Mandava, 1985; Singh et al. 1989).

Plant products have been observed to play an important part in traditional methods of pest control by small-scale farmers. The precise strategy used by different communities varies from place to place and appears to depend partly on the type and efficacy of suitable flora available in different locations. Many of these practices are undocumented and the scientific rationale for their continued use has remained, by and large, uninvestigated (Hassanali et al., 1990).

Substances that are present in plants and affect insects include, those with antifeeding activity, those which interfere with the development of insects (hormone or anti-hormone like substances), attractants and repellents. The mainly terpenoid essential oils (made up of monoterpenes and sesquiterpenes) comprise volatile substances responsible for characteristic scent, odor or smell found in many plants and can repel an insect from feeding on those plants. In some countries, extracts containing such substances are used for their insecticidal activities (Hardborne, 1973;

Marinibettolo, 1983). In this aspect, infestation can be minimized by treating the paking materials with materials which are toxic or repellant (Halliday et al., 1986).

Pest management programmes may include methods of pest monitoring in conjunction with the application of environmentally safe and less toxic natural products such as repellent or antifeedant plant compounds. However, such combinations do not appear to have been explored. A number of authors have compared deleterious effects of plant essential oils on insect pests (Marinibettolo, 1983; Hassanali et al., 1990). However, the effectiveness of plant materials and their essential oils have not been adequately evaluated.

The ability to detect pest insects is fundamental to most recent strategies of stored product insect pests control. Early warning of pest presence can be used to prevent damage and an efficient detection programme can lead to a reduction in losses and pesticide use. Enhancement of physical traps by the use of attractant chemicals is a worthwhile objective and over the last decade many pheromones have been identified from stored products pests for this purpose (Pinniger and Chambers, 1988). The use of pheromones in traps provides several benefits such as: early detection of infestation, accurate assessment of severity of infestation, accurate location of infestation, optimal timing of control measures and evaluation of efficiency of

control measures (Barak and Burkholder, 1976; Pinniger and Chambers, 1988). In this regard, it is possible to employ pheromone traps in evaluating the efficiency of methods of applying plant metabolites for pest control.

Storage insect pests such as maize weevil (*Sitophilus zeamais* Mots.), lesser grain borer (*Rhizoperta dominica* F.) and the angoumois grain moth (*Sitotroga cerealella* (Olive.)) cause considerable damage to stored maize and wheat grains (Cotton, 1963; Hall, 1970; Obeng-Ofori, 1990). The distribution of these pests is cosmopolitan and their damage has been reported to be severe in Africa throughout maize and wheat producing areas (Hall, 1970).

In the control of these pests, fumigant and dust insecticides have been extensively utilized in big commercial stores, while the use of these insecticides is sporadic by small scale farmers. The bulk of African grain production comes from small scale farmers where many farmers utilize plant materials for the control of storage insect pests. The scientific bases of these methods have not been established even though various compounds have been isolated from botanical sources and reported to have anti-insect properties.

Various methods have been employed to apply plant extracts for the control of storage insect pests. The question of efficient and appropriate technology for the

application of plant materials and extracts (essential oils in particular) has not been adequatly addressed.

For these reasons this study has been initiated with the following objectives:-

1. To evaluate and compare the effect of the essential oils and plant materials of the three plant species, namely, *Ocimum suave* Willd. *Ocimum kenyense* Ayobangira and *Ocimum kilimandscharicum* Guerke, on mortality, survival, feeding, reproduction (on number of F1 progeny) and repellency of the three storage insect pests, *S. zeamais, R. dominica* and *S. cerealella;*
2. To determine the effects of the essential oils and plant materials on the viability of maize and sorghum seeds;
3. To evaluate persistence of the toxic effects of the plant materials and essential oils against the three storage insect pests;
4. To evaluate an effective and appropriate application of the plant products for the control of the insects in bulk grain as monitored by pheromone and grain probe traps;
5. To analyze the chemical composition of the effective plant materials and identify the active chemical constituents of the essential oils.

CHAPTER 2

LITERATURE REVIEW

2.1 The role of secondary plant metabolites in insect pest control.

Plants have evolved over some 400 million years and to combat insect attack they have developed a number of protective mechanisms such as repellency and insecticidal action. Thus a large number of different plant species contain natural anti-insect materials. Some of these have been used by man as insecticides since very early times although many of them can not profitably be extracted. However, several of these extracts have provided valuable contact insecticides which possess the advantage that their use does not appear to result in the emergence of resistant insect strains to the same degree as in the application of synthetic insecticides (Cremlyn, 1978).

Plant resistance, one of the main factors of the survival of wild plants, can be attributed to several chemical mechanisms. These include the action of toxic substances, hormone mimics, anti-hormones, anti-feedants and repellents (Marinibettolo, 1983). Most insect species are phytophagous but, a particular insect does not feed on all

plant species. Some plants contain substances which are toxic or repellent to some insects species, while harmless, attractive or feeding stimulants for others. For example, eugenol was found to be highly attractive to *Diabrotica cristata* (Harris) and *D. barberi* Smith (Yun-Tai, 1984; Yaro et al., 1987; Lance, 1988), while it was repellent to *S. zeamais* (Hassanali et al., 1990; Grossman, 1993).

The toxic substances are, by and large secondary plant metabolites and have been utilized for centuries in crude forms to control insect infestation. Based on metabolic pathway from primary metabolites these secondary plant metabolites are classified as phenylpropanes, acetogenins, terpenoids, steroids, flavonoids, phenolic compounds or alkaloids (Geissman and Crout, 1969; Whittaker and Feeny, 1971). These metabolites are natural products that are commonly recognized as substances of limited molecular weights (normally < 300 daltons) and of great structural variety (Williams et al., 1989).

The most important botanical insecticides which have been used for very long time and survived until today are nicotine (alkaloid), rotenoids, (flavonoids) and pyrethrum (terpenoid) (Jacobson, 1989). According to Cremlyn (1978), as early as 1690 water extracts of tobacco leaves were being used to kill sucking insects on garden plants. Nicotine functions as a non-persistent, contact insecticide against aphids on variety of crops. However, it is being replaced by

synthetic insecticides because of its high mammalian toxicity and its lack of effectiveness in cold weather. Nicotine kills vertebrates because it mimics acetylcholine by combining with acetylcholine receptor at the neuro muscular junction (Cremlyn, 1978).

Rotenoids are a group of insecticidal compounds occurring in the roots of *Derris elliptica* and species of *Lonchocarpus*. Derris powder is manufactured by grinding up the roots and mixing the powder with a clay diluent. Rotenoids are toxic to fish and many insects, but are almost harmless to most warm blooded animals. The biochemical mode of action as an insecticide appears to involve the inhibition of mitochondorial electron transport system (Fukami and Nakajima, 1971; Cremlyn, 1978).

Recently, the antifeedant activity of rotenone and *iso*-rotenone against *Tribolium castaneum* and *Spodoptera litoralis* has been described. Rotenone and five of its derivatives showed the strongest deterrent activity against *Sitophilus granarius, Tribolium castaneum* and *Trogoderma granarium* more than *Azadirachtia indica* (Nawrot et al., 1989).

Pyrethrum is another contact insecticide which is obtained from the flower heads of *Chrysanthemum cinerariaefolium* and owes its importance to its outstanding

knock down action on flying insects combined with a very low mammalian toxicity due to its ready metabolism to non toxic products (Matsui and Yamamoto, 1971). It is used to control pests in stored foods and against house hold and industrial insect pests. However, a major disadvantage of pyrethrum, especially for use against agricultural insect pests, lies in its lack of persistence due to its inability to kill insects in the presence of air and light (Cremlyn, 1978).

2.2 **Use of terpenoids (essential oils) in pest control**

Repellent plant substances are made of volatile, often aromatic terpenoid constituents (essential oils) which prevent attack and infestation by insects and, on long exposure causes deleterious physiological effects. Such substances are extracted in some countries for use as insecticides. The powder of heart wood of *Juniperus recura* in Nepal is an example (Marinibettolo, 1983). The use of certain plant substances as insect repellents led directly to the extraction and identification of several essential oils that are fairly efficient repellents. Two examples are oil of citronella and oil of camphor oil (Rice, 1983). A chemical class conspicuous among plant secondary plant metabolites and containing chemicals inimical to insects are the terpenoids (Ryan and Byrne, 1988). Large number of plant substances are included under the term terpenoid which

is used to indicate that all such substances have common biosynthetic origin from isoprene molecules. Plant families particularly rich in terpeniod oils include the Compositae, Labiatae, Myrtacae and some others. Chemically, the terpene essential oil constituents can be divided into mono and sesquiterpenes built in 10 and 15 skeleton of carbon units respectively, and with boiling temperature ranges of 140-180^{O}c and > 300^{O}c respectively. Simple monoterpenes are widespread and tend to occur as components of the majority of essential oils (Hardborne, 1973). Essential oil components are often found in the glands or intercelular spaces in plant tissues which may be concentrated in seeds or flowers (Pavia et al., 1988). A common structural feature of terpenoids is their hydrocarbon skeleton, which in turn confers on them a common property of hydrophobicity. Many hydrophobic compounds are associated with protein deactivation and enzyme inhibition and particularly one enzyme, estrase, which is present in the neuro muscular junction (Ryan and Byrne, 1988). Apart from their insecticidal activity, terpenes as components of characteristic odor produced by plants can have ecological adaptation to repel phytophagous insects and these insects can develop resistance to this defensive odor; however, storage insects rarely fly to the field and do not have the chance to undergo coevolutionary process with the plant and develop resistance to plant repellent constituents (Kumar,

1984). In recent years, increasing attention has been given to the control of storage insect pests through the use of oils including vegetable oils, essential oils and mineral oils (Yun Tai and Burkholder, 1981; Kumar and Okonronkwa, 1991).

Research into repellents began with the outbreak of the second world war, when it became necessary to protect the fighting forces from biting flies, especially mosquitoes. Since then over 15,000 compounds, synthetic and natural, were tested but few qualified for use; of these the synthetic compound DEET (diethyl toluamide) met with stringent requirements for skin or close use (Bell and Carde, 1984).

A number of plant oils have insect repellent activities and these activities decrease through time as it has been observed in repellent activities of Turmeric, *Curcuma longa* L. and sweet flag *Acorus calamus* (L.) against red flour beetle *Tribolium castaneum* Herbst (Jilani et al., 1988). Other essential oils have been found to have insecticidal, acaricidal and fungicidal activity (Raghavaria and Javaramaiab, 1989).

Shaaya et al. (1991) investigated the potential use of essential oils extracted from various species and herb plants and some of their major constituents as fumigants. The fumigant toxicity of 26 essential oils and some of their

major constituents was assessed for adults of *Rhizoperta dominica, Oryzaephilus surinamensis, Tribolium castaneum* and *Sitophilus oryzae* (all storage insects). Three groups of active materials were distinguished according to their toxicity against the insects tested. In addition, based on the structure of the effective constituents it was possible to synthesize and select a compound ZP51 with a potency of 5-10 fold greater relative to all other compounds assayed. A concentration of 1.5 µl/1 liter of air was enough to achieve 100% mortality against *S. oryzae* and 3 µl/1 liter of air against *O. surinamensis, T. cataneum* and *R. dominica*. It should be noted that a concentration of 20-30 mg/1 liter of air of methyl bromide is recommended for the control of stored product insect pests. Essential oils of *Callistemon lanceolata* DC. and *Eupatorium capllifolium* L. have also been found toxic to pulse beetle (*Callosobruchus maculatus*) (Mishra et al., 1990).

Reproduction retarding, fumigant toxicity and grain protection capability of 31 naturally occurring essential oils of plant origin at the rate of 1000 ppm were studied in the laboratory against *S. oryzae* (Singh et al., 1989). The essential oil obtained from *Pinus longifolia* Roxburgh reduced the population of *S. oryzae* by 37.51%, 75.21% and 86.82% compared with controls 30, 60 and 90 days after treatment respectively, indicating strong reproduction-retarding property of the oil. In contrast, essential ial

oils from *Callicarpa macrophylla* Vahl and *Zanthoxylum alatum* Roxburgh increased the insect population 1.5 and 2 times more than controls in 90 days after treatment respectively. Only *Mentha citrata* Ehrhart oil showed significant toxicity (Singh et al., 1989).

Roger et al. (1993) tested the bioactivity of 22 essential oils from aromatic and medicinal plants on *Acanthoscelides obtectus* Say (a pest of kidney bean, *Phaseolus vulgaris* L.). The insecticidal effect was evaluated by determining LC_{50} after 24 and 48 h of exposure. The most effective essential oils were extracted from plants belonging to the Labiatae family. *Origanum majorana* and *Thymus sephyllum* essential oils were the most toxic. *Origanum majorana* essential oil contained terpinene-1-4 ol (20.6%), linalool (15.3%) and terpinene (10.3%)

Linalool is a colorless liquid with fresh-wood odor. It occurs naturally in essential oils from more than 200 herbs, leaves and wood. It is used in perfumes and flavoring and an intermediate in synthesis of vitamins A and E. It is less toxic to vertebrates than insecticides currently recommended for the control of fleas on dogs and cats. LC_{50} experiments demonstrated that linalool is more toxic to adult fleas than D-limonene, a registered natural product for flea control on dogs and cats. Accordingly, linalool has a major potential for use in control programmes for cat fleas (Hink et al., 1988).

Interestingly, *Ocimum canum* Sims which is usually added to stored food stuff, in Rwanda, to prevent insect damage contains linalool in 60-90% in the total volatiles. Linalool is also one of the major (36.8%) constituents of *O. fischeri* Guerke (Mwangi et al., 1988). Direct exposure of adults of *Zobrates subfasciatus* (Bohem) to dried and milled *O. canum* leaves resulted in 100% mortality of males and 50% mortality of females after 48 h of treatment. Dose response curves of linalool isolated from *O. canum* were investigated. The LC_{50} values, on filter paper, were 428 $\mu g/cm^2$ for *Z. subfasciatus*, 405 $\mu g/cm^2$ for *Acanthoscelides obtectus* 428 $\mu g/cm^2$ for *R. dominica* and 427 $\mu g/cm^2$ for *S. oryzae*. Air exposure of linalool treated papers for up to 24 h significantly reduced toxicity to both sexes of *Z. subfasciatus* (Weaver et al., 1991). The toxicity of linalool could be attributed to its chemical structure as it has been reported by Cremlyn (1978) that the combination of unsaturation and cyclic alcohol give rise to stronger insecticidal, fungicidal and bactericidal activity, unlike the saturated aliphatic alcohols.

Vapors of different monoterpenes from the essential oil of *Pinus ponderosa*, host of the western pine beetle *Dendroctonus brevcomis* LeConte, varied in their toxicity to this insect. The order of toxicity was limonene > carene > myrcene > ß-pinene = Á-pinene > control (Smith, 1965).

D-limonene, a monoterpene is a natural product found in the volatile oil expressed from fresh peel of ripe oranges and other citrus fruits. The D-limonene content of orange peel oil is about 98% and it can be purified by steam distillation of the oil from the pressed peels. It is used as a base for soaps and perfumes. D-limonene was found to be toxic to all life stages of the cat flea (*Clenocephalides felis)* in contact toxicity test. It is available commercially as a shampoo, dip and aerosol for flea control (Hink and Fee, 1986).

Ryan and Byrne (1988) tested the effect of five monoterpenes representing five functional groups (citral, an aldehyde; pulegone, a ketone; linalool, an alcohol; -(-) bornyl acetate, an ester; and cineole, an ether) and a single diterpene alcohol (gossypol) on Acetyl Cholin Estrase (AChE) inhibition of non adapted storage insect *Tribolium castaneum*. They found that the five monoterpenes were reversible competitive inhibitors probably occupying the hydrophobic site of the enzyme's (AChE) active center. Gossypol was an uncompetitive inhibitor of the enzyme.

According to Roger et al. (1993), the most effective essential oil extracts on *Acanthoscelides obtectus* contain oxygenated functions (alcoholic or phenolic) within the chemical skeleton of their constituents. Microsomal cytochrome P-450 monooxygenases play an important role in the detoxification of plant toxins for herbivorous insects,

their activity being generally higher in the generalist than in semi specialist. Thus, where as the former group would be relatively resistant to oxygenated compounds the later, like *A. obtectus* might be more susceptible to such compounds. However, toxicity of terpenoids to insects do not appear to be related to the presence of oxygen (Coats et al., 1991).

The bioactivity of a terpenoid against an insect may not necessarily associate with their toxicity. Thus, last instar southern army worm, *Spodoptera eridonia* (Carmer), larvae fed on diets containing up to 0.1% of pulegone developed into reproducing adults. A 0.2% pulegone containing diet retarded development and inhibited reproduction. Last instar larvae accepted a single small meal loaded with up to 4% pulegone, which was acutely toxic to them only at concentration far exceeding those occurring naturally. Accordingly, the effectiveness of pulegone as defensive chemical in natural concentration has been attributed to its interference with feeding behavior, development and reproduction, rather than to its toxicity (Gunderson et al., 1985).

In assessing potential insecticidal activity of the essential oil of *Dennettia tripetala* Baker (Anonaceae) samples of the oil obtained by steam distillation from edible fruits were tested against nymphs and adults of the American cockroach, *Periplaneta americana* (L.) and grass

hopper *Zonocerus variegatus* (L.). Irrespective of species, stage of development, or exposure technique, the oil was significantly toxic to all the insects than diazinon, lindane and propoxur insecticides. The toxicity of the oil was attributed to ß-phenylnitroethane, which comprises nearly 80% of the oil (Iwuala et al.,1981).

Colin (1990) compared the efficiency of Labiate essential oils with classical aerosol treatments including the acaricidal substance "Amitraz" at 0.25% concentration. The essential oils were also injected into each colony by the aerosol way for one minute. It was found that there was no difference between the two treatments for the moderately infested colonies.

The acaricidal effect of essential oils of Labiatae has been reported by Mansour et al. (1986). The effects of essential oils of 14 species of Labiatae collected in Israel on adult female of *Tetranychus cinnabarinus* was mortality at 0.1 to 2% and induced repellency within 48 h of placing adult females on treated discs. Egg laying was reduced and 7 days old residues had the same effect.

Apart from their insecticidal activity some essential oils are also toxic to microorganisms. The essential oil of *O. basilicum* at 1.5 ml/liter completely inhibited the mycelial growth of 22 fungi species, including the mycotoxine producing species of *Aspergillus flavus* and *A. parasiticus*. The toxic dose was much lower than commercial

fungicides (Dube, et al., 1989). When volatiles from the aqueous filtrates of macerated leaves of 16 plant species were tested against *Rhizoctonia solani*, those from three plant species, including *O. canum*, proved most active. The volatile oils of the three species applied in pairs were more toxic to fungi than individual oils (Dubey and Kishore, 1987).

Yun et al. (1993) tested the effect of essential oil extract of *Artemisia princeps* var *orientalis* on the seed germination of *Chrysanthemum boreale*, *Plantago asiatica*, *Diarrena japonica* and *Achyranthes japonica*. They found that germination of all the seeds was suppressed by the essential oils.

2.2.1 *Ocimum* plant species and use of their essential oils

According to Paton (1991) 16 species of *Ocimum* on main land of Africa have been recognized and 4 new species have been described.

The genus *Ocimum* L. consists of 160 species occurring in the warmer parts of the tropics, up to altitude of 2900 meters above sea level. *Ocimum* plant species which belong to the Labiate family have medicinal and insecticidal values which are being widely used by the small scale farmers (Nigist and Abegaz, 1989). They are rich in volatile

essential oils where these may be used for their medicinal value or insecticidal effect. Basil oil from *O. basilicum* L. and *O. gratissimum* are well known for aroma of chemicals used in flavor and fragrance industry. Bioactivity studies conducted on *Ocimum* species have also shown anti-tubercular, insecticidal, fungicidal anti-asmathic and antimicrobial properties (Chaven et al., 1983; Nigist and Abegaz, 1989). The main components in essential oils from 4 species of *Ocimum* (*O. canum, O. americanum, O. gratisimum* and *O. trichodon*) and chemotypes of *O. urticifolium* showed antimicrobial activities against *Escherichia coli, Bacillus subtilies, Staphylococcus aureus* and others. All samples were found to show antimicrobial activity (Janssen et al., 1989).

Basil plant (*Ocimum basilicum*) is one of the species of Labiate widely grown in Africa. Its essential oil is also source of potent juvenile hormone mimic (Bowers and Nishinda, 1980). In Kenya it is used to keep away mosquitoes by laying the branches in the house. Fun et al. (1990) and Gaydou et al. (1990) reported on the composition of essential oil of *O. basilicum* var. *canum* and *O. gratissimum* and concluded that they belong to the methyl cinnamate chemotype. Both contain varying amounts of eugenol which is the main volatile component having insecticidal property (Gaydou et al., 1990).

Growing conditions have significant effect on the composition of essential oils of *Ocimum* plants (Nyakaenen, 1989). Essential oil content and composition of basil (*O. basilicum*) were compared by Morales et al. (1993) under field condition and green house using cloned methyl sinnamate basil lines. The essential oil content, and amount of *cis*-methyl cinnamate, linalool and 1,8-cineole were significantly higher in field grown plants than those grown in the green house while, only *cis*-methyl cinnamate was higher in green house grown plants.

2.2.1.1 Holy bassil (*Ocimum suave* Willd. also *O. gratissimum* var.*gratissimum* Paton)

The holy bassil is 60-250 cm tall aromatic perennial herb. It is widespread in the tropics from India to West Africa, as far south as Namibia and Natal and naturalized in tropical South America (Paton, 1991).

The tropical shrub, *O. suave*, of the family Labiatae is native to Africa and India. It grows extensively in Tanzania and has various medicinal value. It is frequently used as mosquito repellent, where branches are burned or placed on the roof and walls of huts. The leaves are reputed to be insecticidal to mosquitoes, flies and other insects (Chogo and Crank, 1981).

The holy basil has strongly scented leaves which are reported to be of medicinal value (Kokwaro, 1976). The leaves are also used for abdominal pains, sore eyes, ear trouble and for cough. An infusion of leaves acts as disinfectant and insecticidal (Kokwaro, 1976). It yields the highest herbage and essential oil among the *Ocimum* plant species (Trivedi et al., 1981). It is an erect shrub that is commonly found in upland forest areas of East Africa and has been traditionally used as insect repellent, particularly against mosquitoes and as grain protectant (Kokwaro, 1976; Hassanali et al., 1990).

Hassanali et al. (1990) isolated the essential oil of *O. suave* by steam distillation and further analyzed the oil by GC-MS. As a result, a blend of monoterpene and sesquiterpene and eugenol were isolated. When the repellency bioassay of these constituents were performed against the maize weevil (*Sitophilus zeamais*) eugenol was found to be the most potent and was more repellent than N,N-diethyltoluamide (DEET), a synthetic commercial insect repellent. They concluded that there may be scientific basis for the traditional use of *O. suave* as a grain protectant. *O. suave* is also among the indigenous plants of Cameroon commonly used by the farmers for the control of insect pests on stored maize,

beans and cowpeas. Leaf branches of the plant are mixed with the grains for the control of storage insects and this practice has been passed on for generations (Parh et al., 1990).

The essential oil composition of flowering shoots of 40 *O. suave* plants growing at 9 sites in Rwanda in 1981 was determined using GC, GC-MS. A total of 9 constituents were found, their presence and percentage varying among the sampled plants. In all but 3 cases eugenol was predominant, its percentage content ranging from 9.2 to 80.9%. This variation is suggested to be due to the presence of 3 chemo sub-speceis within the country (Teteny et al., 1986). Eugenol was also reported to be the main component (70%) of the essential oil of *Ocimum sanctum* (Lal et al., 1978; Malik et al., 1986).

O. suave was identified as *O. gratissimum* by Paton (1991). Similarly, *O. viride* was identified as *O. gratissimum* by some authors, although the chemical composition of analysis of the essential oils of the two plants were different (Ekundayo, 1986). The three may well be chemotypes.

Nandi and Chatterjee (1986) studied the effect of altitude (100-500 ft), photoperiod and micronutrients (Zn, Mo and B) on the growth and essential oil formation of *O. gratissimum*. Long day (16 h) and the micronutrients treatments showed beneficial effect on the essential oil

yield. Apart from these, inter breeding or cross breeding had significant effect on the composition of essential oils (Sobti et al., 1978). Selected plants from true breeding strains of *O. suave* (*O. gratissimum*, *O. viride*) were crossed. The F1 hybrids and the allopolyploids subsequently synthesized gave, on average, higher yields of both herbage and essential oil. Evidence was obtained which suggested that phenols and sesquiterpenes are inherited independently of each other (Khoshla et al., 1985).

2.2.1.2 *Ocimum kenyense* Ayobangira (O. sp A Agnew)

O. kenyense is 10-30 cm tall aromatic annual or perennial herb. It grows in uplands of Kenya, and northern Tanzania in wet, seasonally waterlogged places (Paton, 1991). It is rhizomatous herb with ascending stems and subsessile ovate to elliptic leaves, simple racemes, and terminal similar to those of *O. kilmandscharicum* but, more slender. Locally abundant in black-cotton and water logged soils (Agnew, 1974).

Mwangi et al. (1994) identified the chemical composition of the essential oil of *O. kenyense*. 22 compounds were identified accounting for 86.8% of the oil. 1,8-cineole (38.4) and methyl chaavicol (23.9%) were the major constituents of the oil.

2.2.1.3 *Ocimum kilimandscharicum* Guerke (*O. johnstonii* Baker, *O. tortuosum* Baker)

O. kilimandscharicum is a shrub with maximum height of 2 meter and grows in Uganda, Kenya, Tanzania and introduced to the Sudan (Paton, 1991). It is an erect branching pubescent shrub, with ovate to elliptic leaves and simple terminal racemes of rather distant whorls of small white to pinkish flowers (Agnew, 1974).

The steam of boiled leaves of *O. kilimandscharicum* is used to cure serious colds in the countrysides of Kenya. The leaves may be simply rubbed between palms and snuffed for colds and coughs. Leaves are also dipped into water and the liquid drunk for abdominal pains. They are also used to cure measles and diarrhea in small children (Kokwaro, 1976).

Oils distilled from the leaves of *O. kilimandscharicum* and 2 other *Ocimum* species and clove oil were more active against 11 gram positive bacteria than 7 gram negative bacteria. There was also considerable activity against 13 fungi, notably 5 dermatophytes. The *Ocimum* oil compared well with clove oil (Prasad et al., 1986).

Charles and Simon (1990) extracted essential oils of leaves, flowers and stems of *O. basilicum*, *O. kilimandscharicum* and *O. micranthum* by hydrodistillation and the oil was analyzed by GC and GC-MS for composition. No

significant difference were observed in the essential oil yield and relative concentration of major constituents using fresh or dry samples and using sample sizes of 75 to 10 g of dry plant tissue.

2.3 The storage insect pests

Most storage insect pests belong to the order coleoptera. One or several species may be found attacking the same commodity at the same time. These may live as primary or secondary pests. The most important Coleopterous storage insect pests are among the Bostrichidae, Curculionidae, Dermistidae and Tenebrionidae families. The other most important storage insect pests are from the Lepidoptera (Gelechidae) group and most have been recognized as primary pests (Cotton, 1963).

2.3.1 Maize weevil (*Sitophilus zeamais* Motschulsky)

This insect is very much like the rice weevil (*S. oryzae*) and widely distributed in all warm and tropical parts of the world. It has a characteristic rostrum and elbowed antennae (Cotton, 1963).

The adults are long lived (several month to one year) and eggs are laid throughout the adult life, although about 50% may be laid in the first four to five weeks. The eggs are laid individually in small cavities chewed into cereal grains by the female. Each egg is protected by a waxy secretion produced by the female. The incubation period is about six days at 25^{O}C and above 32^{O}C and below 12% moisture content (Howe, 1952). Rates of oviposition are low below 20^{O}C and above 32^{O}C and below 12% moisture content of the seeds (Birch, 1944).

Upon hatching the larva feeds inside the grain, excavating a funnel as it develops. There are four larval instars. At 25^{O}C and 70% relative humidity pupation takes place in about 25 days, although development periods increase at low temperature (for example, 98 days at 18^{O}C and 70% relative humidity). The larva pupates within the grain and the newly developed adult chews its way out leaving a large characteristic emergence hole. Total development period ranges from about 35 days under optimum conditions to over 110 days at sub-optimal conditions (Longstaff, 1981).

The actual length of life cycle under a given set of conditions depends on the quality of grain being infested. The maize weevil is predominantly found associated with maize however, it is capable of developing on all cereal grains and cereal products (Walgenbach and Burkholder, 1986;

Walgenbach et al., 1987; Obeng-Ofori, 1989). It is a most destructive pest of rice, corn, other grains and their processed products and its distribution is world wide (from tropical areas to temperate zones) (Tipping et al., 1987; Maeshima et al., 1985). It causes serious damage to maize grains in maize growing areas of Africa. Hall (1970) reported that the insect causes 20% weight loss in Ghana alone. In addition, the insect causes heavy reduction in viability of stored seeds by feeding on the endosperm and embryo.

2.3.2 The Lesser Grain Borer, *Rhizoperta dominica*, (Fabricius)

The Lesser Grain Borers gained much prominence during world war I, when shipment of Australian wheat developed heavy infestation because of delay by submarine warfare. When it was introduced into the southern United States via California, where it was known as the Australian wheat weevil. As the species is able to attack sound, dry grain in storage it is regarded as a primary pest (Khorramsha and Burkholder, 1981).

This insect causes serious damage to stored maize and wheat in tropical countries. Both the adult beetle and larvae cause serious damage in warm climate attacking a great variety of grains, even resistant to other storage

insects. The destruction may be to the extent of reducing grain to mere shell by the feeding of the beetle and the grabs. In addition to its destructiveness in stored grains, it is not uncommon to find this beetle breeding in wheat and maize flour that has been held in storage for some time (Cline, 1972). Hall (1970) reported that this insect causes about 34% weight loss in Nigeria alone.

According to Cotton (1963), this insect is distinguished from other grain insect pests by its slender, cylindrical form and small size. It is polished dark brown or black with somewhat rough surface and about one-eighth of an inch long. It belongs to the originally wood-boring group (family Bostrichidae) of the Coleoptera (beetles) that have the head turned down under the thorax and are armed with powerful jaws with which they can cut directly into wood or other tough vegetable materials.

The females lay from 300 to 500 eggs each, dropping them singly or in clusters in crevices or the rough surface of seeds. The eggs hatch in few days and the small whitish grabs crawl actively and start to bore into the grains or feed on flour produced by the adult beetles. They complete their growth either within the grain or in the grain dust, transform to white pupae and, in time, change to adult beetles. The period from egg to adult in summer is said to be about one month (Back and Cotton, 1938). Larval development occurs more rapidly on whole grains than on

flour. On a diet of whole wheat at the optimum temperature of 34°C and 70% relative humidity larvae develop to pupae in about 17 days (Obeng-Ofori, 1990).

The lesser grain borer is one of the most resistant of the stored product insect species to gamma radiation (Tilton et al., 1966), infrared treatment (Tilton and Schrooder, 1963) and various insecticides.

2.3.3 **The Angoumois Grain Moth, *Sitotroga cerealella* (Oliver)**

The angoumois grain moth is the second most important insect pest following rice and granary weevils as a pest of stored grain. Its distribution is cosmopolitan and it flies to the fields of ripening corn and wheat as they are nearing maturity and lays eggs up on the wheat heads or corn kernels.

These initial infestations take place in the grain when it is in or passing the milk stage and usually involve small percentage of the kernels. From the time of harvest until wheat is threshed and stored, infestation by the moth increases with great rapidity. When the wheat is in straw it is easy for the moths to make their way from one wheat head to another with the result that infestation is unimpeded. After the grain is threshed it is impossible for the soft-bodied moths to make their way below the surface of

the grain and infestation is restricted to the surface of the grain (Cotton, 1963).

Each female lays on average about 40 eggs, although as many as 389 eggs may be laid by a single moth. The eggs which are laid on or near the grain hatch into white caterpillars that bore into the kernels of grain and feed on the content. When fully grown, each caterpillar eats a channel to the outside of the seed but leaves a thin layer of seed coat intact. It then changes to reddish brown pupa and later the adult moth emerges pushing aside the thin section of seed coat that covers the exit from the channel. Development from egg to adult may take five weeks (Simmons and Ellington, 1953; Mills, 1965; Mills and Wilburn, 1967). It has been reported to cause 2.5, 14 and 4-5 percent grain weight loss in Egypt, Sudan and Kenya respectively Hall, 1970).

CHAPTER 3

MATERIALS AND METHODS

3.1 General materials and methods

3.1.1 Collection of plant materials, preparation of plant extracts and seeds treatment

O. suave (Plate 3.1) was collected from "Kabete" area (Nairobi), *O. kenyense* (Plate 3.2) from "Kasarani" (Nairobi) and *O. kilimaɑscharicum* (Plate 3.3) from "Siaya" district. Only the aerial part of the plant were collected and brought to the laboratory and stored at 40°C. Maize and sorghum seeds purchased from "Kisii" and "Kitale" farmers were disinfested in an oven at 40°C and 250 g of each were put into 1 liter volume glass jars.

The fresh plant materials comprising leaves, inflorescence and succulent stems (all together) were subjected to steam distillation to obtain the essential oils. The distillation process was carried out using clavenger type of apparatus, (Plate. 3.4), Gunter (1946). The condensing oils were collected in hexane solvent. The hexane solution was filtered through a filter paper containing anhydrous sodium sulfate in a funnel to remove any remaining traces of water. Hexane was then removed by distillation at 60°C from 'Contes' short path

distillation apparatus (Plate 3.4). When hexane was completely removed (condensation stoped) the oil was collected and weighed. The oil was applied to the seeds at 0.012, 0.06 and 0.3% (30, 150 and 750 mg/250 g of seeds) corresponding with the method used by Jadhav and Jadhav (1983). Application was done in glass jars (1 liter) in 10 ml of 95% n-hexane (Aldrich HPLC grade) and the contents were shaken throughly to ensure uniform distribution. The seeds were kept aside for 24 hours to allow the hexane to evaporate before bioassays were conducted.

Fresh leaves, inflorescence and succulent stems of the meristematic region of the plants were also used for fresh plant material treatments. These were mixed with the seeds in the glass jars at three levels of treatment based on their essential oil content (Table 3.1). Thus, O. suave fresh plant materials were applied at the rates of 4 .3, 21.4 and 107 g per 250 g of seeds corresponding to the essential oil doses of 30, 150 and 750 mg/250 g of seeds respectively. Similarly, *O. kenyense* was applied at the rates of 6, 30 and 150 g per 250 g of seeds, and *O. kilimandscharicum* at the rates of 5, 25 and 125 g /250 g of seeds corresponding to the same relative doses of their essential oil (Table 3.1).

Plate 3.1 Holy bassil (*Ocimum suave* Willd. also *O. gratissimum* var *gratissimum* Paton)

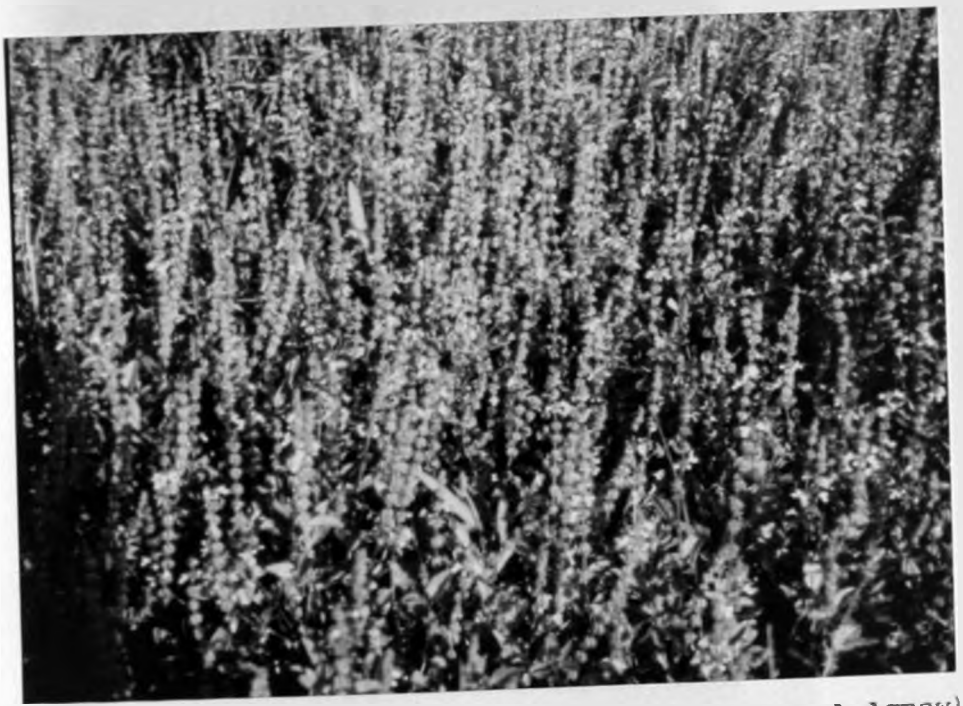


Plate 3.2 *Ocimum kenyense* Ayobangira (*O.* sp A Agnew)

Plate 3.3 *Ocimum kilimandscharicum* Guerke (*O. johnstonii* Baker, *O. totuosum* Baker)

(a)

(b)

Plate 3.4 Set up (a) steam distillation (b) isolation of the essential oil

Table 3.1 List of different treatments used in the different experiments

Essential oil treatments (mg/250 g seeds)			
	O. suave	*O. kenyense*	*O. kilimandscharicum*
level 1 (0.012%) =	30.0	30.0	30.0
level 2 (0.06%) =	150.0	150.0	150.0
level 3 (0.30%) =	750.0	750.0	750.0
Fresh plant materials treatments (g/250 g seeds)			
level 1 =	4.3	6.0	5.0
level 1 =	21.4	30.0	25.0
level 3 =	107.1	150.0	125.0
Dry plant materials treatments (g/250 g seeds)			
level 1 =	1.1	1.2	1.0
level 2 =	5.4	6.0	5.0
level 3	26.8	30.0	25.0
Ground plant materials treatments (g/250 g seeds)			
level 1 =	1.1	1.2	1.0
level 2 =	5.4	6.0	5.0
level 3 =	26.8	30.0	25.0

Note; levels of fresh, dry and ground doses were determined based on equivalent doses of the essential oils.

Some of the fresh plant materials were kept in a well ventilated room (under shade) for dry and ground plant materials treatments. After 3-5 days (depending on weather condition) the dry matter content of the plants was calculated. This was done after putting a known weight of fresh plant material in the drying room and weighing the plant materials after drying to constant weight (dry weight/fresh weight X 100). Three levels of dry and ground plant materials were determined on the basis of their dry matter contents. In the case of O. suave, this was worked out to 1.1, 5.4 and 26.8 g/250 g of seeds for the three respective levels of dry and ground treatments; for *O. kenyense* these were 1.2, 6 and 30 g respectively and for *O. kilimandscharicum* these were 1, 5 and 25 g/250 g of seeds, respectively. Ground plant material was obtained by grinding dried plant materials in "Janke and Kunkel IKA" laboratory mill. The fineness of the powder was determined using sieve size of 1 mm.

3.1.2 Mass rearing of the test insects

Mass rearing of the test insects was done following the method of Santhoy and Rejesus (1975).

The three insect pests (*S. zeamais, R. dominica* and *S. cerealella*) were obtained from the original stock at ICIPE and KARI laboratories. Maize and sorghum seeds (local varieties) were obtained from "Kisii", "Ugunja" and "Kitale" farmers. The seeds were disinfested by keeping them in an oven at 40 ^{o}c for 4 hours (Santhoy and

Rejesus, 1975). To glass jars of 1 liter volume were introduced 250 g of the disinfested maize or sorghum seeds. Test insects were sexed following the methods of Halstead (1963) and Stenley Wilbur (1966). 20 pairs of *S. zeamais* and 40 adults of *S. cerealella* were introduced into separate glass jars containing disinfested maize seeds. Like wise, 20 pairs of *R. dominica* were introduced into the glass jars containing disinfested sorghum seeds.

The jars were then covered with nylon mesh and held with rubber bands. All the jars were then kept in a room maintained at 60 ±5% rh and 26 ±1°C using heaters and water troughs. The S. zeamais, *R. dominica* and *S. cerealella* were allowed to stay in the glass jars for 30, 15 and 7 days respectively, their optimum oviposition time, after which they were removed and discarded. The seeds were kept in the room until the emergence of first progeny. After 35, 27 and 35 days for *S. zeamais*, *R. dominica* and *S. cerealella* respectively, the newly emerged progenies were transferred into their respective disinfested grains in the glass jars daily until 65, 42 and 42 days after introduction of the insects. A sieve of mesh number 10 (sieve size 2 mm, Endecotts LTD, London) was used in sieving *S. zeamais* and *R.*

dominica and a glass box and a vial were used in transferring adults of *S. cerealella*. By daily sieving and transferring the newly emerged adults the ages of the insects were determined.

3.2 Evaluating toxicity of the three plant materials and essential oil against the three insects

The experiment was conducted following the method of Mukherjee et al. (1990). After treating 250 g of seeds in 1 liter volume glass jar with the three levels of fresh, dry, ground plant materials and essential oils, 10 pairs of 4-8 days old *S. zeamais* or *R. dominica* or 3-5 days old *S. cerealella* adults were introduced into the glass jars containing the treated seeds. As control, 10 ml hexane (the amount used to dissolve the essential oils) treated 250 g of maize and sorghum seeds were infested with the three insects. Hexane alone treated seeds were also treated and infested similary. The glass jars were then covered with nylon mesh held with rubber bands. After 24 hours, the adults of *S. zeamais* and *R. dominica* were sieved out while *S.cerealella* were transferred into a glass box. The dead ones were counted and discarded, but live ones were returned to the glass jars. This process was repeated after 48, 72 and 96 h of treatments. After 96 h the live adults were returned to the glass jars which were left under the ambient laboratory conditions until the optimum oviposition time of the insects as indicated in mass rearing of the

insects. Treatments where 100% mortality occurred were also kept under the same conditions.

After 30, 15 and 7 days (optimum oviposition period) for *S. zeamais*, *R. dominica* and *S. cerealella*, respectively, the adults were removed and the rate of survival was determined by counting the total number of dead and live adults.

3.3 Evaluation of the effect of the plant materials and essential oils on the first filial generation

The number of F1 progenies produced were counted on 62, 42 and 42 days after the first introduction of the 10 pairs of the adults of *S. zeamais*, *R. dominica* and *S. cerealella* respectively (section 3.2). These periods were determined based on the life cycle of the insects to avoid overlapping of generation.

3.4 Effect of the three plant materials and essential oil on feeding of the insects (Damage assessment)

On the days the number of F1 progenies were counted, damage assessment was conducted on the treated and untreated seeds. To determine weight loss, 100 seeds were taken and damaged (seeds with a characteristic hole) and undamaged (seeds with no sign of damage) were separated and counted. Average weight of one undamaged kernel was calculated by dividing the total weight of the

undamaged seeds by total number of seeds. Percent weight loss was calculated using the method of FAO (1985).

Percent weight loss was compared with percent number of damaged seeds in case some insects may prefer feeding on larger seeds to small ones.

3.5 Effect of the plant materials and essential oil on germination potential of maize and sorghum seeds.

Maize and sorghum seeds used for germination tests were disinfested in an oven as previously described. The seeds were then treated with the different plant materials and essential oil extracts of the three plants at 3 levels of application and were kept under previously described conditions until the days of F1 progeny count (62 and 42 days for maize and sorghum seeds, respectively). Untreated seeds were also kept under similar conditions as control.

The effects of the different treatments on the viability of the seeds were assessed using the method of the Anonymous (International Seed Testing Association) (1966). Samples of 50 seeds were taken from maize and sorghum seeds treated with the different plant materials and essential oil. The seeds were placed on moist "Whatman No 1" filter paper (11 cm diameter) in plastic petridishes. Maximum humidity was maintained by daily provision of water when necessary. Germination was determined by observing the emergence of the radicle.

Germinated seeds were counted from the second to the fifth day after the start of the experiment.

3.6 To test repellent effect of the three plant materials and essential oils against the three insects

Repellent effects of fresh, dry, ground and essential oils of the three plant species to the three insect pests was conducted with a choice bioassay system. The system consisting of two 1 liter glass jars were connected together at their rims by means of a 34 X 10 cm nylon mesh tube (Fig. 3.1). A 5.0 cm diameter circular hole was cut at the middle of the upper side of the nylon mesh for the introduction of the test insects. 250 g of disinfested maize (*for S.zeamais* and *S. cerealella*) and sorghum (for *R.dominica*) seeds were placed in each of the two 1-liter volume glass jars. The seeds in one of the bottles were treated with the plant materials and essential oil extracts, while the seeds in the other bottle were untreated. 50 adults of *S. zeamais* or *R. dominica*, or 20 adults of *S. cerealella* of mixed age and sexes were released into the nylon mesh tube through the circular hole using a funnel of size 15 cm diameter with stem diameter of 1.5 cm.

After introducing the insects the funnel was removed and the hole on the nylon mesh was sealed with a cellophane tape. Each bottle was covered with a card

board box to maintain dark conditions as the insects demonstrate photonegative responses (Hassanali et al.1990). The middle of the nylon mesh was lighted with a fluorescent lamp to encourage the insects to migrate to either side. After 24 hrs, the number of insects in treated and untreated bottles was counted.

All the bioassays were carried out in a laboratory maintained at 26 ± 5^{o}C and 60 ± 5 rh. Percent repellency was calculated using the method of Hassanali et al. (1990).

Preliminary assays were conducted to compare results obtained with the device with those of "Y" olfactometer using essential oils extracts of the plants. With this device it was possible to test larger quantities of plant materials compared to the classical "Y" olfactometer as well as allowing combined assessments of sources of repellents and attractants in a push and pull situation.

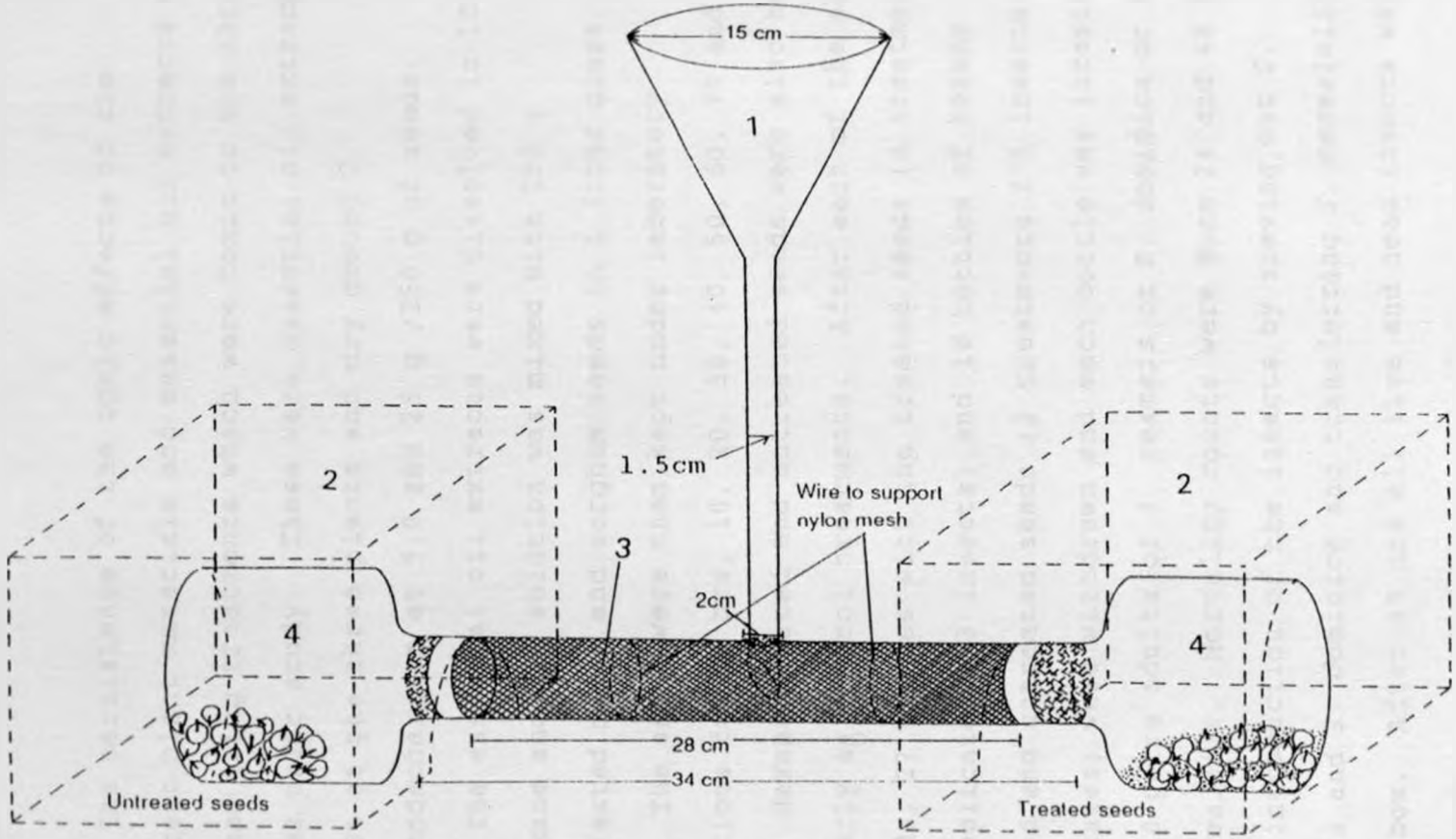


Fig. 3.1 Apparatus used to carry out repellency bioassay. 1, funnel for the introduction of the test insects; 2, card board used to cover the bottles; 3, nylon mesh tube used for the free movement of the insects and 4, glass jars with treated and untreated seeds.

3.7 Testing persistence of the toxic treatments against the three insect

The persistence of the toxic effects of the different plant materials and essential oil extracts was assessed using treatments which were toxic to the three species under study. These were essential oil extracts at 0.3% of the three plants and dry ground *O. kilimandscharicum* at 5.0 and 25 g /250 g of seeds.

The essential oil extracts were dissolved in 10 ml of hexane and the solution was mixed with 250 g disinfested maize and sorghum seeds in 1 liter glass jars. The seeds were then kept under laboratory conditions for 24 hrs, 10, 20, 30, 40, 50, 60, 70 and 80 days. Hexane treated and untreated seeds were also kept similarly as control treatments. After each of the above periods, 27 bottles with the treated seeds (3 treatments X 3 replicates X 3 insects) and 18 bottles of hexane treated and untreated seeds (2 treatments X 3 insects X 3 replicates) were withdrawn and each bottle was infested with 10 pairs adults of *S. zeamais* or *R. dominica* or 20 *S. cerealella*. Mortality counts were done 24 and 48 h after introduction of the insects by sieving out *S. zeamais* and *R. dominica* and transferring *S. cerealella* to glass box. After 48 hrs all live and dead insects were discarded.

Similar series of experiments were done with dry ground *O. kilimandscharicum* plant materials. Infestation and mortality counts were done at each time as described above.

Because treatments with the essential oil of *O. kenyense* and all treatments of *O. kilimandschricum* did not induce any mortality of the insects after 10 days, the experiments with these treatments were repeated and mortality counts were carried out daily instead of every 10 days.

3.8 Large scale evaluation of the effective treatments and pheromone monitoring

This study was carried out to evaluate the effectiveness of some of the plant products for large scale protection of grains against insect infestation.

Ground plant materials of *O. kilimandscharicum* was selected for the study because of its toxicity in previous experiments and potential for application by rural farmers. *S. zeamais* and *R. dominica* which are the major storage insect pests in "Siaya" district where the seeds were purchased and also relatively less susceptible to various treatments in our studies were selected as the target insects.

Sorghum and maize seeds (purchased from around Ugunja town) were disinfested and 25 kg of each type of seed were kept in jute bags, a common method of grain storage by farmers. Based on the result of toxicity test on the two beetles, ground plant materials were applied at the levels 2 and 3 (2% w/w and 10% w/w) against *R. dominica* and *S. zeamais*, respectively. The freshly collected plant materials were allowed to dry in a well ventilated room, under shade. After 4 days, leaves, succulent stems and inflorescence were ground to fine powder with "Janke and Kunkel IKA" laboratory mill. Sorghum and maize seeds were treated with the powder at the rate of 500 g and 2.5 kg per 25 kg of seeds respectively. Each treatment was replicated 3 times. Three replicates each (25 kg per each jute bag) of untreated sorghum and maize seeds were also prepared. After treatment, 50 pairs of 5-8 days old adults of *R. dominica* and *S. zeamais* were introduced into each treated and untreated sorghum and maize seeds respectively, in the jute bag.

After 24 and 48 hours, the live and dead insects were counted by:

1. taking 6 samples of (280 g each) seeds randomly and sieving out adults;
2. trapping adults using 6 pheromone baited corrugated traps;
3 trapping adults by 6 unbaited and pheromone baited grain probe traps.

Sticky surface corrugated card board traps modified

from Burkholder and Ma (1985) and Trematera and Gergenti (1989) were baited with pheromone dispensers and used in trapping adults of *R.dominica*. The card board measured 14 X 2.5 cm. The trapping area was prepared by gluing sticky "Afritape" to the surface with "Pritt glue" and allowing the sticky surface of the tape to face up. Male produced aggregation pheromone dispensers,purchased from AgriScense-BSC LTD, USA, were then placed at the middle of the sticky surface where the corrugations converge. The traps were placed in the bags (2 traps/bag) at the opposite sides of the bag.

For grain probe traps the pheromone dispenser was suspended at the middle of the trap using thin wire attached to the cover of the trap. The number of adults trapped were counted 24 h after the traps were placed in the bags.

3.9 Chemical analysis of the essential oils for the purpose of identifying the toxic constituents.

To identify the chemical components of the essential oil of the three plant species a VG analytical organic mass spectometery (VG12-250) GC-MS equipped with data system and Hewlett Packard 5790 GC was used. Gas chromatography (GC) analyses were performed using Hewlett Packard 5890 A gas chromatograph equipped with splitless injector and flame ionization detector. The identity of the compounds were confirmed by comparison of retention

times and peak enrichment (co-injection) of the authentic compounds with those of essential oil components. Helium and nitrogen gases were used as carrier gases for GC-MS and GC respectively. In both GC-MS and GC analysis, a fused silica capillary column (Hewlett Packard 50m long, 0.22mm id) coated with carbowax (0.32 μm film) was used.

The column temperature programme used in GC-MS was also used in the GC analysis. This included: initial temp. 60^0C for 5 min. and temp. rise to 175^0C at the rate of 5^0/min., a hold at 175^0C for 5 min. and another rise to 220^0C at the rate of 10^0C/min. and final hold at 220^0C for 10 min.

3.10 **Toxicity test of essential oil constituents**

This was conducted using a Watman No. 1 filter paper (9 cm dia.) in pyrex glass petri dishes (10 cm dia.) (Weaver et al. 1991). A sample of each essential oil of the three plants was weighed and dissolved in 1 ml of acetone (Merck 95-97%). The solution was then delivered to the filter paper. Acetone was allowed to evaporate for 20 min. Prior to the addition of the insects. Acetone alone treated filter papers were used as control treatment. 10 pairs of *S. zeamais* and *R. dominica* were introduced into the petri dishes which were then kept in the laboratory (26 ± 1^0c and 60 ± 5% rh) for 24 hr.

The number of dead insects were counted after 24 hr of treatment. Insects were considered dead if they

became immobile and did not react to three probings with a blunt dissecting probe.

The experiment was repeated with blends of synthetic constituents in their proportions in the natural oils with each component subtracted in turn to determine its relative contribution to the overall toxicity of the natural oil (Owaga et al. 1988). Percent composition of each particular compound was determined by gas chromatography. The doses of the compounds were calculated from the minimum doses of the essential oils which gave 100% mortality of each insect (Table 3.2). Finally, for each component which demonstrated toxicity in the subtraction bioassay dose-toxicity tests were undertaken to determine their LD_{50} and LD_{99}.

Table 3.2 List of compounds tested and their respective doses obtained based on their proportion in the essential oils.

1 *O.suave* oil constituents	Dose levels	
	S. zeamais	*R. dominica*
ß-Pinene	3.10	1.10
trans & ß-Ocimene	30.71	10.60
Linalool	2.61	0.92
ß-Cubebene	9.70	3.42
Eugenol	111.00	39.00
trans-Caryophellene	6.11	2.15
Isoeugenol	13.90	4.90
2 *O. kenyense* oil constituents	Dose levels	
	S. zeamais	*R.dominica*
Ethylisovalerate	3.80	1.20
ß-Pinene	3.63	1.16
α-Terpineole	0.60	0.20
1,8-Cineole	46.25	14.80
trans-Caryophellene	1.75	0.56
4-Terpineole	0.80	0.24
Methyl chavicol	16.13	5.16
α-Humulene	3.50	1.12
Isoeugenol	10.63	3.40
Allyl phenol	0.63	0.20

List of compounds contd..

3 *O. kilimands charicum* oil constituents	Dose levels	
	S. zeamais	*R.dominica*
Camphene	4.60	2.60
Limonene	7.30	4.10
1,8-Cineole	7.20	4.01
Camphor	63.40	35.22
Linalool	0.81	0.45
4- Terpineole	2.25	1.25
trans-Caryophellene	2.52	1.40
α-Terpineole	0.99	0.60
Endoborneol	0.84	0.50
Myrtenol	1.26	0.70

Note. Dose levels were obtained by multiplying %area of each constituent, in GC profiles, by the minimum doses of the essential oils used to give 100% mortality) of each insect.

3.11 Evaluating source of toxicity of ground plant materials.

Freshly collected plant materials of *O. suave* and *O. kilimandscharicum* were dried in a well ventilated room (under shade) and ground to fine powder as described in section 3.1.1. The ground plant materials (108 g and 100 g for *O.suave* and *O. kilimandscharicum*, respectively) were steam distilled following the method of Gunter (1946). The rates were determined based on level 3 rate of application of the ground plant materials which produced 100% mortality of *S. zeamais* and *R. dominica*. The essential oil free powder (residue) was then allowed to dry in the lab. When the powder was completely dry it was divided into 4 equal parts for the 4 replications. Each part (about 27 g and 25 g for *O. suave* and *O. kilimandscharicum*, respectively) was mixed with disinfested maize and sorghum seeds. Undistilled ground plant materials were also similarly mixed with the seeds. 10 pairs of 5-8 days old *S. zeamais* and *R. dominica* were then introduced into the treated and untreated seeds. Mortality count was done for 24, 48, 72 and 96 h after treatment.

3.12 Data analysis

In cases of toxicity, survival, reproduction and damage assessment, the experiments were laid out in completely randomized design with three replications, while experiments on germination and repellency were replicated 4 times. The experiments involving assessment of toxicity of essential oil constituents were layed out in completely randomized design with 6 replications. The data obtained were analyzed using analysis of variance following the method of Gomez and Gomez (1984). Appropriate data transformation methods (arc sine and square root) were applied when necessary. Probit analysis for the determination of LC50 and LC99 was based on the methods of Busvine (1971) and Finney (1978).

In case of damage assessment %weight loss was calculated using the method of FAO (1985) of weight loss assessment:

$$\% \text{ weight loss} = \frac{UaN-(U+D)\ X\ 100}{UaN}$$

where:

U = weight of undamaged fraction in a sample

N = total number of grains in a sample

Ua = average weight of one undamaged kernel

D = weight of damaged fraction in the sample.

Percent repellency of the plant materials and essential oils was calculated following the method of

Hassanai et al. (1990) as shown below:

$$\frac{(\text{Cont} - \text{Trt})}{(\text{Cont} + \text{Trt})} \times 100$$

where: cont = control
trt = treated.

When mortality of *S. cerealella* was observed in the untreated seeds (control) 72 h after introduction of the adults into the treated and untreated seeds, Abbot's (1925) formula for correction of control mortality as shown below was used.

$$PT = \frac{Po - Pc}{100 - Pc} \times 100$$

where: PT = %corrected mortality
Po = %observed mortality
Pc = %control mortality

In the case of large scale treatment, the number of adults found in the traps and samples were pooled together and analysis of variance was performed on the arc sine transformed values.

In all cases, the data obtained were analyzed using analysis of variance in Completely Randomized Design (CRD) and mean comparison was done using the (Student-Newman-Keuls) SNK method.

CHAPTER 4

RESULTS

4.1 Effect of *O. suave* on *S. zeamais*, *R. dominica* and *S. cerealella* in terms of toxicity, survival, reproduction, feeding and repellency

4.1.1 Toxicity effect of *O. suave* against the three storage insects

All levels of fresh, dry and ground plant materials of *O. suave* failed to cause any mortality of *S. zeamais*. Essential oil levels 2 and 3, however, caused significant mortality of this weevil. The maximum mortality recorded was 88.35% in grains treated with 0.3% w/w (level 3) of essential oil 96 hours after treatment (Fig.4.1).

Essential oil and ground plant materials of this plant at levels 2 and 3 were toxic to *R. dominica*. 100% mortality was observed in grains treated with 11% w/w (level 3) ground plant materials and 0.3% W/W (level 3) of essential oil after 96 h exposure (Fig.4.1). Like *S. zeamais*, fresh and dry plant materials treatments did not cause any mortality of this grain borer.

S. cerealella was the most susceptible insect to

all treatments (Fig.4.2). Significant rate of mortality was observed in all treatments related to hexane and control treatments (Fig. 4.2). 100% mortality was observed in grains treated with 27 g of ground plant materials (level 3), 0.06 and 0.3% w/w (levels 2 and 3) essential oil after 48 h of exposure (Fig.4.2 b). Similarly, essential oil level 1 and ground plant materials level 2 caused significant ($p < 0.05$) mortality 96 hrs after treatment (Fig.4.2 d). Generally, essential oil treatments were more toxic than the ground plant materials irrespective of dosage applied when observed 96 h after treatment.

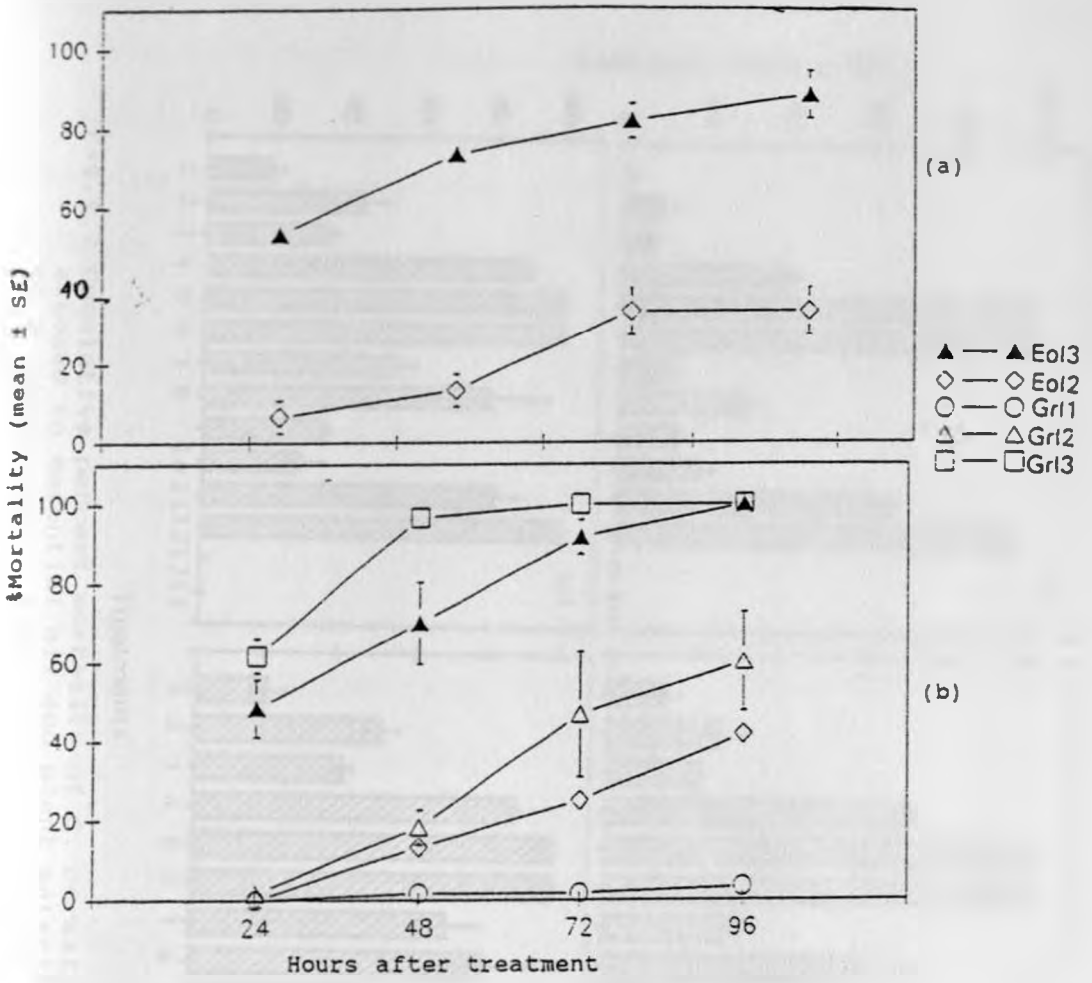


Fig.4.1 Cumulative percent mortality of *S. zeamais* (a) and *R. dominica* (b) exposed to essential oil and ground plant materials of *O. suave* 24, 48, 72 and 96 hrs after treatment. Eol3, essential oil level 3; Eol2, essential oil level 2; Grl1, ground level 1; Grl2, ground level 2; Grl3, ground level 3.

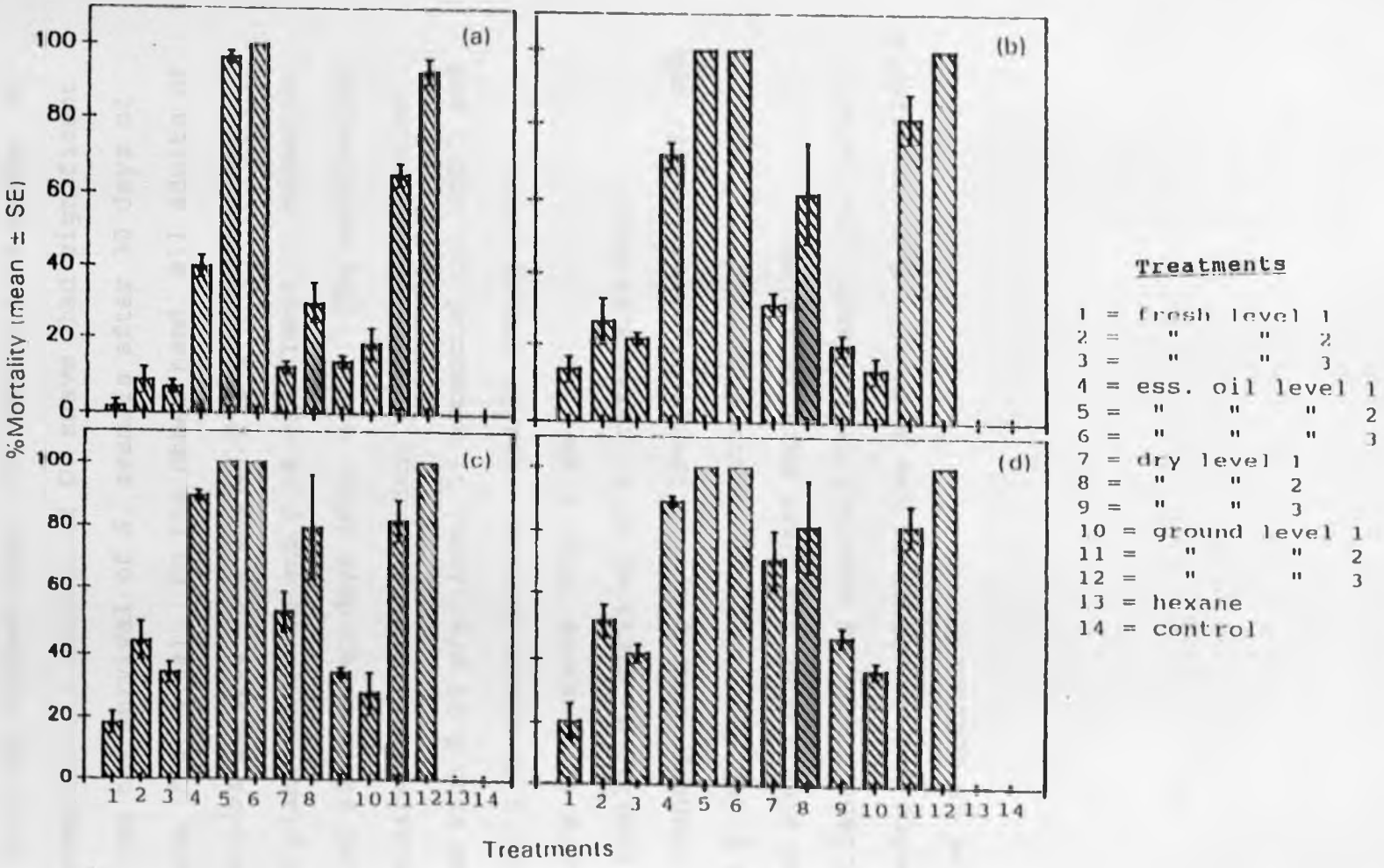
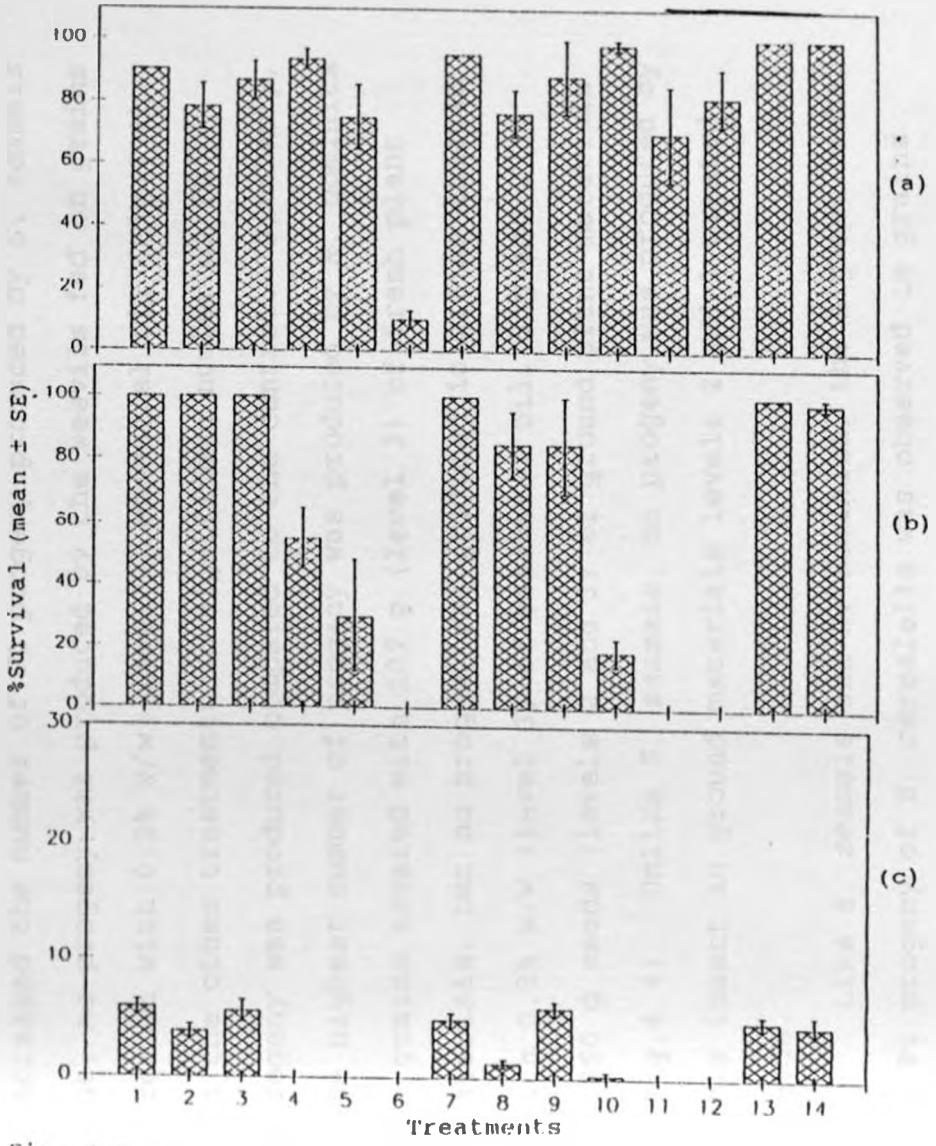


Fig.4.2 Cumulative percent mortality of *S. cerealella* exposed to essential oil and plant materials of *O. suave* 24(a), 48(b), 72(c) and 96(d) h after treatment.

4.1.2 Effect of *O. suave* on the long term survival of the three insect species

Only the highest dose (750 mg/250 g of seeds) of the essential oil extract of *O. suave* had significant effect on the survival of *S. zeamais* after 30 days of exposure (Fig. 4.3a). On the other hand, all adults of *R. dominica* exposed to 5 and 27 g (levels 2 and 3) ground plant materials and 0.3% w/w (level 3) essential oil died after seven days (Fig. 4.3b). The survival of *R. dominica* was also significantly affected in grains treated with 0.3% w/w (level 3) essential oil and 5 and 27 g/250 g of seeds (levels 2 and 3) ground plant materials of *O. suave* (Fig. 4.3b)

Similarly, survival of *S. cerealella* was significantly reduced by all levels of essential oil and ground plant materials treatments (Fig.4.3c). Grains treated with 5 g of dry plant materials also significantly reduced survival of the moth. All other treatments had no effect on the survival of *S. cerealella* relative to control..



Treatments

1 = fresh level 1
2 = " " 2
3 = " " 3
4 = ess. oil level 1
5 = " " " 2
6 = " " " 3
7 = dry level 1
8 = " " 2
9 = " " 3
10 = ground level 1
11 = " " 2
12 = " " 3
13 = hexane
14 = control

Fig. 4.3 Survival of *S. zeamais* (a), *R. dominica* (b) and *S. cerealella* (c) on *O. suave* essential oil and plant materials treated seeds as observed at the end of their optimum oviposition time.

4.1.3 Effect of *O. suave* on the reproduction of the three insect species (F1 progeny production)

Fresh plant materials of *O. suave* applied at 107 g/250 g of seeds (level 3) significantly ($p < 0.05$) increased the number of F1 progeny produced by *S. zeamais* but, no progeny was produced by the weevils fed on grains treated with 0.3% w/w (level 3) essential oil (Fig.4.4). In the other treatments no significant number of F1 progeny was produced compared to the control. Similarly, the highest number of progeny was produced by *R. dominica* in grains treated with 107 g (level 3) of fresh plant materials, but no progeny was produced in grains treated with 0.3% w/w (level 3) of essential oil, 5 g and 27 g/250 g seeds (levels 2 and 3) of ground plant materials (Fig.4.4). Unlike *S. zeamais*, no progeny was produced by this insect in ground materials levels 2 and 3 treated seeds.

Like *S. zeamais* and *R. dominica*, the highest number of F1 progeny of *S. cerealella* was observed in grains treated with 107 g (level 3) of fresh plant materials (Fig.4.4). The number of progeny produced by *S. cerealella* which were fed on seeds treated with either the ground plant material or essential oil extract of *O. suave* at levels 2 and 3 was significantly reduced related to the control. All moths fed on grains treated with

0.06% and 0.3% w/w of essential oil and 5 and 27 g/250 g of seeds (levels 2 and 3) of ground plant materials died after treatment and therefore did not reproduce (Fig. 4.4).

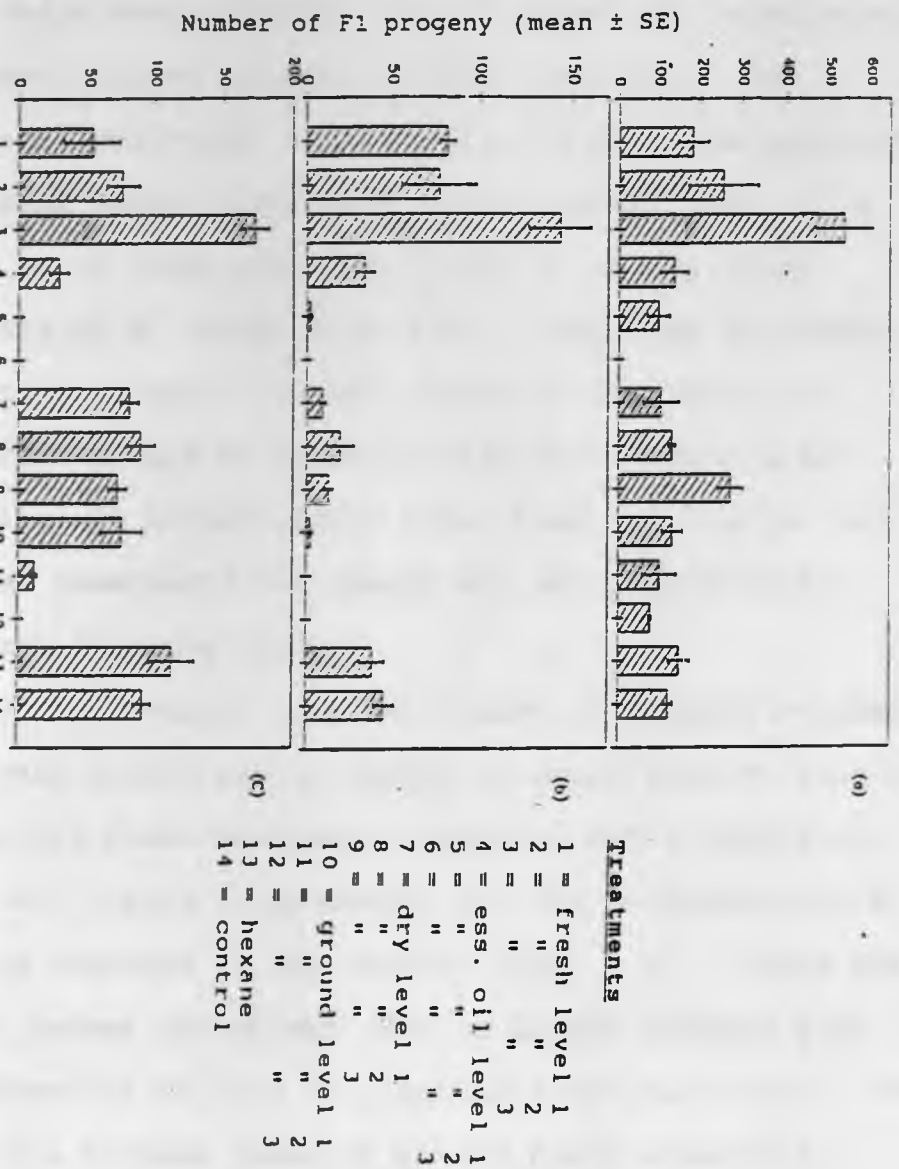


Fig.4.4 Number of F1 progeny produced by *S. zeamais* (a), *R. dominica* (b) and *S. cerealella* (c) fed on seeds treated with *O.suave*.

4.1.4 Effect of *O suave* on the feeding activity of the three insects (weight loss and number of damaged seeds)

Maize seeds treated with *O. suave* and infested with *S. zeamais* showed variable weight loss due to the different treatments. The highest weight loss and number of damaged seeds occurred in seeds treated with 107 g (level 3) of fresh and 27 g (level 3) of dry plant materials of *O. suave* (Fig. 4.5). There was no damage in seeds treated with 0.3% w/w (level 3) essential oil. Similarly, damage of seeds treated with ground plant materials was significantly lower than the control but, in other treatments the damage was not significantly different from the control.

Percent weight loss and number of damaged sorghum seeds were significantly higher in seeds treated with 1 g and 5 g dry plant materials (levels 1 and 2) and with 0.012% w/w (level 1) essential oil due to feeding by *R. dominica* compared to the control (Fig. 4.6). There was no seed damage and weight loss in grains treated with 0.3% essential oil and 27 g ground plant materials. The medium and highest doses of ground plant materials provided adequate protection against damage by *R. dominica*.(Fig. 4.6). The larvae of *S. cerealella* did not cause any damage to maize seeds treated with 0.06%

and 0.3% essential oil (Fig. 4.7). Feeding of this insect, however, was stimulated by the lowest and the medium doses of fresh and dry plant materials. However, the number of damaged seeds and percent weight loss were not correlated in all cases. For example, weight loss in 5 g (level 2) treated seeds was higher than those treated with 1 g (level 1) ground plant materials, but the reverse was observed in the number of damaged seeds.

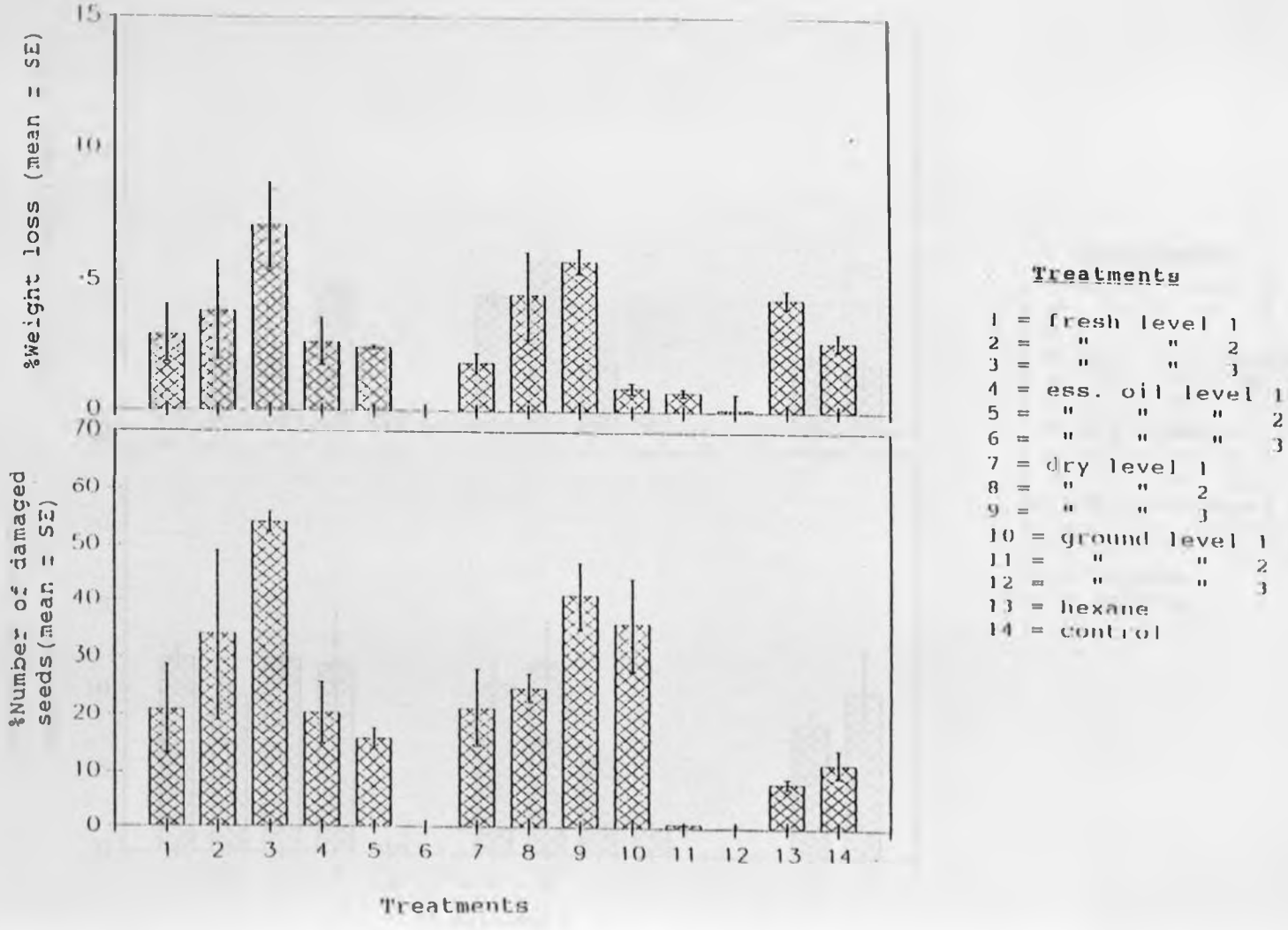


Fig. 4.5 Percent weight loss and percent number of damaged seeds of *O. suave* treated maize seeds due to feeding by *S. zeamais*.

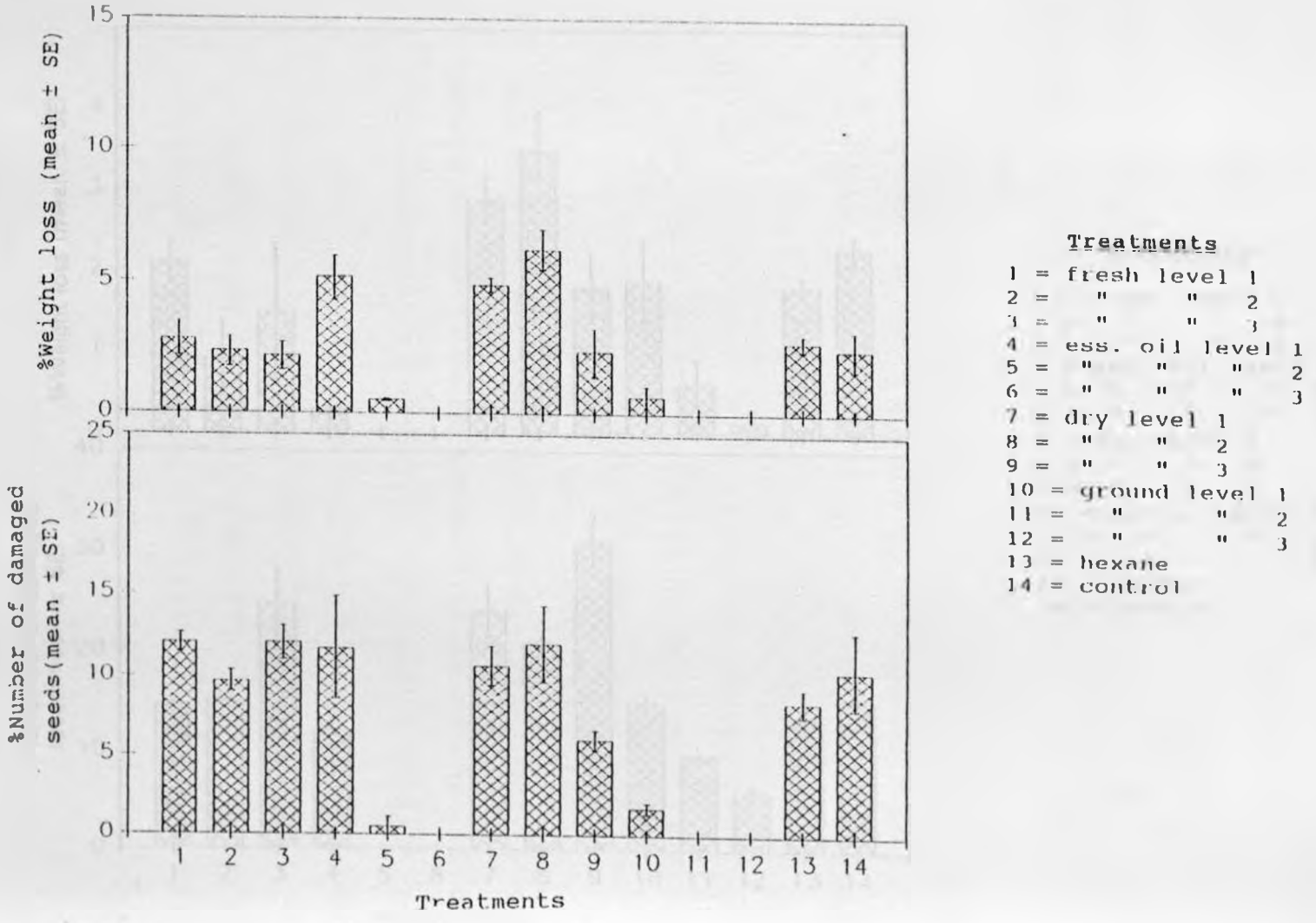
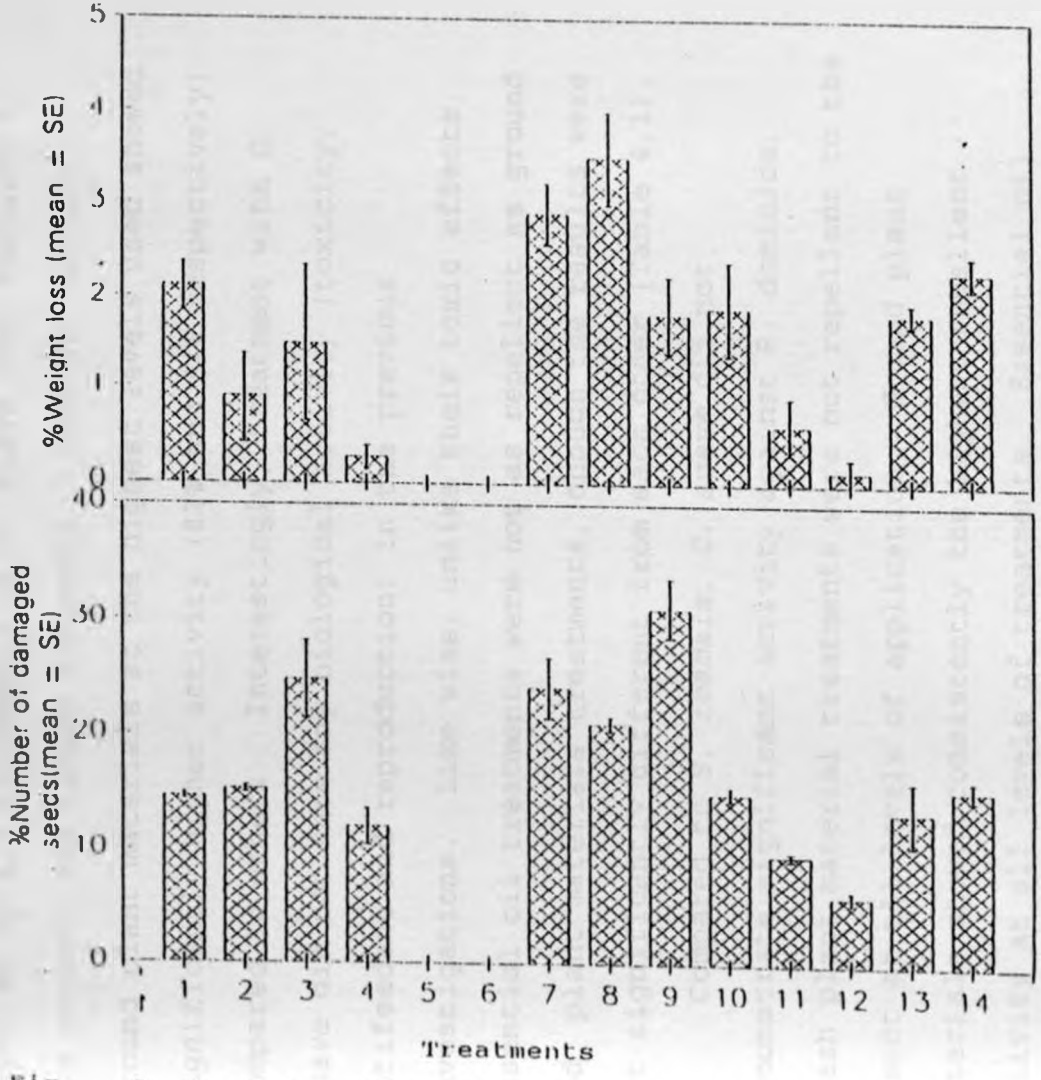


Fig. 4.6 Percent weight loss and percent number of damaged seeds of *O. suave* treated sorghum seeds due to feeding by *R. dominica*.



Treatments

1 = fresh level 1
2 = " " 2
3 = " " 3
4 = ess. oil level 1
5 = " " " 2
6 = " " " 3
7 = dry level 1
8 = " " 2
9 = " " 3
10 = ground level 1
11 = " " 2
12 = " " 3
13 = hexane
14 = control

Fig. 4.7 Percent weight loss and percent number of damaged seeds of *O. suave* treated maize seeds due to feeding by *S. cerealella*

4.1.5 Repellent effect of *O.suave* against the three insects

The maize weevil, *S. zeamais*, was significantly repelled by all treatments of *O. suave* when compared to the control and hexane treatment. However, dry and ground plant materials at the highest levels used showed significantly higher activity (82% and 84% respectively) compared to others. Interestingly, treatment with *O. suave* did not show any biological activity (toxicity, antifeedant and reproduction) in the previous investigations. Like wise, unlike their toxic effects, essential oil treatments were not as repellent as ground and plant materials treatments, though the results were not significantly different from each other (Table 4.1).

Compared to *S. zeamais*, *O. suave* did not demonstrate significant activity against *R. dominica*. Fresh plant material treatments were not repellent to the insect at all levels of application. Ground plant materials showed consistently the highest repellent activity at all levels of treatments. Essential oil treatments which were toxic to the insect were not as repellent. During the observation time some of the test insects were attracted to the essential oil treated seeds and were later found dead showing that toxicity and repellency do not necessarily go together.

The angoumois grain moth (*S. cerealella*) was mildly attracted by all fresh plant materials, dry and ground plant materials level 1 treatments (Table.4.1). Significantly higher and similar repellent activities were obtained with the highest dose (level 3) dry and ground plant materials and the essential oil. Essential oil level 2 repellent activity was also significantly higher compared to the control and hexane treatment. All other treatments gave results which were not significantly different from the controls.

Table 4.1 Repellency (attraction) of *O. suave* against the three storage insect pests

Treatments	Mean %repellency ± SE [1,2] *		
	S.zeamais	*R. dominica*	*S. cerealella*
fresh lev 1	60.0±4.10abc	0.0±0.00c	-48.3±3.21d
fresh lev 2	49.0±5.32c	0.0±0.00c	-49.3±11.21d
fresh lev 3	51.0±2.63bc	0.0±0.00c	-54.3±9.63d
ess.o lev 1	60.0±6.22abc	35.0±5.74a	11.7±9.56c
ess.o lev 2	40.0±4.55c	16.0±5.42ab	43.3±16.65b
ess.o lev 3	69.0±4.57abc	29.0±9.98ab	6.7±9.99a
dry lev 1	66.0±1.30abc	12.0±9.52ab	0.0±0.0c
dry lev 2	62.0±3.00abc	65.0±12.48a	6.7±4.71c
dry lev 3	82.0±2.08a	17.0±10.12ab	78.3±4.19a
grd lev 1	60.0±2.08abc	28.0±13.56ab	0.0±0.00c
grd lev 2	66.0±1.30abc	54.0±20.68a	6.7±4.71c
grd lev 3	84.0±2.58a	59.0±10.75a	78.3±4.19a
hex.	1.0±0.08d	0.0±0.00c	0.0±0.00c
cont	0.0±0.00d	0.5±0.00c	1.0±0.89

[1] Average of 4 replications, each replicate consisting of 60 insects (*S. zeamais & R. dominica*)

[2] Average of 4 replicates, each replicate consisting of 20 insects (*S. cerealella*).

* Means with the same alphabet within the column are not significantly different from each other, $p < 0.05$, SNK.

4.1.6 Toxicity persistence of *O. suave* against the three insect species

The toxicity of *O. suave* essential oil level 3 against *S. zeamais* was effective for 60 days after treatment. Mortality declined to less than 50% 20 days after treatment and afterwards until only 5 % mortality was observed at day 60. Mortality was about 100 % for the first 10 days after treatment (Table 4.2).

Similar effect was observed in the case of *R. dominica*. Mortality rates were significantly higher at day 10 and started to decline 20 days after treatment. The toxic effect of the oil was not significantly different from the control (which was zero) 60 days after treatment and there after.

The angoumois grain moth was the most susceptible insect compared to the other two. Mortality of the insect was significantly high (above 50 %) until 50 days. The toxic effect of the oil against *S. cerealella* was significant 80 days after treatment though the effect was diminishingly small (Table 4.2).

In all cases, no mortality was observed in untreated (control) and hexane treated seeds.

Table 4.2 Residual toxicity of *O. suave* essential oil level 3 against the three storage insect pests

Days after treatment	% mean mortality ± SE [1*]		
	S. zeamais	*R. dominica*	*cerealella*
1	100.0±0.00a	100.0±0.00a	100.0±0.00a
10	98.3±0.96a	88.3±1.96a	100.0±0.00a
20	23.3±3.47b	48.3±3.85b	98.3±0.96a
30	15.0±0.00bc	30.0±3.30c	100.0±0.00a
40	16.7±3.85bc	23.3±2.55c	75.0±1.67b
50	8.3±0.96cd	6.7±1.92d	70.0±2.89b
60	5.0±0.00de	1.3±0.33de	35.0±1.67c
70	0.0±0.00e	0.0±0.00e	18.3±1.67d
80	----------	---------	8.3±1.67e
90	----------	---------	0.0±0.00ef

1 Average of three replicates, each consisting of 10 pairs of adult insects.

*. Means with the same letter within the column are not significantly different from each other, SNK, $P < 0.05$.

4.2 Effect of *O. kenyense* on *S. zeamais*, *R. dominica* and *S. cerealella* in terms of toxicity, survival, reproduction, feeding and repellency

4.2.1 Toxicity of *O.kenyense* against the three insect pests

Grains treated with *O. kenyense* were found to be the least toxic to all the three insects, particularly to *S. zeamais*. The maximum mortality recorded for *S. zeamais* was 30% in grains treated with 0.3% w/w (level 3) essential oil 96 h after treatment (Fig.4.8). Very low mortality of the weevil was observed with essential oil level 2 (0.06% w/w) and level 1 (0.012 w/w). None of the other treatments caused any mortality to *S. zeamais*.

Similar effects were obtained for *R. dominica* although this insect was more susceptible to the highest dose of the essential oil which killed 96.33% of the borer after 96 h of exposure (Fig.4.8). Fresh and dry plant materials and succulent plant parts of *O. kenyense* were not toxic to *R. dominica* but, essential oil at levels 1 and 2 and ground plant materials at level 3 showed very low toxicity effects (Fig.4.8).

Unlike *S. zeamais* and *R. dominica*, all the plant materials and essential oil of *O. kenyense* were toxic to

S. cerealella (Fig.4.9). Significantly higher mortality ($p < 0.05$) was observed in treatments invclving essential oil levels 2 and 3 and ground plant materials level 3, 24 and 48 h after treatment. On the other hand, fresh plant materials were less toxic than other treatments at all levels of application. Ground and dry plant materials at level 2 were also toxic to the insect as observed 96 h after treatment (Fig. 4.9).

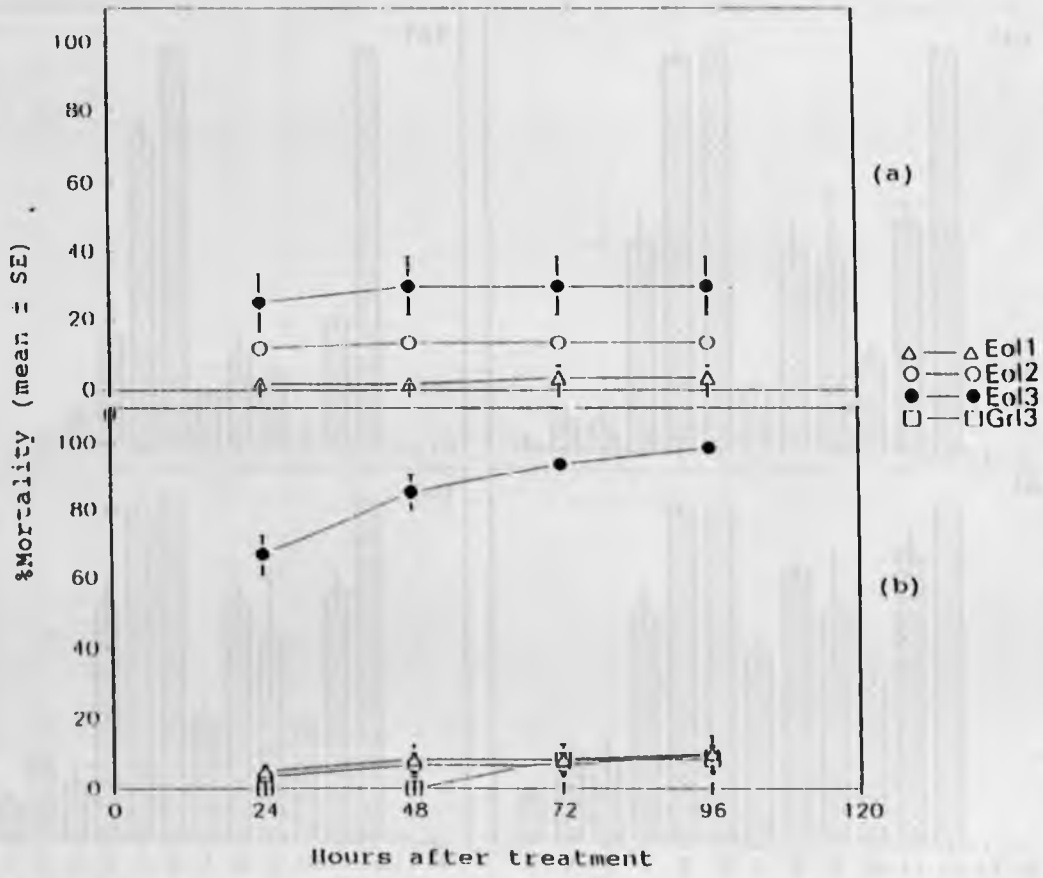


Fig.4.8 Cumulative percent mortality of *S. zeamais* (a) and *R. dominica* (b) exposed to essential oil and ground plant materials of *O. kenyense* after 24, 48, 72 and 96 hrs after treatment. Eol1, essential oil level 1; Eol2, essential oil level 2; Eol3, essential oil level 3; Gr13, ground level 3.

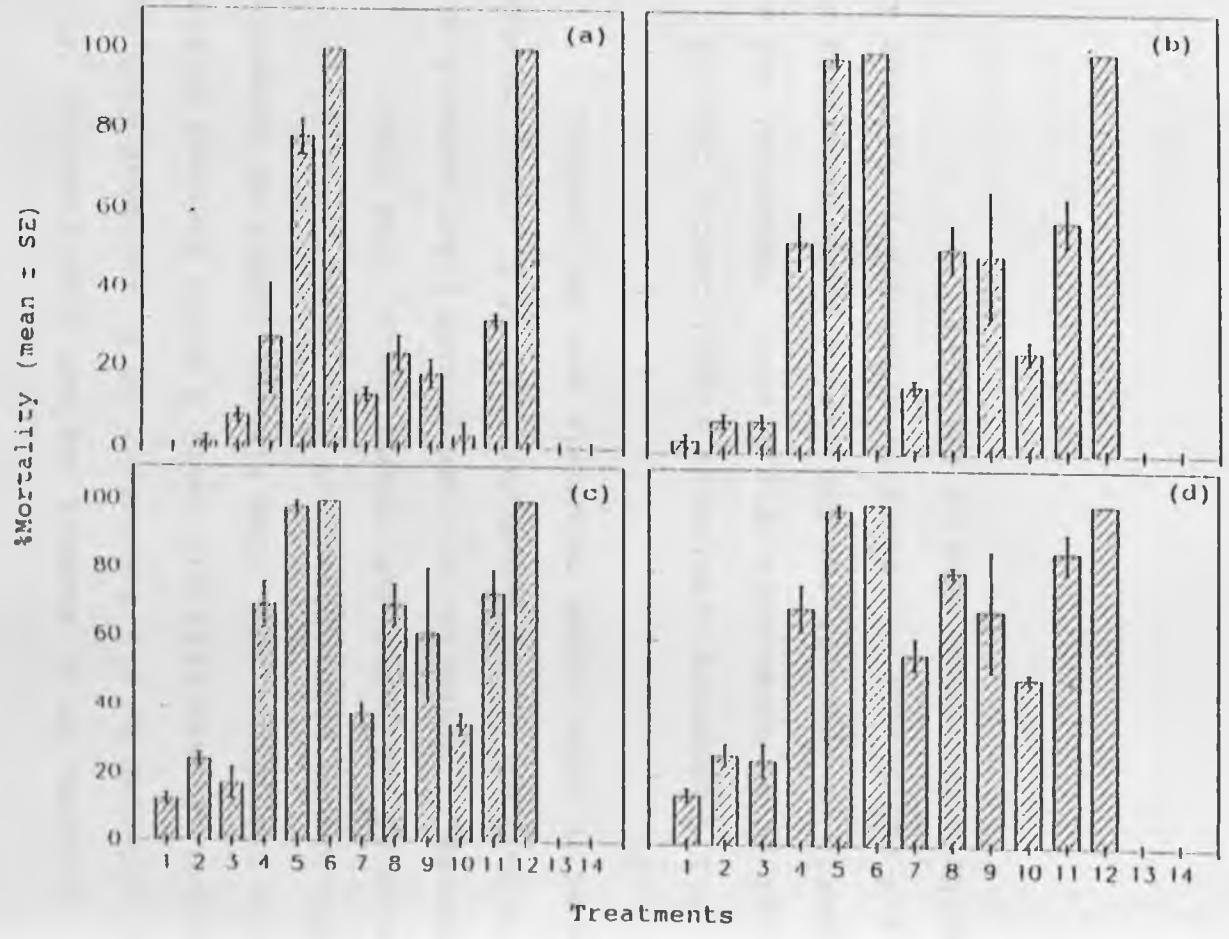
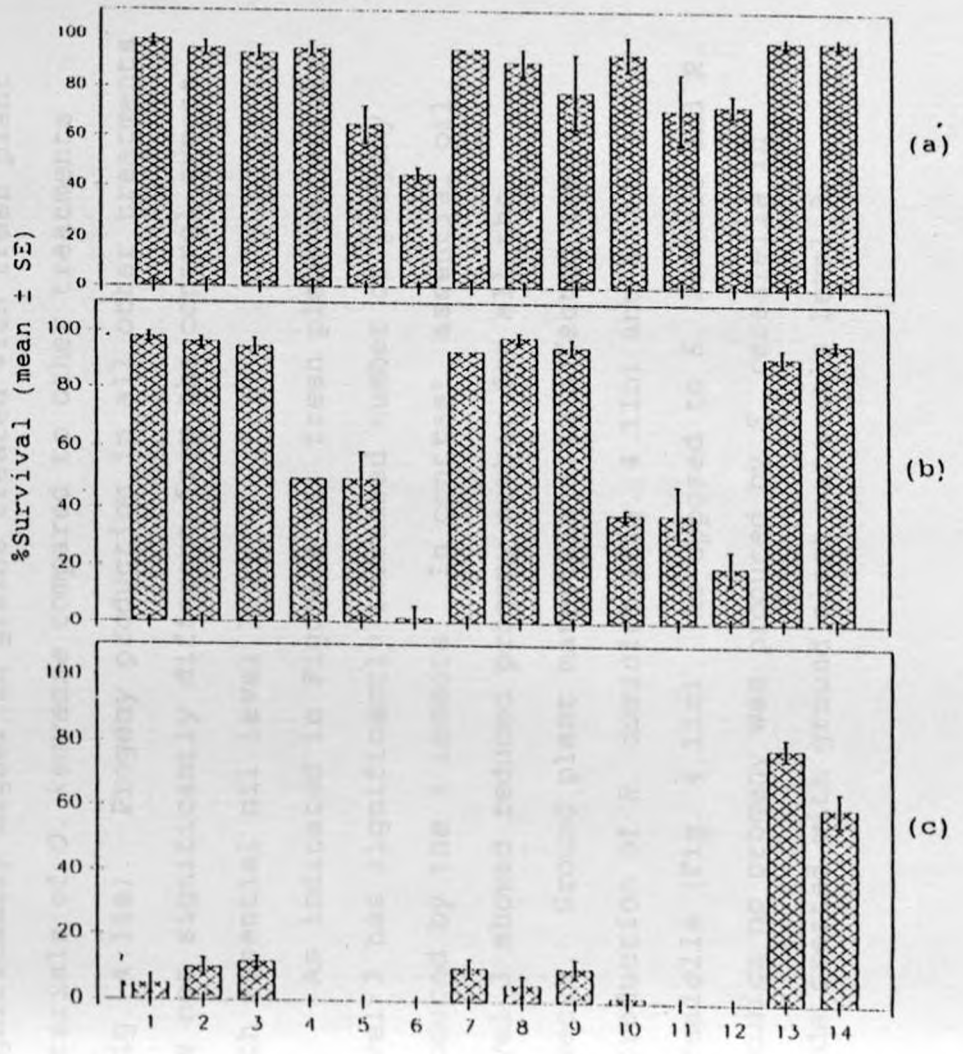


Fig.4.9 Cumulative percent mortality of *S. cerealella* exposed to essential oil and plant materials of *O. kenyense* 24(a), 48(b), 72(c) and 96(d) h after treatment.

4.2.2 Effect of *O. kenyense* on the long term survival of the three insects

Survival of *S. zeamais* was not significantly ($P < 0.05$) affected by all treatments of *O. kenyense* with the exception of essential oil level 3 which reduced survival rate to 45% (Fig.4.10). The highest dosage of essential oil and ground plant materials reduced survival of *R. dominica* to 1.67% and 20% respectively. The other treatments of essential oil and ground plant materials also significantly affected survival of *R. dominica* when compared to other plant materials and the control (Fig.4.10).

All *O.kenyense* treatments significantly reduced survival of *S. cerealella* (Fig.4.10c). Essential oil at all levels of applications and ground plant materials at levels 2 and 3 were particularly effective in killing all the moths after seven days of exposure.



Trreatments

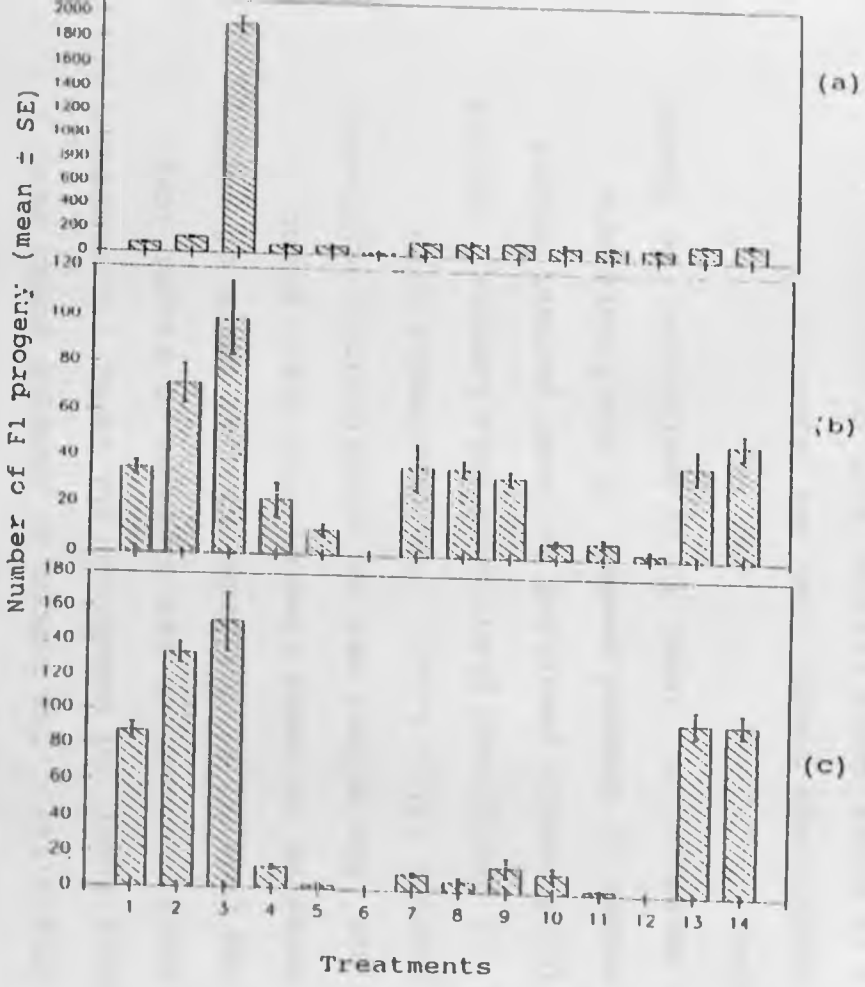
1 = fresh level 1
2 = " " 2
3 = " " 3
4 = ess. oil level 1
5 = " " " 2
6 = " " " 3
7 = dry level 1
8 = " " 2
9 = " " 3
10 = ground level 1
11 = " " 2
12 = " " 3
13 = hexane
14 = control

Fig. 4.10 Survival of *S. zeamais* (a), *R. dominica* (b) *S. cerealella* (c) on *O. kenyense* essential oil and plant materials treated seeds as observed at the end of their optimum oviposition time.

4.2.3 Effect of *O. kenyense* on the reproduction of the three insects (F1 progeny)

The number of F1 progeny produced by *S. zeamais* was significantly higher in grains treated with fresh plant materials of *O. kenyense* compared to other treatments (Fig. 4.11a). Progeny production in all other treatments was not significantly different from the control except with essential oil level 3.

As indicated in Figure 4.11, fresh plant materials level 3 has significantly increased number of progeny produced by the 3 insects. In contrast, essential oil level 3 showed reduced progeny number for all the insects. Ground plant materials also affected the reproduction of *R. dominica* (Fig. 4.11b) and *S cerealella* (Fig. 4.11c). As opposed to *S. zeamais* and *R. dominica* no progeny was produced by *S. cerealella* in seeds treated with ground plant materials level 3.



Treatments

1 = fresh level 1
2 = " " 2
3 = " " 3
4 = ess. oil level 1
5 = " " " 2
6 = " " " 3
7 = dry level 1
8 = " " 2
9 = " " 3
10 = ground level 1
11 = " " 2
12 = " " 3
13 = hexane
14 = control

Fig.4.11 Number of F1 progeny produced by *S. zeamais* (a), *R. dominica* (b) and *S. cerealella* (c) fed on seeds treated with *O.kenyense*.

4.2.4 Effect of *O. kenyense* on the feeding activity of the three insects

S. zeamais caused significantly higher percent weight loss and number of damaged seeds when fed on maize seeds treated with 150 g fresh plant materials of *O. kenyense* (Fig.4.12). The number of damaged seeds were significantly fewer for essential oil level 3 treatment but, no significant effect was observed on weight loss.

In the case of *R. dominica*, similar trend of weight loss was observed in seeds treated with fresh plant materials but, the effect was not significantly different from the control (Fig. 4.13). The treatments with essential oil and ground plant materials however, reduced feeding significantly resulting in lower percent weight loss and number of damaged seeds. In the treatments involving essential oil level 3, in particular, no damage was observed.

No significant weight loss was caused by *S. cerealella* to maize seeds treated with fresh plant materials compared to the control. However, the number of damaged seeds were significantly higher in seeds treated with 150 g of fresh plant materials. Ground plant materials and essential oil treatments reduced weight loss of the seeds (Fig.4.14). No weight loss or

seed damage was observed in seeds treated with 0.3% essential oil (Fig. 4.14).

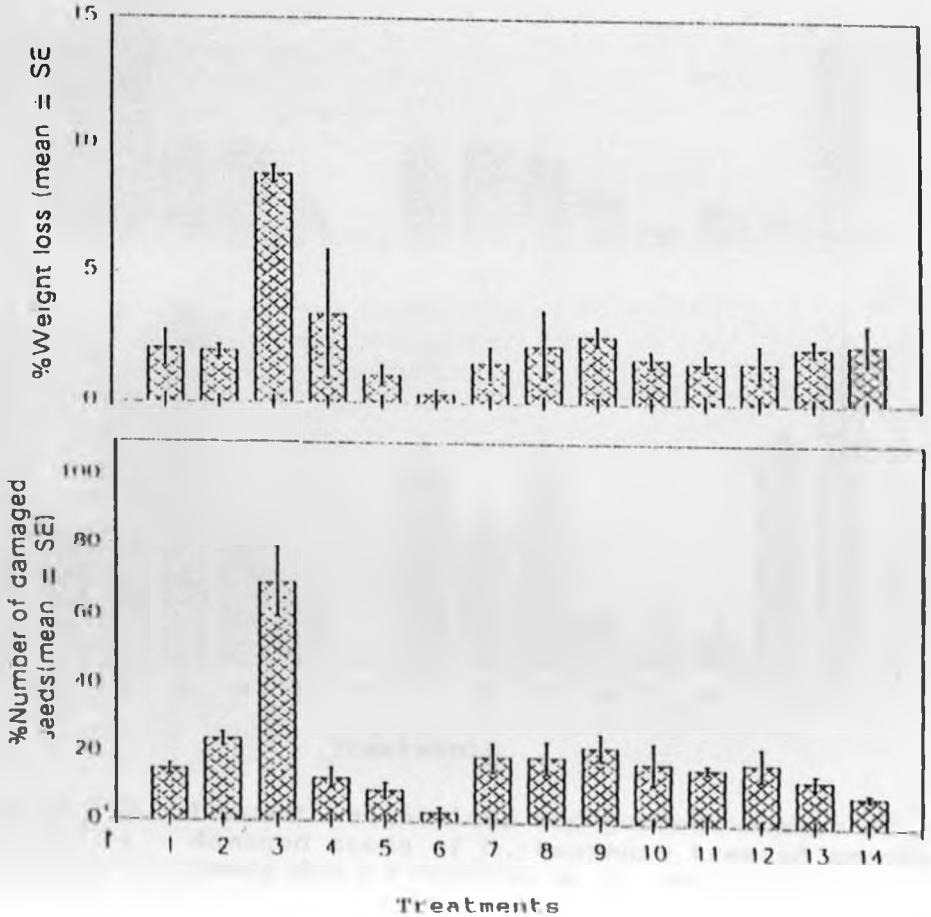
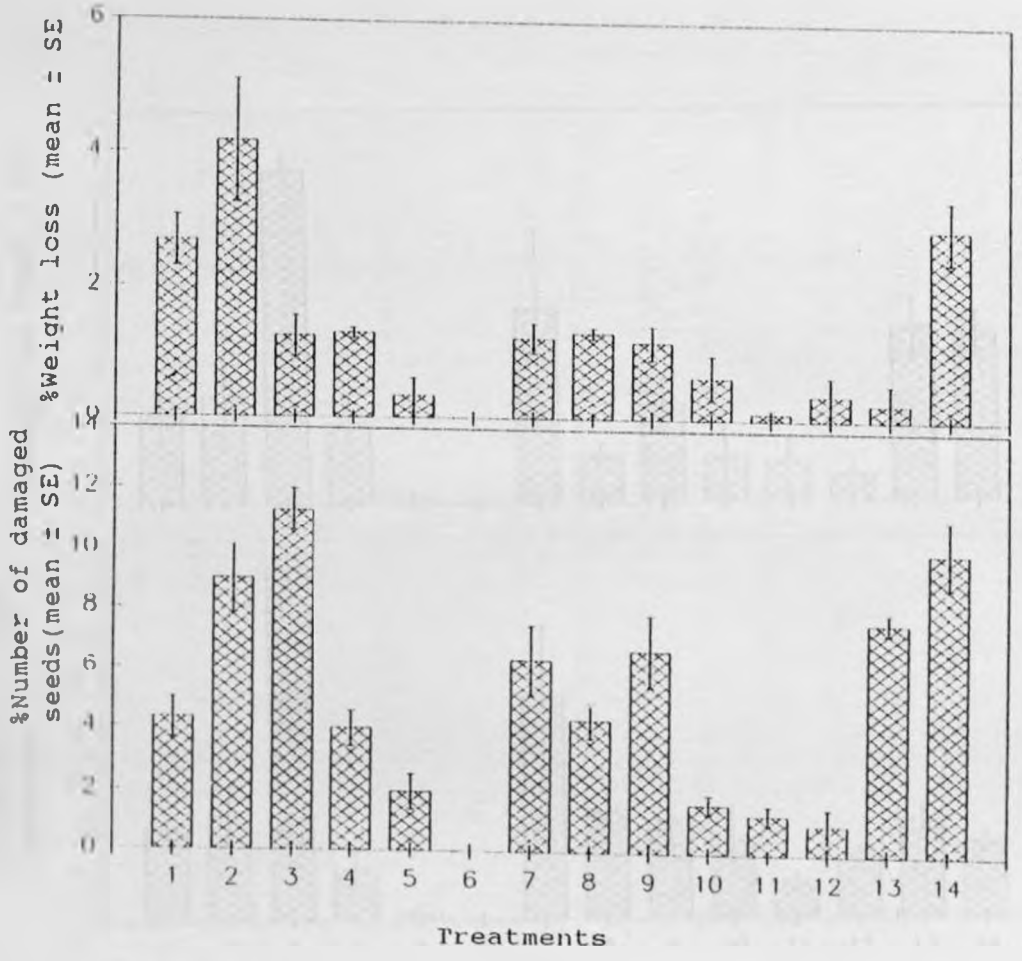


Fig. 4.12 Percent weight loss and percent number of damaged seeds of *O. kenyense* treated sorghum seeds due to feeding by *S. zeamais*.



Treatments

1 = fresh level 1
2 = " " 2
3 = " " 3
4 = ess. oil level 1
5 = " " " 2
6 = " " " 3
7 = dry level 1
8 = " " 2
9 = " " 3
10 = ground level 1
11 = " " 2
12 = " " 3
13 = hexane
14 = control

Fig. 4.13 Percent weight loss and percent number of damaged seeds of *O. kenyense* treated sorghum seeds due to feeding by *R. dominica*.

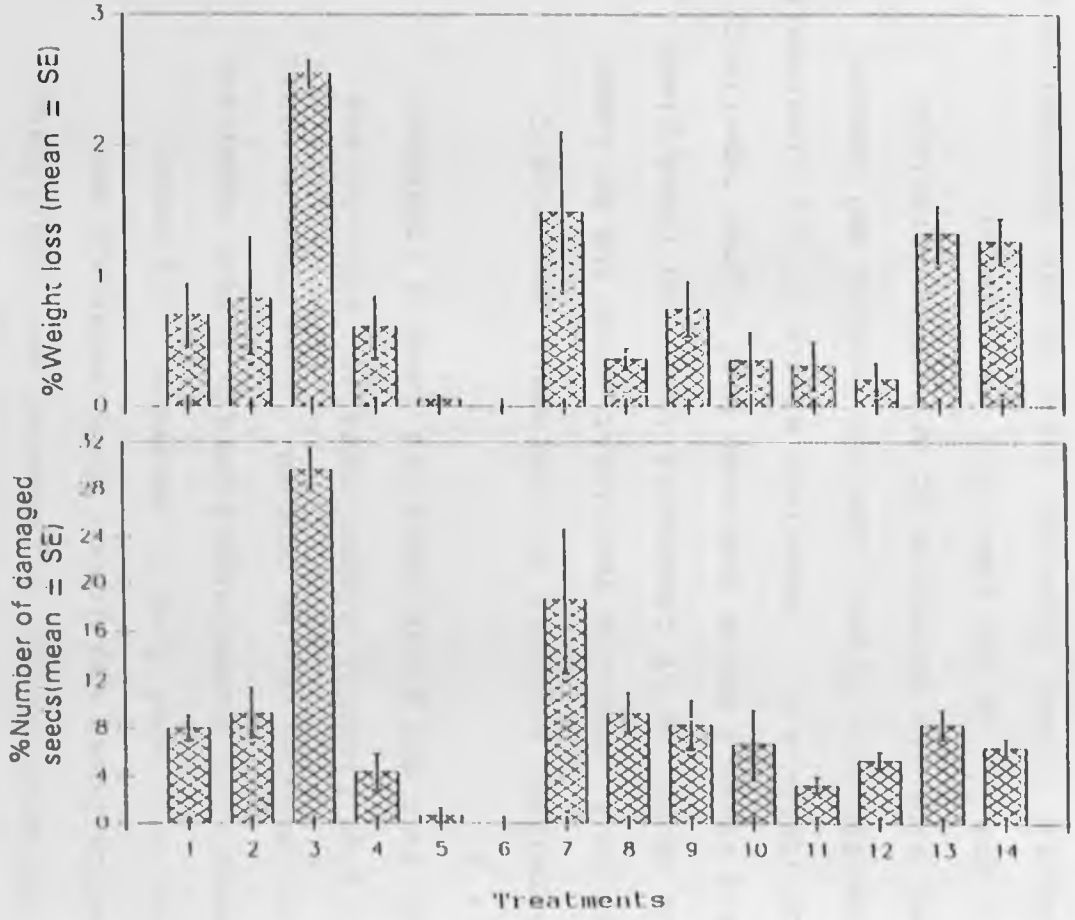


Fig. 4.14 Percent weight loss and percent number of damaged seeds of *O. kenyense* treated maize seeds due to feeding by *S. cerealella*.

4.2.5 Repellency of *O. kenyense* to the three insect species

The maize weevil (*S. zeamais*) was significantly repelled by fresh and ground plant materials and essential oil level 3 of *O. kenyense*. All other treatments also showed significantly higher repellent activity compared to the control. Like the two other plants, significantly higher repellent activity was obtained with dry plant materials level 3 treatment (Table 4.3).

Repellent activity of treatments involving *O. kenyense* to *R. dominica* was either about 50% or lower with the exception of treatments involving fresh plant materials level 3 which gave some what higher repellency activity (Table 4.3). Repellent effect of all treatments were significantly higher than the control and hexane treatment with the exception of dry plant materials levels 1 and 2. In the case of *S. cerealella*, significantly higher repellent activity was obtained only with fresh plant materials levels 1 and 3 (Table 4.3).

Table 4.3 Repellency of *O. kenyense* against the three storage insect pests

Treatments	Mean % repellency ± SE[1, 2, *]		
	S.zeamais	*R. dominica*	*S.cerealella*
fresh lev 1	48.0±4.32c	51.0±6.19a	45.0±1.29ab
fresh lev 2	66.0±2.63b	36.0±7.48ab	22.5±2.06bc
fresh lev 3	74.0±2.62a	65.0±8.69a	57.5±1.26a
ess.o lev 1	56.0±6.71c	21.0±7.00ab	10.0±0.82c
ess.o lev 2	58.0±8.54c	18.0±4.16ab	20.0±1.83bc
ess.o lev 3	73.0±5.74b	50.0±9.02a	27.5±1.50bc
dry lev 1	22.0±2.52de	4.0±4.00bc	15.0±1.29c
dry lev 2	34.0±5.21d	3.0±1.19bc	25.0±1.29bc
dry lev 3	65.0±6.83b	25.0±11.59ab	5.0±1.29c
grd lev 1	42.0±8.22c	39.0±14.82a	10.0±0.82c
grd lev 2	55.0±5.74c	35.0±17.08ab	22.5±1.50bc
grd lev 3	73.0±5.22b	51.0±6.61a	22.5±2.22bc
hex.	0.0±0.00e	0.0±0.00c	0.0±0.00c
cont	0.0±0.00e	0.0±0.00c	1.0±0.89c

[1] Average of 4 replications, each replicate consisting of 60 insects (*S. zeamais* & *R. dominica*)

[2] Average of 4 replicates, each replicate consisting of 20 insects (*S. cerealella*).

* Means with the same letter within the column are not significantly different from each other, $p < 0.05$, SNK.

4.2.6 Toxicity persistence of *O. kenyense* against the three insects

The essential oil of *O. kenyense* at level 3 (750 mg per 250 gms of seeds) was not toxic at day 10 after treatment to all insects. Compared to the case of *O. suave*, that of this plant was much less toxic to *S. zeamais* even 24 hrs after treatment and the effect did not persist for more than 3 days (Table 4.4).

A similar effects of the oil was observed in the case of *R. dominica*. However, unlike the above 2 insects, 100 % mortality of *S. cerealella* was observed 24 hrs after treatment. This effect declined rapidly until no significant mortality was observed at day 4 after treatment (Table 4.4).

Table 4.4 Residual toxicity of *O. kenyense* essential oil level 3 against the three storage insect pests

Days after treatment	% mean mortality ± SE[1] *		
	S. zeamais	*R. dominica*	*S. cerealella*
1	66.3 ± 2.77a	70.0 ± 2.70a	100.0 ± 0.00a
2	16.3 ± 1.57b	12.5 ± 0.72b	50.0 ± 1.02b
3	5.0 ± 0.57c	6.0 ± 0.50c	17.8 ± 1.20c
4	0.0 ± 0.00cd	0.0 ± 0.00cd	3.3 ± 2.88d
5	-	-	0.0 ± 0.00d

1 Average of three replicates, each consisting of 10 pairs of adult insects.

*. Means with the same letter within the column are not significantly different from each other; SNK $P < 0.05$.

4.3 Effect of *O. kilimandscharicum* on *S. zeamais*, *R. dominica* and *S. cerealella* in terms of toxicity, survival, reproduction, feeding and repellency

4.3.1 Toxicity of *O. kilimandscharicum* against the three insects

Fresh and dry plant materials of *O. kilimandscharicum* were not toxic to *S. zeamais*. However, ground plant materials and essential oil treatments caused significantly higher mortality of *S. zeamais* 24 h after treatment. Grains treated with 0.3% w/w (level 3) essential oil caused 100% mortality after 48 h of exposure, while levels 1 and 2 were not as effective (Fig. 4.15). Ground plant materials were equally effective with the highest level of application inducing 100% mortality of the insect after 72 h of treatment. Similarly, grains treated with ground plant materials levels 1 (5 g/250 g of seeds) and 2 (25 g/250 g of seeds) caused significant mortality of the weevil. The essential oil and the ground plant materials of *O. kilimandscharicum* treatments were more toxic against *S. zeamais* compared with those of *O. suave* and *O. kenyense*.

The highest dosage of essential oil and ground plant materials of *O. kilimandscharicum* caused 100% mortality of *R. dominica* 48 h after treatment (Fig.4.15). Ground plant materials level 2 treatment was more toxic

than essential oil level 2 treatment. The lowest dosages of essential oil and ground plant materials were, however, less toxic to the insect indicating a dose dependent relationship.

The angoumois grain moth (*S. cerealella*) was susceptible to all levels of ground and essential oil treatments. All levels of ground plant materials induced 100% mortality 48 h after treatments, while such rate was observed only at level 3 of essential oil treatment (Fig.4.16). Fresh plant materials level 3 was also equally effective as the oil and ground plant materials. Dry plant materials did not cause any mortality of the insect 24 h after treatment but, killed some of the moths 72 and 96 hrs after treatment (Fig.4.16 c, d).

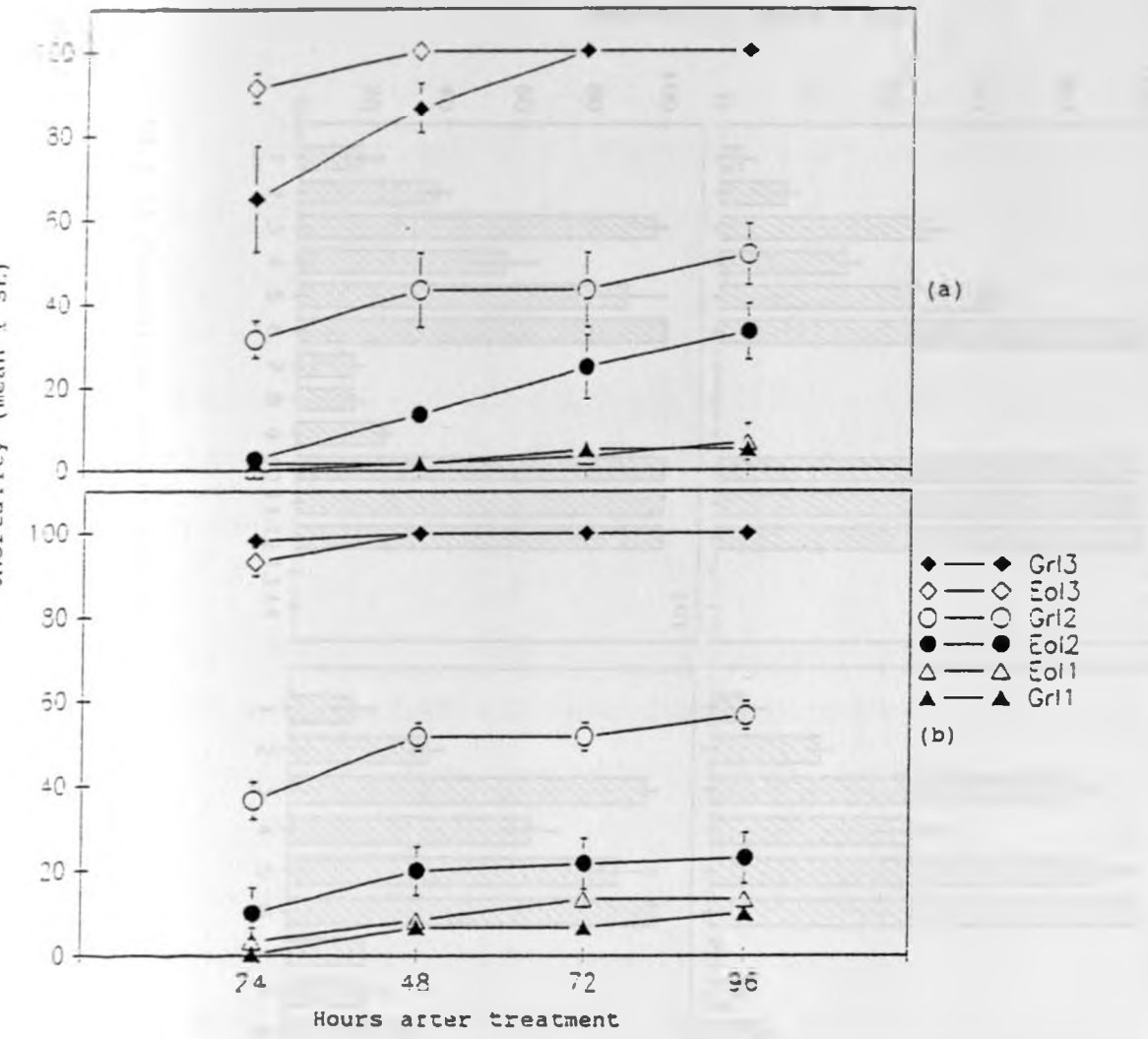


Fig.4.15 Cumulative percent mortality of *S. zeamais* (a) and *R. dominica* (b) exposed to essential oil and ground plant materials of *O. Kilimandscharicum* after 24, 48, 72 and 96 hrs of treatment. Eo11, essential oil level 1; Eo12, essential oil level 2; Eo13, essential oil level 3; Gr11, ground level 1; Gr12, ground level 2; Gr13, ground level 3.

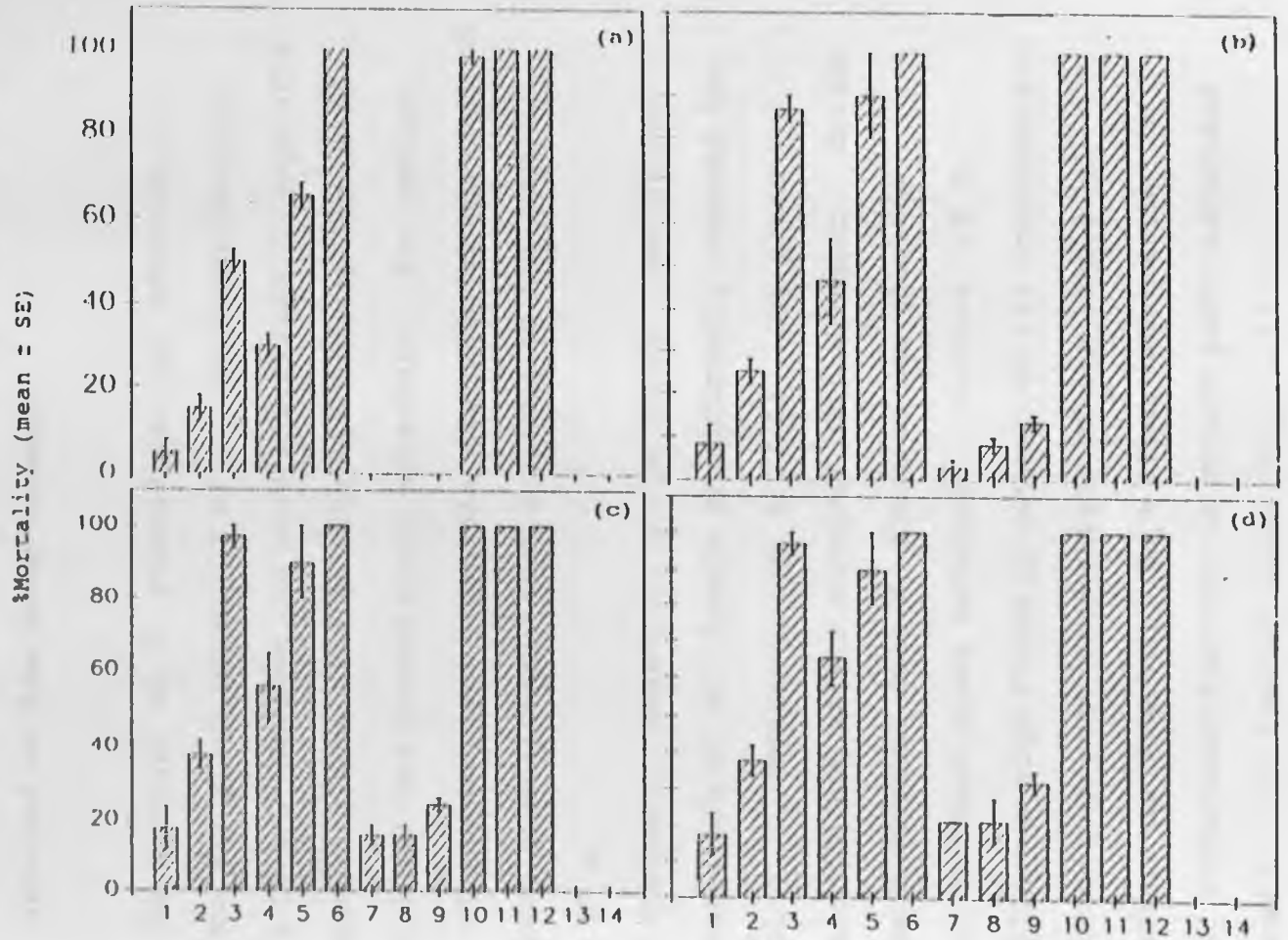


Fig.4.16 Cumulative percent mortality of *S. cerealella* exposed to essential oil and plant materials of *O. kilimandscharicum* 24(a), 48(b), 72(c) and 96(d) h after treatment.

4.3.2 Effect of *O. kilimandscharicum* on the long term survival of the three insects

The survival of *S. zeamais* was not affected by fresh and dry plant materials of *O. kilimandscharicum* (Fig. 4.17 a). However, no weevil survived 30 days after treatment in grains treated with the highest dosage of essential oil and ground plant materials. The medium dosage of ground plant materials also reduced survival of the insect significantly when compared to the control (Fig.4.17 a).

Similarly, essential oil at level 3 and ground plant materials at all levels significantly reduced the survival of *R. dominica* (Fig. 4.17 b). Dry plant materials did not affect survival of the insect. Unlike *S. zeamais* survival rate *R. dominica* was slightly affected by fresh plant materials. Survival of *S. cerealella* was significantly reduced by all treatments of *O. kilimandscharicum* irrespective of the levels of applications as observed 7 days after treatments. No adult *S. cerealella* survived on ground plant materials and essential oil treated seeds (Fig. 4.17 c).

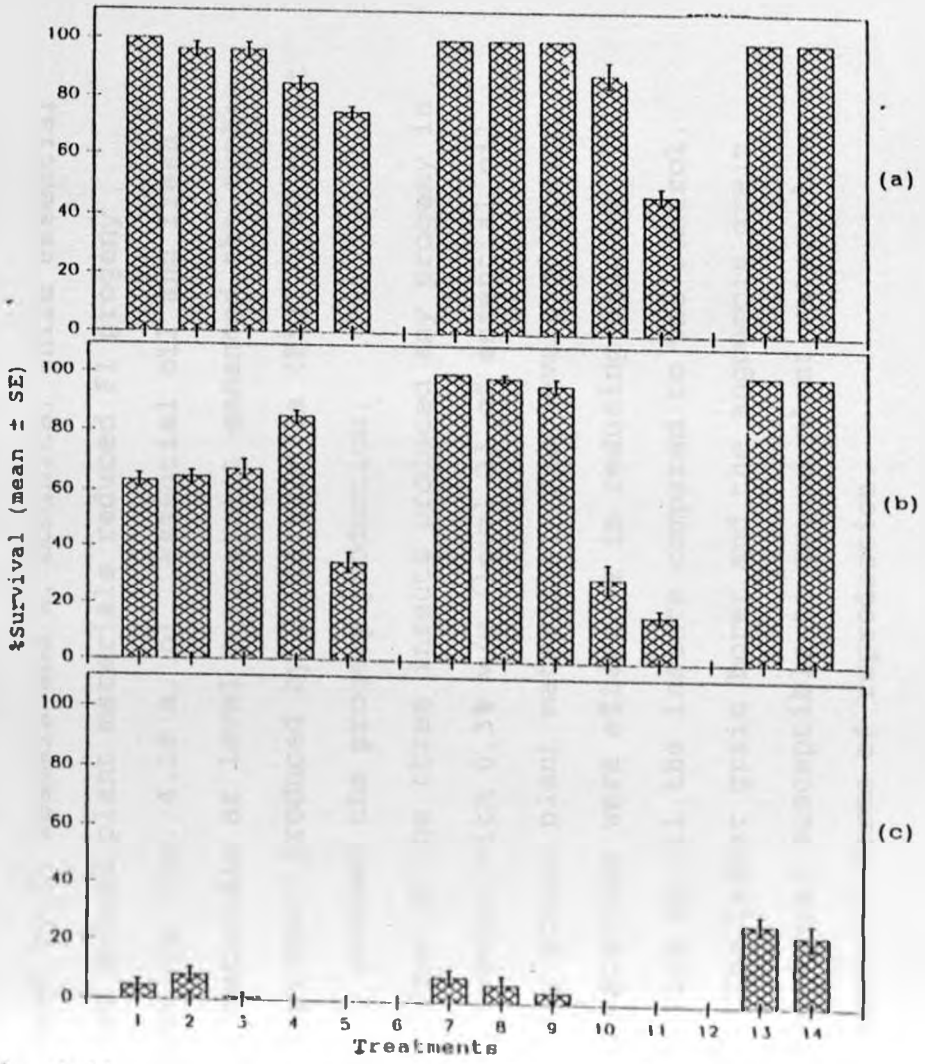


Fig. 4.17 Survival of *S. zeamais* (a), *R. dominica* (b) and *S. cerealella* (c) on *O. kilimandscharicum* essential oil and plant materials treated seeds as observed at the end of their optimum oviposition time.

4.3.3 Effect of *O. kilimandscharicum* on the reproduction of the three insects (f1 progeny)

Fresh and dry plant materials of *O. kilimandscharicum* increased the number of F1 progeny produced by *S. zeamais* and *R. dominica*, while essential oil and ground plant materials reduced F1 progeny production (Fig. 4.18 a, b). Essential oil and fresh plant materials at level 1 treatment enhanced the number of F1 progeny produced by *S. cerealella* (Fig. 4.18 c) but level 3 reduced the progeny production.

None of the three insects produced any progeny in seeds treated with 0.3% w/w (level 3) of essential oil and 25 g ground plant materials. All levels of ground plant materials were effective in reducing progeny production by all the insects compared to the control.

The lesser grain borer and the angoumois grain moth were more susceptible to ground plant materials treatments in terms of reproduction.

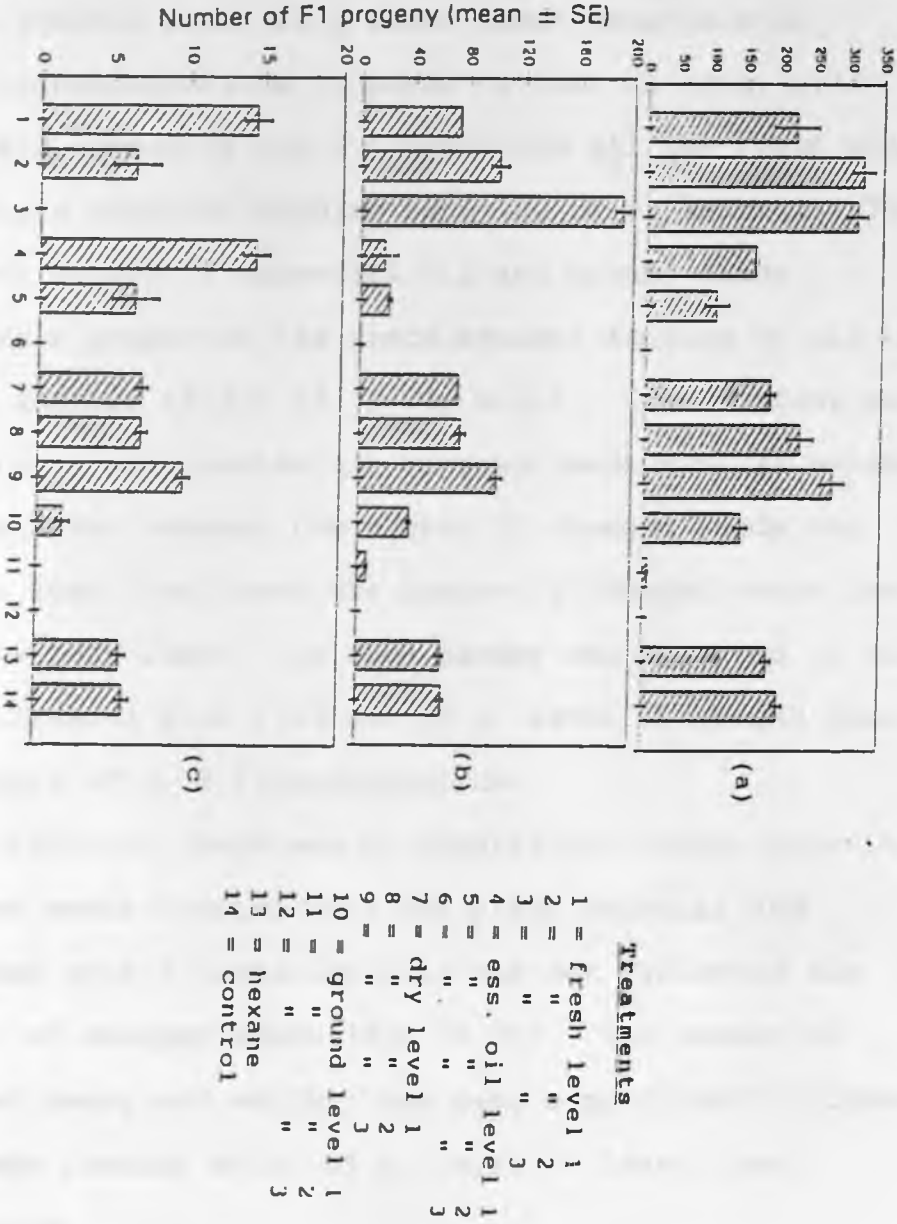


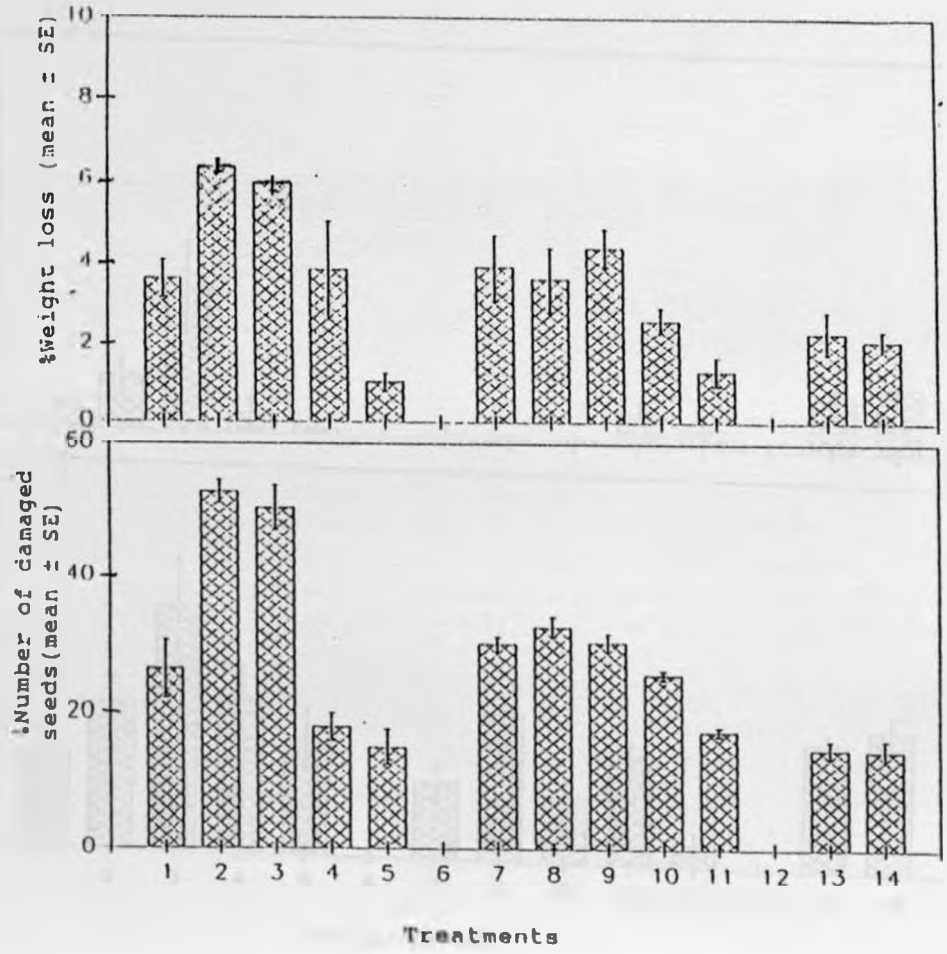
Fig. 4.18 Number of F1 progeny produced by *S. zeamais* (a), *R. dominica* (b) and *S. cerealella* (c) fed on seeds treated with *O. kilimandscharicum*.

4.3.4 Effect of *O. kilimandscharicum* on the feeding activity of the three insects (%weight loss and number of damaged seeds)

Weight loss and number of damaged seeds induced by *S. zeamais* and *R. dominica* were significantly higher in seeds treated with 125 g fresh plant materials of *O. kilimandscharicum* compared to control (Fig. 4.19). Unlike *R. dominica* and *S. cerealella* all the fresh plant materials enhanced feeding activity of *S. zeamais*. The highest dosage of essential oil and ground plant materials protected the seeds against feeding by all the three insects (Fig.4.19; 4.20; 4.21). In essential oil and ground plant materials treated seeds similar tendency was obtained between the number of damaged seeds and weight loss (the lower the number of damaged seeds the lower weight loss). No seed damage was observed in maize seeds treated with 0.3% and 25 g (level 3) ground plant materials of *O. kilimandscharicum*.

Although there was no significant weight loss in sorghum seeds treated with dry plant material and infested with *R. dominica* this was not reflected the number of damaged seeds (Fig. 4.20). The number of damaged seeds and weight loss were significantly higher in seeds treated with 125 g (level 3) fresh plant materials.

S. cerealella did not cause any damage to seeds treated with the highest dosage of fresh plant materials, and the medium and highest dosages of essential oil and ground plant materials (Fig. 4.21). The highest number of damaged seeds was observed in seeds treated with fresh plant materials level 1; however, the percent weight loss was not significantly different compared to the control (Fig. 4.21).



Treatments

1 = fresh level 1
2 = " " 2
3 = " " 3
4 = ess. oil level 1
5 = " " 2
6 = " " 3
7 = dry level 1
8 = " " 2
9 = " " 3
10 = ground level 1
11 = " " 2
12 = " " 3
13 = hexane
14 = control

Fig. 4.19 Percent weight loss and percent number of damaged seeds of *O. kilimandscharicum* treated maize seeds due to feeding by *S. zeamais*

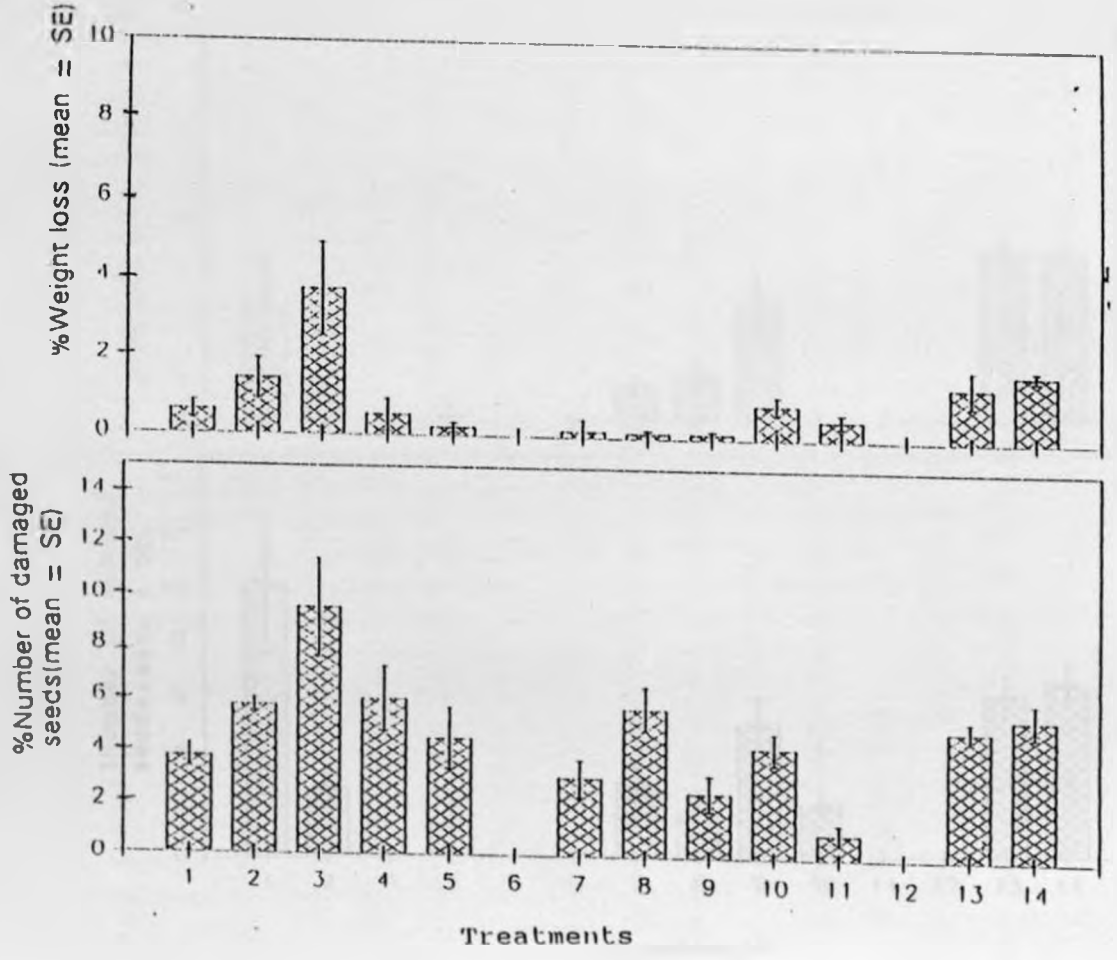


Fig. 4.20 Percent weight loss and percent number of damaged seeds of *O. kilimandscharicum* treated sorghum seeds due to feeding by *R. dominica*.

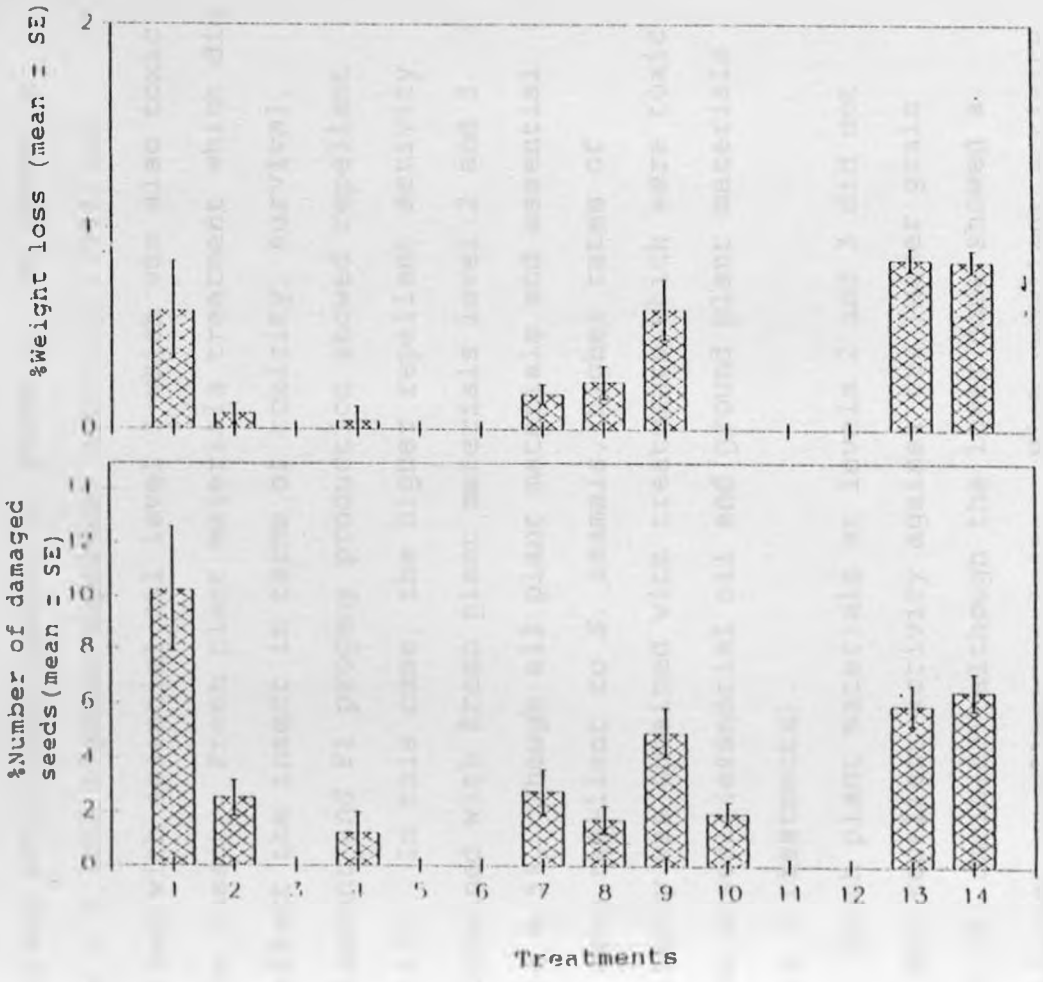


Fig. 4.21 Percent weight loss and percent number of damaged seeds of *O. kilimandscharicum* treated maize seeds due to feeding by *S. cerealella*

4.3.5 Repellent effect of *O. kilimandscharicum* against the three insects

All *O. kilimadscharicum* treatments showed some repellent activities against *S. zeamais*. As shown in Table 4.5, the highest repellent activity (79%) was obtained with essential oil level 3 which was also toxic to the insect. Fresh plant materials treatment which did not affect the insect in terms of toxicity, survival, antifeedant and F1 progeny production showed repellent activity. In this case, the higher repellent activity was obtained with fresh plant materials level 2 and 3 (Table 4.5). Though all plant materials and essential oils were repellent to *S. zeamais*, higher rates of repellency was obtained with treatments which were toxic to the insect (essential oil and ground plant materials levels 3 treatments).

Fresh plant materials at levels 2 and 3 did not show any repellent activity against the lesser grain borer (*R. dominica*) although the lower dose showed a significant repellent activity. High repellent activity was observed with essential oil treatments (Table 4.5).

Essential oil levels 2 and 3 showed the highest repellency to the angoumois grain moth (*S. cerealella*). Fresh plant materials were repellent at the lowest rate of application. As the rate of application was increased

percent repellency was observed to decline. Dry plant materials was the least repellent compared to all other treatments (Table 4.5).

Table 4.5 Repellency of *O. kilimandscharicum* against the three storage insect pests

Treatments	Mean % repellency ± SE [1,2] *		
	S.zeamais	*R. dominica*	*S. cerealella*
fresh lev 1	28.0±1.41c	41.5±14.86ab	45.0±1.29a
fresh lev 2	73.0±4.99a	0.0±0.00c	15.0±1.29b
fresh lev 3	58.0±1.29ab	0.0±0.00c	10.0±1.41b
ess.o lev 1	34.0±5.25bc	21.0±6.81b	56.3±1.49a
ess.o lev 2	52.0±4.97abc	67.0±5.00a	70.0±0.82a
ess.o lev 3	79.0±2.06a	55.0±13.12ab	65.0±0.96a
dry lev 1	57.0±0.96ab	36.0±9.79ab	12.5±0.96b
dry lev 2	59.0±1.71ab	54.0±7.75ab	12.5±0.96b
dry lev 3	34.0±6.45bc	23.0±8.39b	5.0±1.29b
grd lev 1	55.0±4.57ab	17.0±3.42b	2.5±0.96b
grd lev 2	59.0±1.29ab	49.0±4.12ab	12.5±0.96b
grd lev 3	66.0±1.29a	50.0±3.83ab	45.0±1.29a
hex.	0.0±0.0d	1.0±1.00c	0.0±0.00b
cont	0.0±0.0d	0.0±0.00c	1.0±1.29b

[1] Average of 4 replications, each replicate consisting of 60 insects (*S. zeamais & R. dominica*).

[2] Average of 4 replicates, each replicate consisting of 20 insects (*S. cerealella*).

* Means with the same alphabet within the column are not significantly different from each other, $p < 0.05$, SNK.

4.3.6 Toxicity persistence of *O. kilimandscharicum* against the three insects

As with the essential oil of *O. kenyense*, the oil of *O. kilimandscharicum* at level 3 was not significantly toxic to *S. zeamais* 2 days after treatment. The oil was toxic to *R. dominica* and *S. cerealella* up to 4 and 5 days respectively as observed 24 hrs after introduction of the insects in to the treated seeds. In both cases mortality rates decreased to less than 50 % starting at day 3 and onwards (Table 4.6).

Ground plant materials of *O. kilimandscharicum* at level 3 showed similar effect on the 3 insects as its essential oil level. However, the effect of the ground plant material against *S. cerealella* and R. *dominica* persisted for 1 more day than the effect of the oil (Table 4.7).

Ground plant materials of the plant at level 2 was not toxic to *S. zeamais* at day 1 after treatment. The effect of this treatment was observed only against *R. dominica* and *S. cerealella*. The toxic effect of the treatment against *R. dominica* was observed only for 2 days . Mortality rate of the insect declined from 59 to 26 % within 2 days and no mortality was observed on day 3 after treatment. Mortality rate of *S. cerealella* unlike

the above insect was 100 % at day 1 and declined to 8 % 5 days after treatment (Table 4.7).

Table 4.6 Residual toxicity of *O. kilimandscharicum* essential oil level 3 against the three storage insect pests

Days after treatment	% mean mortality ± SE[1] *		
	S. zeamais	*R. dominica*	*S .cerealella*
1	75.0±4.30a	100.0±0.00a	100.0±0.00a
2	17.8±1.59b	62.5±3.97b	73.3±1.92a
3	0.0±0.00c	25.0±4.91c	46.7±4.19b
4	-------	6.7±1.92d	16.7±2.55c
5	-------	0.0±0.00e	8.3±1.67cd
6	-------	--------	0.0±0.00d

1 Average of three replicates, each consisting of 10 pairs of adult insects.

*. Means with the same letter within the column are not significantly different from each other; SNK, $P < 0.05$.

Table 4.7 Residual toxicity of *O. kilimandscharicum* ground plant materials against the (a) three and (b) two storage insect pests at two levels of treatments

a. Ground plant materials level 3

Days after treatment	% mean mortality ± SE[1]*		
	S. zeamais	*R. dominica*	*S.cerealella*
1	60.0±2.76a	100.0±0.00a	100.0±0.00a
2	23.8±1.87b	62.5±2.97b	77.5±2.60b
3	6.7±3.33c	25.0±3.68c	55.0±3.68c
4	0.0±0.00cd	6.0±0.50d	21.3±1.19d
5	-------	0.0±0.00e	18.3±1.67d
6	------	--------	3.3±1.67ef
7	------	--------	0.0±0.00f

b. Ground plant materials level 2

Days after treatment	% mean mortality ± SE1	
	R. dominica	*S.cerealella*
1	58.8±1.05a	100.0±0.00a
2	26.3±4.38a	66.3±2.14b
3	0.0±0.00b	35.0±0.07c
4	------	17.8±1.20cd
5	------	8.3±0.58d
6	------	0.0±0.00e

1 Average of three replicates, each consisting of 10 pairs of adult insects.

*. Means with the same letter within the column are not significantly different from each other; SNK, $P < 0.05$

4.4 Effect of the three plant species on germination of maize and sorghum seeds

4.4.1 Effect of *O. suave* on the germination of the seeds

Germination of maize seeds treated with *O. suave* was not affected by all treatments except with the lowest dose of essential oil (level 1) (Table 4.8). Higher germination of the seeds was observed with treatments of dry and ground plant materials. The effect of dry plant materials level 1 treatment was observed to act through out the observation time. Similarly, higher germination rates and persistent effect was observed in seeds treated with 5 g and 27 g (levels 2 and 3) dry and ground plant materials. The lowest dosage of the essential oil (level 1) treatment reduced germination of maize seeds compared to the control (Table 4.8).

Germination of sorghum seeds treated with *O. suave* was not affected by all treatments except with essential oil level 3 treatment through out the observation time (Table 4.9)

Table 4.8 Cumulative percent germination of *O. suave* treated maize seeds [1,2]

Treatments	Days after germination set up			
	2	3	4	5
Fresh lev 1	41.3 ± 3.83b	63.8 ± 5.92abc	68.8 ± 5.53abcd	70.0 ± 6.12abc
Fresh lev 2	46.3 ± 5.13ab	67.5 ± 2.23abc	68.8 ± 3.14abcd	68.8 ± 3.12abc
Fresh lev 3	47.8 ± 7.80ab	63.8 ± 6.23abc	65.0 ± 7.33cd	65.0 ± 7.33abc
E.oil lev 1	6.3 ± 1.25d	13.8 ± 6.82d	27.5 ± 5.23e	38.8 ± 3.12d
E.oil lev 2	38.8 ±10.32b	47.8 ± 8.23abc	55.0 ± 7.24bcd	58.8 ± 7.12bcd
E.oil lev 3	26.3 ± 4.74c	35.0 ± 5.42c	51.3 ± 9.44cd	55.0 ±10.83cd
Dry lev 1	62.5 ± 8.53a	91.3 ± 5.12a	92.5 ± 4.73a	92.5 ± 4.73a
Dry lev 2	52.5 ± 4.33a	76.3 ± 5.51ab	80.0 ± 5.43ab	80.0 ± 5.44abc
Dry lev 3	61.3 ±10.52a	87.5 ± 6.23ab	90.0 ± 7.12a	90.0 ± 7.12ab
Gr.lev 1	35.0 ±13.12b	56.3 ± 6.64abc	73.8 ± 9.44abc	73.8 ± 9.44abc
Gr.lev 2	42.5 ± 5.84b	77.5 ± 4.40ab	88.8 ± 3.12a	88.8 ± 3.12ab
Gr.lev 3	42.5 ± 6.33b	77.5 ± 6.23ab	87.5 ± 4.74a	91.3 ± 3.73a
Hexane	26.3 ± 3.14c	42.5 ± 4.73bc	42.5 ± 4.80d	55.0 ± 2.00cd
control	23.8 ± 3.72c	46.3 ± 7.42abc	46.3 ± 7.42cd	62.5 ± 6.64abc

[1] Average of 4 replicates (each 50 seeds) ± SE
[2] In column treatment averages followed by the same letter (s) are not significantly different from each other, SNK, $P < 0.05$.

Table 4.9 Cumulative percent germination of *O. suave* treated sorghum seeds [1,2]

Treatments	Days after germination set up			
	2	3	4	5
Fresh lev 1	76.5 ± 4.00a	87.5 ± 1.72ab	89.5 ± 1.52a	89.5 ± 1.52a
Fresh lev 2	75.5 ± 2.62a	83.5 ± 2.53ab	86.5 ± 3.00ab	87.0 ± 3.13ab
Fresh lev 3	73.0 ± 1.73ab	82.5 ± 1.53ab	84.5 ± 1.72ab	84.5 ± 1.72ab
E.oil lev 1	77.5 ± 1.22a	79.0 ± 1.72b	79.0 ± 1.72b	79.0 ± 1.72b
E.oil lev 2	84.0 ± 2.44a	85.5 ± 1.52ab	85.5 ± 1.53ab	85.5 ± 1.53ab
E.oil lev 3	66.0 ± 3.44b	68.5 ± 3.00c	72.5 ± 3.32c	72.5 ± 3.32c
Dry lev 1	78.5 ± 1.53a	89.0 ± 2.62a	90.0 ± 3.00a	90.0 ± 3.00a
Dry lev 2	78.5 ± 1.73a	90.0 ± 2.32a	92.0 ± 3.00a	92.0 ± 3.00a
Dry lev 3	78.5 ± 3.52a	91.0 ± 2.00a	91.0 ± 2.00a	91.0 ± 2.00a
GR lev 1	82.5 ± 2.63a	91.5 ± 2.00a	94.5 ± 2.22a	94.5 ± 2.22a
Gr lev 2	81.0 ± 1.72a	88.5 ± 1.62a	89.5 ± 2.23a	90.0 ± 2.22a
Gr lev 3	72.0 ± 2.00ab	85.5 ± 2.53ab	88.0 ± 2.72a	89.0 ± 3.12a
Hexane	77.5 ± 1.52a	88.0 ± 2.00a	90.5 ± 2.00a	91.5 ± 2.12a
Control	75.0 ± 4.12a	90.0 ± 1.42a	90.5 ± 1.52a	90.5 ± 1.52a

[1] Average of 4 replicates (each 50 seeds) ± SE
[2] In column treatment means followed by the same letter (s) are not significantly different from each other, SNK, $P < 0.05$.

4.4.2 Effect of *O. kenyense* on germination of the seeds

Germination of *O. kenyense* treated maize seeds was not significantly affected by any of the treatments, except with fresh plant materials level 3 and dry plant materials level 1 two days after germination set up (Table 4.10). However, starting with the third day until the end of the observation time (day 5) germination of the seeds was significantly affected only by fresh plant materials level 3 treatment. It was observed in the laboratory that some seeds mixed with fresh plant materials at level 3 started to germinate while they were there in the bottles (treatment time) and germination was interrupted when the leaves started to dry. When germination test of the treated seeds (with no sign of germination) was conducted very low rate of germination was observed (Table 4.10).

Significantly, germination of sorghum seeds was not affected by any of treatments of *O. kenyense* through out the observation time except those treated with fresh plant materials level 3 (Table 4.11).

Table 4.10 Cumulative percent germination of *O. kenyense* treated maize seeds [1,2]

Treatments	Days after germination set up			
	2	3	4	5
Fresh lev 1	67.5 ± 4.84ab	90.0 ± 3.51a	91.3 ± 2.41a	91.3 ± 2.41a
Fresh lev 2	41.3 ± 6.93cde	61.3 ± 3.82ab	68.8 ± 2.42ab	70.0 ± 2.00ab
Fresh lev 3	27.5 ± 6.00e	28.8 ± 7.22c	28.8 ± 7.21c	28.8 ± 7.12c
E.oil lev 1	51.3 ± 3.12abcd	76.3 ± 3.12ab	78.8 ± 4.32ab	80.0 ± 3.52ab
E.oil lev 2	36.3 ± 3.83de	65.0 ± 3.53ab	73.8 ± 4.32ab	76.3 ± 4.31ab
E.oil lev 3	45.0 ± 6.12bcde	70.0 ± 5.44ab	73.8 ± 3.12ab	73.8 ± 3.12ab
Dry lev 1	27.5 ± 6.00e	55.0 ±12.44b	57.5 ±11.62b	58.8 ±11.62b
Dry lev 2	57.5 ± 7.53abcd	86.3 ± 5.23a	91.3 ± 2.41a	92.5 ± 1.41a
Dry lev 3	48.8 ± 3.12abcd	82.5 ± 7.52ab	82.5 ± 7.51ab	83.8 ± 8.00ab
Gr.lev 1	73.8 ± 2.44ab	80.0 ± 0.00ab	80.0 ± 0.00ab	81.3 ± 1.31ab
Gr.lev 2	76.3 ± 4.32a	87.5 ± 5.23a	88.8 ± 4.31a	88.8 ± 4.32a
Gr.lev 3	72.5 ± 7.23ab	80.0 ± 8.43ab	83.8 ± 7.00a	83.8 ± 7.00ab
Hexane	61.3 ± 9.44abcd	67.5 ± 6.00ab	68.8 ± 5.21ab	68.8 ± 5.21ab
control	55.0 ± 8.92abcd	63.8 ± 5.50ab	68.8 ± 5.21ab	68.8 ± 5.21ab

[1] Average of 4 replicates (each 50 seeds+ ± SE
[2] In column treatment means followed by the same letter (s) are not significantly different from each other, SNK, $P < 0.05$.

Table 4.11 Cumulative percent germination of *O. kenyense* treated sorghum seeds [1,2]

Treatments	Days after germination set up			
	2	3	4	5
Fresh lev 1	68.5 ± 1.52b	86.0 ± 2.21ab	86.0 ± 2.21ab	86.0 ± 2.21ab
Fresh lev 2	66.5 ± 2.22b	82.5 ± 1.52ab	85.0 ± 2.00ab	88.5 ± 1.51ab
Fresh lev 3	57.5 ± 2.00c	79.0 ± 1.73b	81.0 ± 2.12b	81.5 ± 2.52b
E.oil lev 1	86.0 ± 0.82a	88.5 ± 0.52ab	88.5 ± 0.51ab	89.5 ± 0.51ab
E.oil lev 2	85.5 ± 3.53a	87.5 ± 3.14ab	88.0 ± 3.00ab	88.0 ± 3.00ab
E.oil lev 3	87.0 ± 3.33a	90.0 ± 3.62ab	90.0 ± 3.61ab	90.0 ± 3.62ab
Dry lev 1	86.0 ± 2.00a	89.0 ± 3.12ab	89.0 ± 3.12ab	89.5 ± 3.12ab
Dry lev 2	86.5 ± 3.62a	89.5 ± 2.62ab	91.0 ± 2.12ab	91.0 ± 2.11ab
Dry lev 3	88.0 ± 1.62a	93.5 ± 1.00a	93.5 ± 1.00a	93.5 ± 1.00a
Gr.lev 1	86.0 ± 1.44a	91.5 ± 1.54a	93.0 ± 2.00a	93.5 ± 2.21a
Gr.lev 2	80.5 ± 2.12a	84.0 ± 2.62ab	86.5 ± 2.21ab	87.0 ± 2.12ab
Gr.lev 3	86.5 ± 3.52a	89.0 ± 3.00ab	90.0 ± 2.41ab	91.0 ± 2.62ab
Hexane	82.5 ± 3.21a	90.5 ± 3.42ab	91.5 ± 2.62ab	92.0 ± 2.41ab
control	87.5 ± 3.21a	89.5 ± 2.41ab	90.5 ± 2.12ab	90.5 ± 2.12ab

1 Average fo 4 replicates (each 50 seeds) ± SE.
2. In columns, treatment averages followed by the same letter (s) are not significantly different from each other ,SNK, $P < 0.05$.

4.4.3 Effect of *O. kilimandscharicum* on germination of the seeds

Maize seeds treated with *O. kilimandscharicum* at 25 and 125 g per 250 g of seeds (levels 2 and 3) of fresh plant materials showed significantly lower germination rate throughout the observation time. Higher germination rates were observed in essential oil and ground plant materials level 3 treatments, although the differences were not significant when compared to the controls. The effect of essential oil level 3 was persistent until the end of the observation time (Table 4.12).

Similarly, viability of sorghum seeds was affected by level 3 treatment involving fresh plant materials as lower rate of germination was observed throughout the observation time. In all other treatments, germination rates were not significantly different from the controls (Table 4.13).

Table 4.12 Cumulative percent germination of *O. kilimandscharicum* treated maize seeds.[1,2]

Treatments	Days after germination set up			
	2	3	4	5
Fresh lev 1	50.0 ± 4.12ab	52.5 ± 5.21c	52.5 ± 5.21c	52.5 ± 5.21c
Fresh lev 2	25.0 ± 7.42c	28.8 ± 7.52d	28.8 ± 7.51d	28.8 ± 7.51c
Fresh lev 3	22.5 ± 8.00c	23.8 ± 7.41d	23.8 ± 7.41d	23.8 ± 7.42c
E.oil lev 1	46.3 ± 7.21ab	65.0 ± 5.41abc	67.5 ± 5.22abc	67.5 ± 5.21ab
E.oil lev 2	52.5 ± 1.41ab	80.0 ± 2.00ab	81.3 ± 2.41ab	82.5 ± 1.42a
E.oil lev 3	60.0 ± 4.62a	86.3 ± 2.41a	86.3 ± 2.41a	86.3 ± 2.41a
Dry lev 1	41.3 ± 4.32ab	57.5 ± 1.41bc	58.8 ± 1.32bc	60.0 ± 0.00ab
Dry lev 2	35.0 ± 3.52bc	51.3 ± 1.32c	53.8 ± 2.41c	53.8 ± 2.04b
Dry lev 3	35.0 ± 3.51bc	51.3 ± 1.32c	52.5 ± 1.41c	52.5 ± 1.41b
Gr lev 1	45.0 ± 3.52ab	53.8 ± 3.12c	53.8 ± 3.12c	53.8 ± 3.12b
Gr lev 2	43.8 ± 2.41ab	48.8 ± 4.32c	53.8 ± 7.00c	53.8 ± 7.00b
Gr lev 3	62.5 ± 1.42a	73.8 ± 5.21abc	77.5 ± 5.21ab	77.5 ± 5.21ab
Hexane	42.5 ± 3.22ab	57.5 ± 3.21bc	60.0 ± 2.01abc	61.3 ± 1.32ab
Control	47.5 ± 3.21ab	58.8 ± 2.41bc	61.3 ± 2.41abc	61.3 ± 2.41ab

[1] Average of 4 replicates (50 seeds each) ± SE
[2] In column treatment means followed by the same letter (s) are not significantly different from each other, SNK, $P < 0.05$.

Table 4.13 Cumulative percent germination of *O. kilimandscharicum* treated sorghum seeds).2

Treatments	Days after germination set up			
	2	3	4	5
Fresh lev 1	81.5 ± 4.72ab	85.0 ± 4.82a	85.0 ± 4.81a	85.0 ± 4.82a
Fresh lev 2	85.0 ± 2.14ab	88.0 ± 2.00a	89.0 ± 2.00a	89.0 ± 2.00a
Fresh lev 3	62.5 ± 1.32c	67.5 ± 1.71b	68.0 ± 2.22b	68.0 ± 2.21b
E.oil lev 1	72.0 ± 3.74b	82.0 ± 3.51a	83.5 ± 3.62a	84.5 ± 3.41a
E.oil lev 2	73.5 ± 5.12b	87.5 ± 2.21a	88.5 ± 2.22a	89.5 ± 1.33a
E.oil lev 3	75.5 ± 1.33ab	85.5 ± 1.00a	86.5 ± 1.00a	87.0 ± 1.32a
Dry lev 1	83.0 ± 1.32ab	85.0 ± 1.71a	86.5 ± 1.73a	86.5 ± 1.72a
Dry lev 2	88.5 ± 2.61a	90.5 ± 2.00a	91.5 ± 2.23a	91.5 ± 2.21a
Dry lev 3	85.5 ± 2.62ab	89.0 ± 3.71a	90.5 ± 3.42a	90.5 ± 3.42a
Gr.lev 1	84.5 ± 3.61ab	85.5 ± 3.00a	86.5 ± 2.51a	87.5 ± 2.11a
Gr.lev 2	86.0 ± 4.32ab	88.5 ± 2.21a	88.5 ± 2.21a	88.5 ± 2.22a
Gr.lev 3	86.0 ± 0.82ab	90.0 ± 0.00a	90.0 ± 0.00a	90.0 ± 0.00a
Hexane	81.5 ± 2.61ab	87.0 ± 3.41a	88.5 ± 3.00a	88.5 ± 3.00a
control	85.5 ± 2.61ab	87.5 ± 1.52a	89.0 ± 1.72a	89.0 ± 1.72a

1 Average of 4 replicates (each 50 seeds) ± SE.
2 In column, treatment means followed by the same letter (s) are not significantly different from each other ,SNK, $P < 0.05$.

4.5 Bulk (large scale) treatment and pheromone monitoring

4.5.1 Comparison of sampling methods

It was impossible to compare the efficiency of sampling methods in treated maize and sorghum seeds as no surviving adults of both *S. zeamais* and *R. dominica* were observed. However, significant difference of observation methods was observed in untreated (control) seeds. Among the two methods used for *S. zeamais* the grain probe was significantly better than sieve sampling in trapping most of the 50 pairs of adults introduced into the untreated seeds in the bags 48 hrs after infestation (Table 4.14). Similar results were obtained in trapping F1 progeny adults.

In the case of *R. dominica*, among the 3 methods used 48 h after treatment, pheromone trapping was significantly efficient in trapping adults introduced into untreated seeds compared to the other methods (Table 4.14). During F1 progeny emergence pheromone baited grain probe (which was not used earlier) was the most efficient in trapping more adults of *R. dominica* from untreated sorghum seeds compared to the other two methods (Table 4.14).

Table 4.14 Comparison of sampling methods for *S. zeamais* (a) and *R. dominica* (b) in untreated maize and sorghum seeds at different times of observations.

A. *S. zeamais* in maize seeds

Sampling method	No of adults trapped after 48 hrs (Mean ± SE)[1] *	No of F1 progeny after 42 days (Mean ± SE)[1] *
Sieve sampling	8.7±0.36b	12.4±0.47b
grain probe	12.9±0.52a	14.5±0.38a

B. *R. dominica* in sorghum seeds

Sampling method	No of adults trapped after 48 hrs Mean ± SE)[1] *	No of F1 progeny 65 days (Mean ± SE)[1] *
Sieve sampling	7.4±0.47b	17.0±0.89c
Pheromone	11.3±0.62a	not applied
Grain probe	3.2±0.26c	29.1±1.30b
Pheromone + Grain probe	not applied	87.8±1.87a

[1]. Average of 6 samples (each 280 g), grain probe, Pheromone and grain probe + pheromone trap catches

* Means with the same letter within column are not significantly different from each other, $P < 0.05$, SNK.

4.5.2 **Effectiveness of the treatment**

In maize seeds treated with ground *O. kilimandscharicum*, (10 % w/w) no live adult *S. zeamais* was trapped by the traps used or observed in sample seeds when sieved out with sieve of size 2 mm, 24 hrs after treatment and during F1 progeny emergence. All of the sieved adults were found dead. Similarly, no adult was trapped by the grain probe trap indicating that the treatment was effective to induce 100 % mortality of the insect (Table 4.15a).

Similarly, no adult of *R. dominica* was observed or trapped from all samples taken with traps employed in seeds treated with ground *O. kilimandscharicum* (2 % w/w) on both occasions of observations mentioned above (Table 4.15b).

In both cases, significant numbers of adults were observed in untreated seeds (control) on both occasions of observations by the different methods used (Table 4.15b).

Table 4.15 Number of live (a) *S. zeamais* and (b) *R. dominica* in untreated seeds and seeds treated with different levels of ground leaves of *O.kilimandscharicum* at different times of observations after treatment

a. *S. zeamais* in maize seeds

Treatment	No of live adults after 48 hrs (Means ± SE)[1]*	No of F1 progeny after 65 days (Means ± SE)[1]*
Treated (10% w\w)	0.0±0.00b	0.0±0.04b
control	10.8±0.08a	13.5±0.08a

b. *R. dominica* in sorghum seeds

Treatment	No of live adults after 48 hrs (Means ± SE)[1]*	No of F1 progeny after 50 days (Means ± SE)[1]*
Treated (2% w\w)	0.0±0.00b	0.0±0.00b
control	7.1±0.11a	44.6±0.59a

1 Average of 6 samples (each 280g), grain probe, pheromone and grain probe + pheromone catches.
* Means with the same letter are not significantly different from each other, P < 0.05, LSD.

4.6 Chemical composition of essential oils of the three *Ocimum* plant species

4.6.1 *O. suave* essential oil chemical composition

Based on the GC profile of *O. suave* (Fig. 4.22) and GC-MS a total of 8 compounds (6 mono terpenes and 2 sesquiterpenes) comprising 93% (relative proportion) of the essential oil were identified (Table 4.16). Most of the compounds are aliphatic and cyclic hydrocarbons except linalool which is alcohol and eugenol which is phenol. Eugenol was identified as the major constituent of the essential oil (~60%). The relative proportion of eugenol was observed to vary from season to season even though the collection place was the same.

4.6.2 *O. kenyense* essential oil chemical composition

The chemical analysis of the essential oil of *O. kenyense* was carried out by GC and GC-MS.

The essential oil constituents of *O. kenyense* (based on the GC profile, Fig.4.23) were identified as shown in Table 4.17. In total 11 compounds (5 monoterpenes, 5 sesquiterpenes and 1 Easter) comprising 93 % of the oil were identified. Among these 5 were identified as alcohols, one as ester (ethyl isovalerate)

while, the remaining compounds were aliphatic and cyclic hydrocarbons. The major constituent of the oil was identified as 1,8-cineole.

4.6.3 *O. kilimandscharicum* essential oil chemical composition

Based on the GC profile of the essential oil of *O. kilimandscharicum* (Fig. 4.24) 10 constituent compounds comprising 95% of the essential oil composition were identified (Table 4.18). These compounds were alcohols (linalool, 4-terpineole, α-terpineole, endoborneole and myrtenol), cyclic hydrocarbons (camphene, limonene and *trans*-caryophyllene), ketone and ether (camphor and 1,8-cineole, respectively) were classified. The major constituent compound (~70%) was camphor. However, the composition of all the compounds varied when the plants were collected from different places (Ngong and Siaya). The composition of camphor was lower (~ 40%) in the Ngong variety. The maximum composition of camphor (70%) was observed in the Siaya variety, which was toxic to all insects tested. All the compounds were monoterpenes with the exception of *trans*-caryophellene.

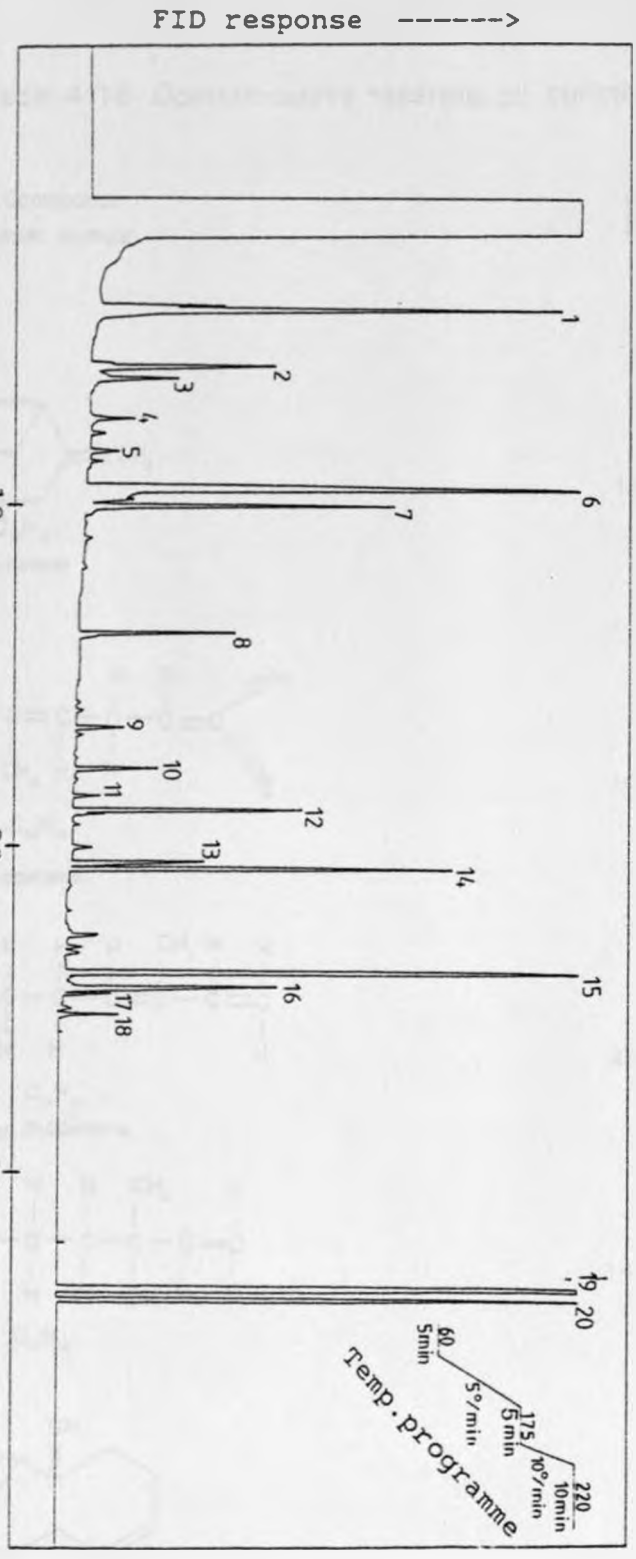
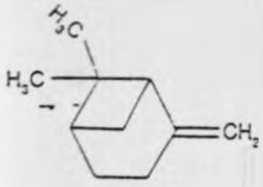
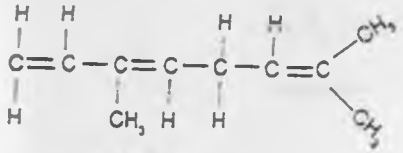
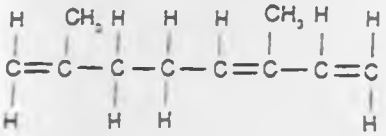
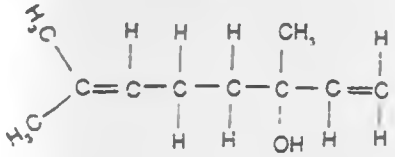
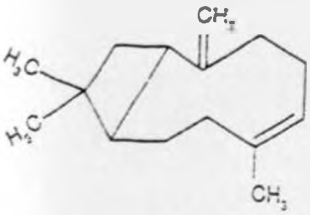
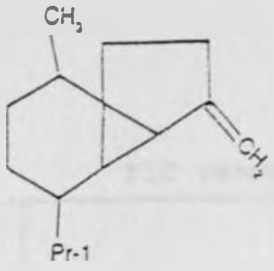


Fig. 4.22 Gas chromatograph profile of *Ocimum suave* essential oil

Table 4.16 *Ocimum suave* essential oil constituent compounds

Peak no.	Compounds (molecular formula)	Composition (% area)
1	 $C_{10}H_{16}$ β-pinene	5.26
		1.66
6	 $C_{10}H_{16}$ *trans*-β-ocimene	14.52
7	 $C_{10}H_{16}$ *trans*-*O*-ocimene	2.14
12	 $C_{10}H_{16}$	1.41
14	 $C_{15}H_{24}$ *trans*-caryophyllene	3.27

15

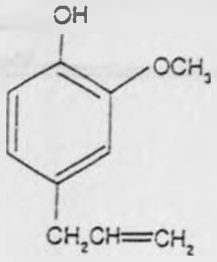


5·3

$C_{15}H_{24}$

β-cubebene

19



57.65

$C_{10}H_{12}O_2$

eugenol

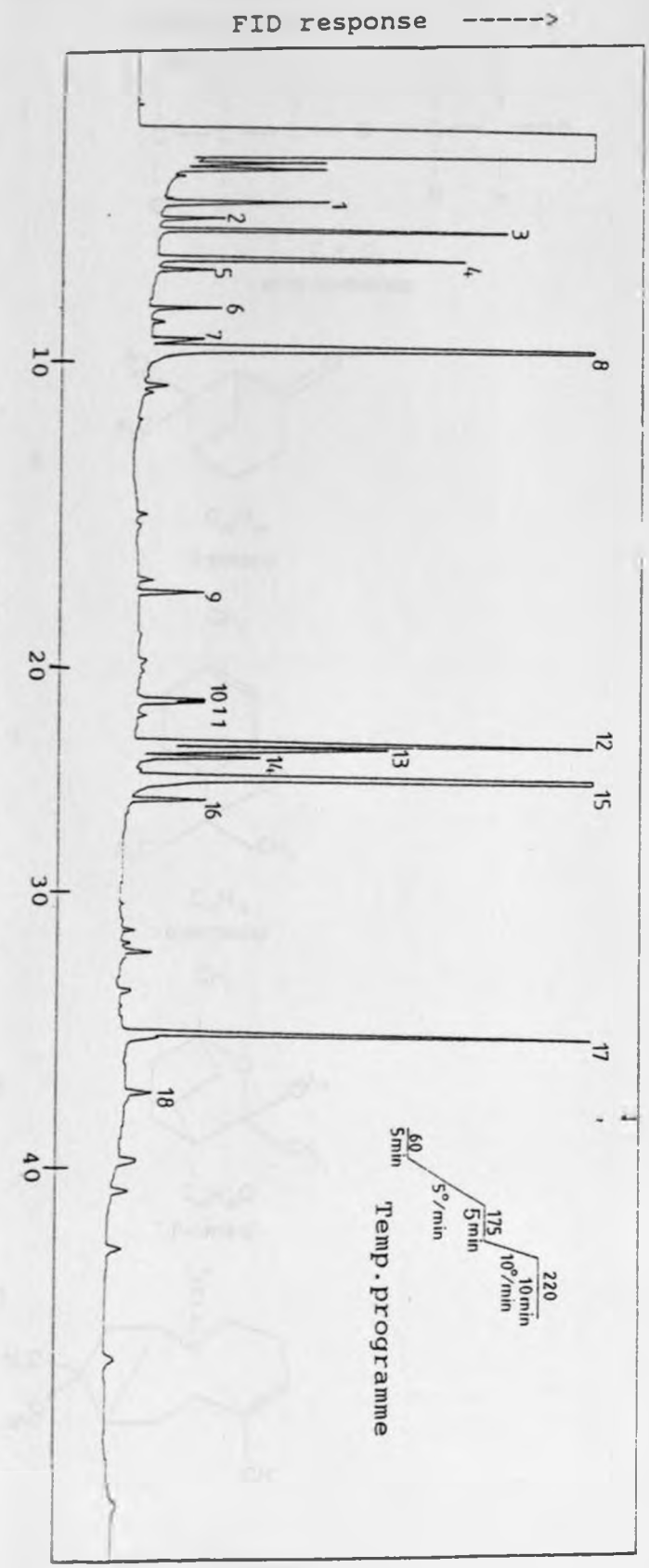
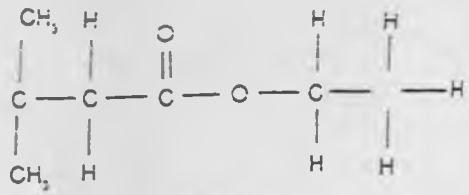
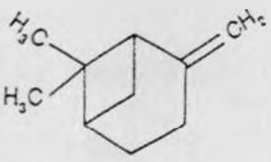
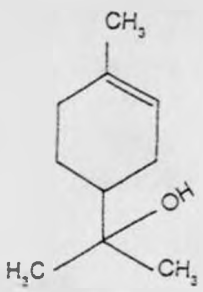
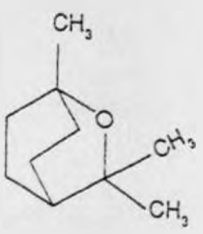
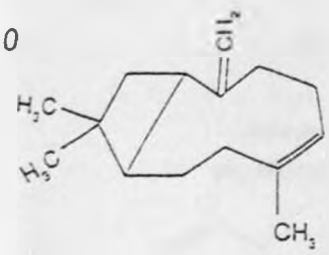
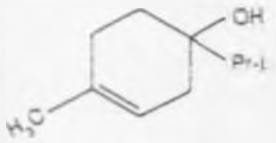


Fig. 4.23 Gas chromatograph profile of *Ocimum kenyense* essential oil

Table 4.17 *Ocimum kenyense* essential oil constituent compounds

Peak no.	Compounds (molecular formula)	Composition (% area)
3	 $C_7H_{14}O_2$ ethyl isovalerate	2.99
4	 $C_{10}H_{16}$ β-pinene	2.86
7	 $C_{10}H_{18}O$ α-terpineol	0.55
8	 $C_{10}H_{18}O$ 1,8-cineol	36.93
10	 $C_{15}H_{24}$ *trans*-caryophyllene	0.66

11

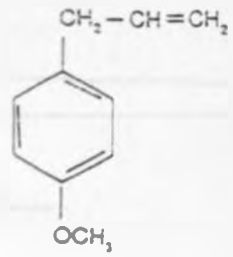


$C_{10}H_{18}O$

4-terpineol

0.79

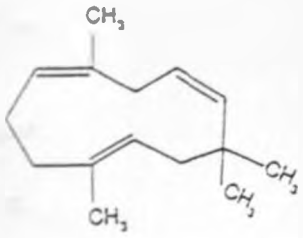
12



12.86

methyl chavicol

13

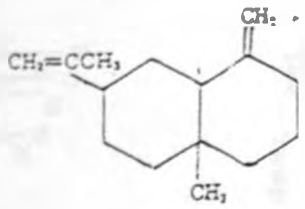


2.78

$C_{15}H_{24}$

α-humulene

15

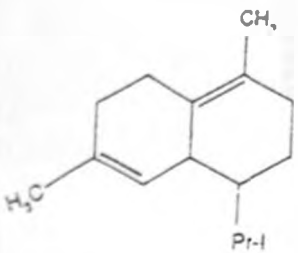


23.57

$C_{15}H_{24}$

β-Selinene

16

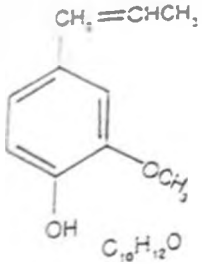


0.78

$C_{15}H_{24}$

γ-cardinene

17



8.46

$C_{10}H_{12}O$

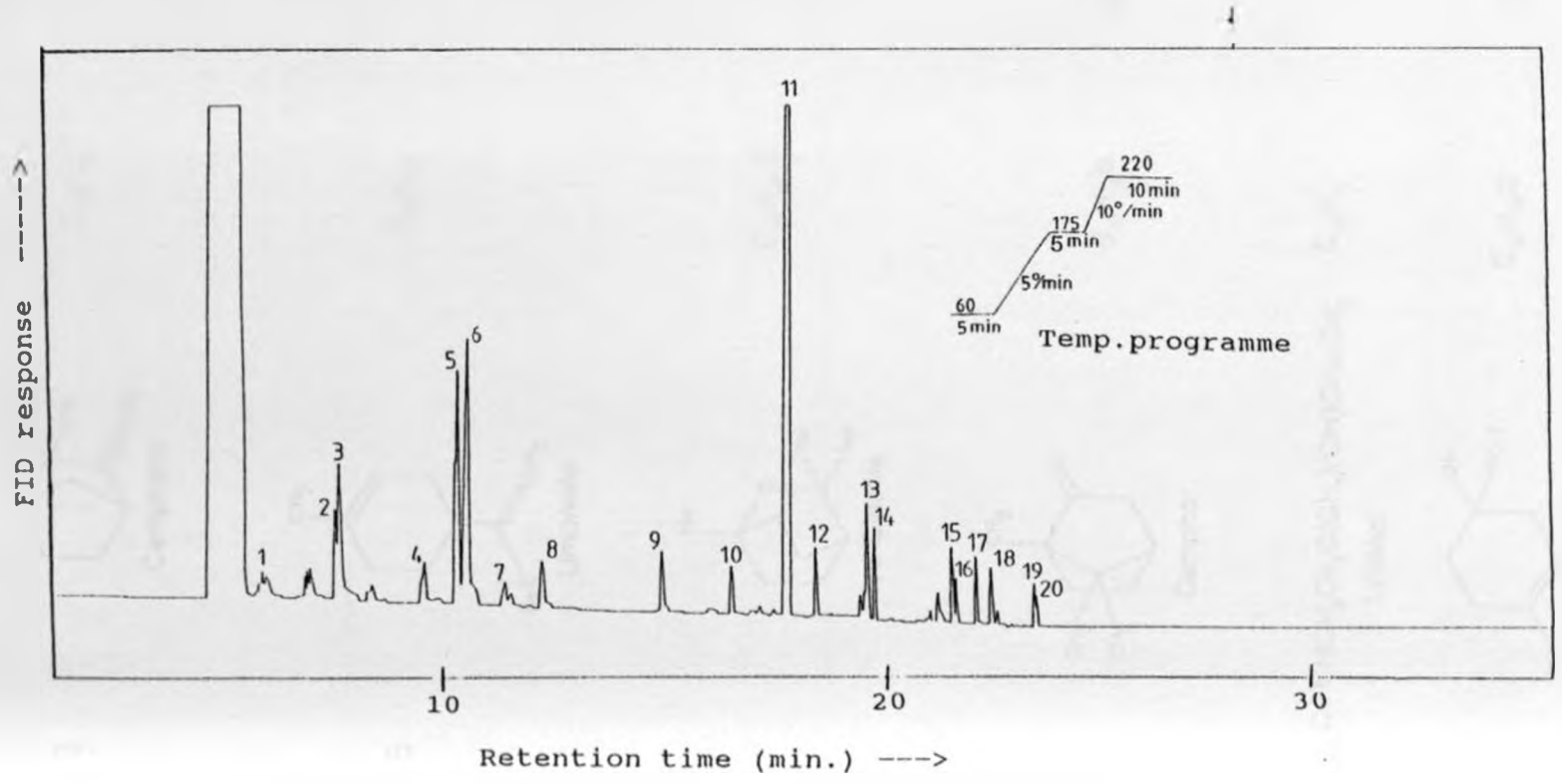
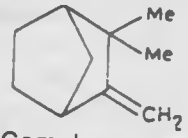
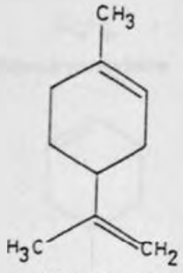
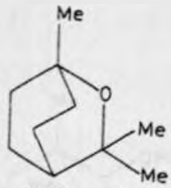
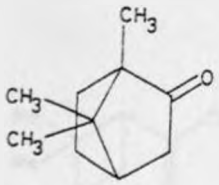
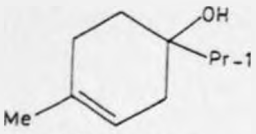
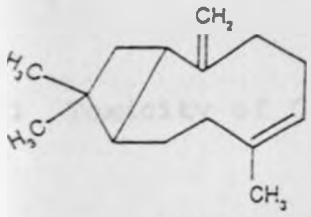
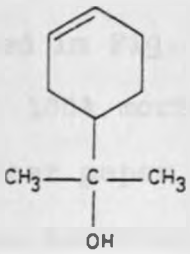
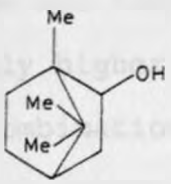
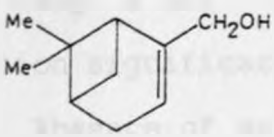


Fig 4.24 Gas chromatograph profile of *Ocimum kilimandscharicum* essential oil

Table 4.18 *Ocimum kilimandscharicum* essential oil constituent compounds

	Compounds	Molecular formula	Composition % area
3	 Camphene	$C_{10}H_{16}$	5.07
5	 Limonene	$C_{10}H_{16}$	6.23
6	 1, 8-Cineole	$C_{10}H_{18}O$	7.20
11	 Camphor	$C_{10}H_{16}O$	70.43
12	$(CH_3)_2 C= CHCH_2CH_2C(CH_3)(OH)CH= CH_2$ Linalool	$C_{10}H_{18}$	0.47
13	 4-Terpineol	$C_{10}H_{18}O$	1.44–

Peak no.	Compounds	Molecular formula	Composition % area
14	 $C_{15}H_{24}$ *trans*-caryophyllene	$C_{15}H_{24}$	2.8
15	 α-Terpineol	$C_{10}H_{18}O$	0.6
16	 Endo-borneol	$C_{10}H_{18}O$	0.6
20	 Myrtenol	$C_{10}H_{16}O$	1.3

4.7 Toxic effect of essential oils of the three plant species and their constituent compounds .

4.7.1 Toxicity of *O. suave essential oil*

Dose response for the toxicity of the essential oil of *O. suave* against S. *zeamais* was established as illustrated in Fig. 4.25. LC_{50} of the oil was 69.6/62.6 cm^2 while, 100% mortality was obtained with 185 mg/62.6 cm^2 of filter paper. At relative amounts present in the dose of the essential oil O. *suave* which resulted in 100% mortality of *S. zeamais*, all identified compounds except eugenol were not toxic to the weevil. Eugenol caused significantly higher mortality rate of the insect (Fig. 4.26). Combination of the 4 major compounds of the essential oil (eugenol, ocimene, cubebene and *trans*-caryphyllene) caused 100% mortality in the adults of *S. zeamais* (Fig. 4.26). Removal of eugenol from the combination significantly reduced mortality of the insect. Absence of any of the other 3 compounds did not have any significant effect on the toxicity of the resulting blend. Eugenol with any one of the other three compounds caused high mortality of the insect.

Similarly, dose response effect of the oil against *R. dominica* was evaluated and the results are summarized

in Fig. 4.27. 100% mortality of the borer was obtained with 40 mg/62.6 cm^2 while, LC_{50} of the oil was 16.7 mg/62.6 cm^2.

Among the constituent compounds, eugenol caused 100% mortality in adults of *R. dominica* while, none of the other identified compounds caused any mortality of the insect at their natural proportion (Fig. 4.28). Combination of the four major constituent compounds similarly induced 100% mortality of the insect. Removal of eugenol from the blend resulted in a very low mortality rate of the insect, while removal of any of the other three compounds did not have any effect on the mortality of *R. dominica*. Blends of the compounds without eugenol resulted in very low or no mortality (Fig. 4.28).

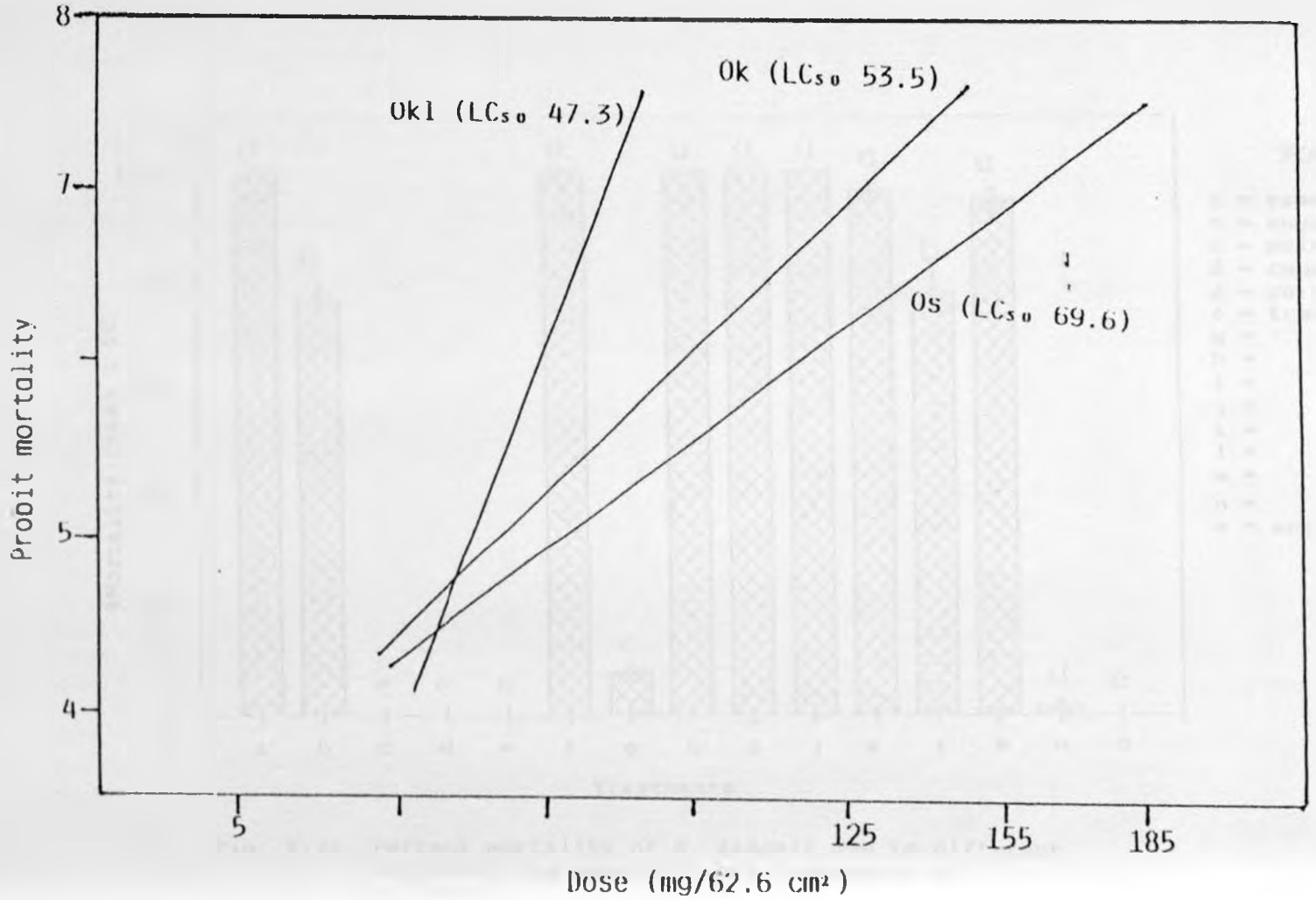


Fig. 4.25 Dose response curve of essential oils of the three plants against *S. zeamais*. Os, *O. suave*; Ok, *O.kenyense*; Okl, *O. kilimandscharicum*.

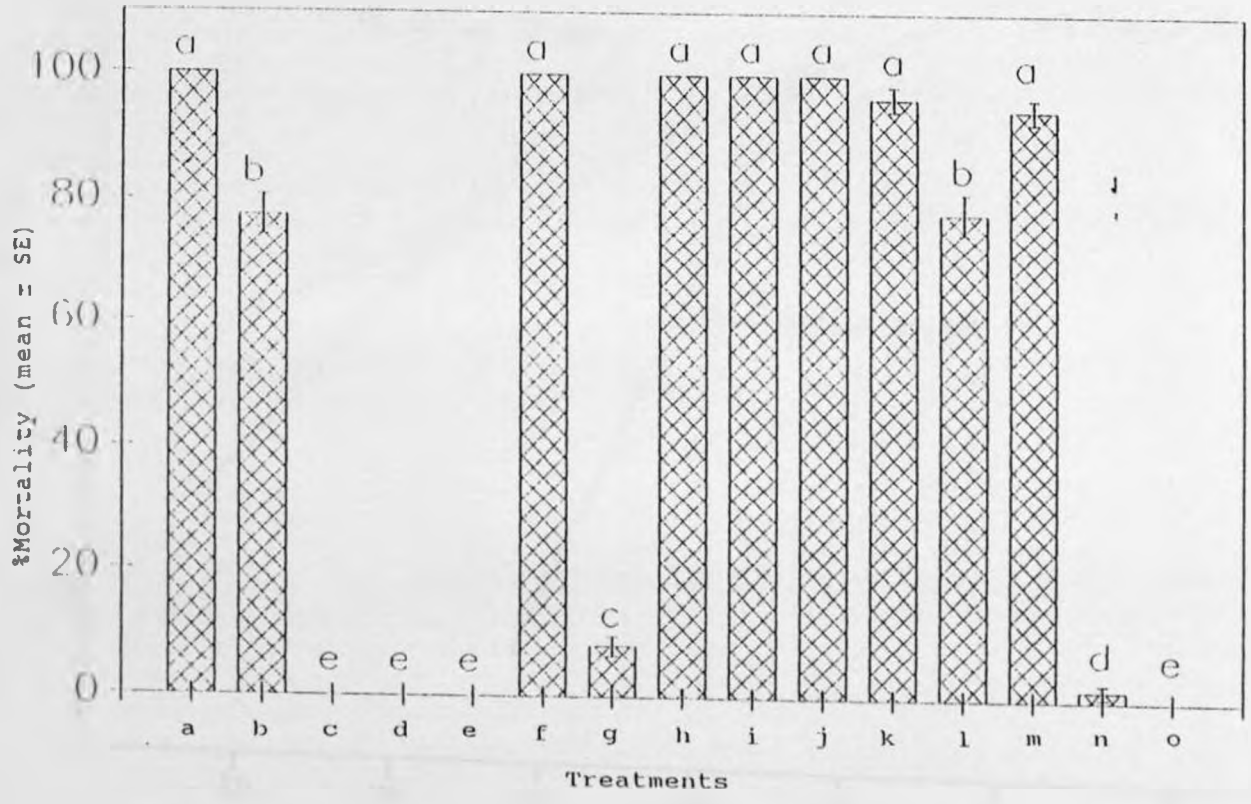


Fig. 4.26 Percent mortality of *S. zeamais* due to different compounds and essential oil treatments of *O. suave*.
Histograms (average of 6 replicates) with the same letter are not significantly different from each other, SNK, $P < 0.05$.

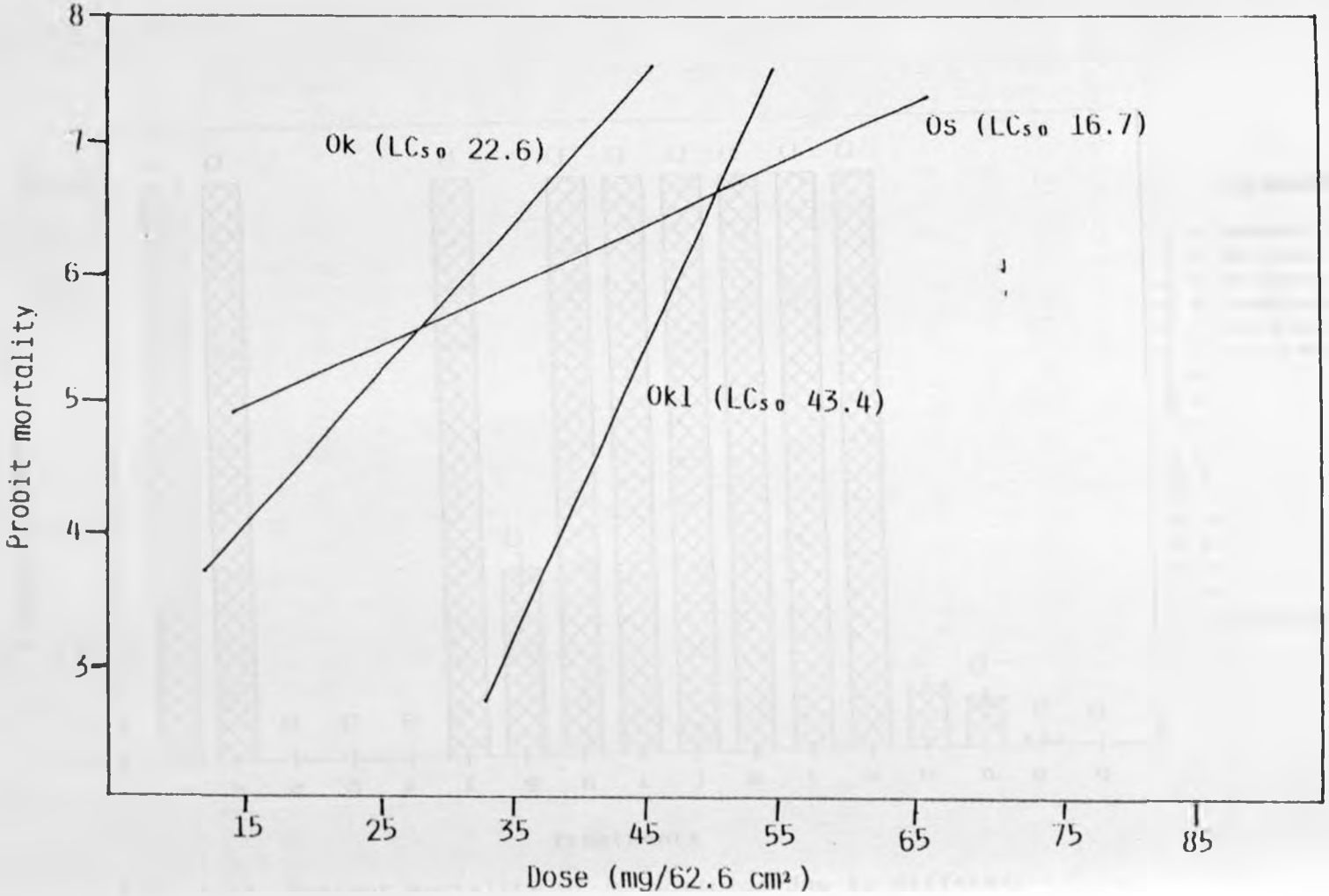


Fig. 4.27 Dose response curve of essential oils of the three plants against *R. dominica*. Os, *O. suave*; Ok, *O.kenyense*; Okl, *O. kilimandscharicum*.

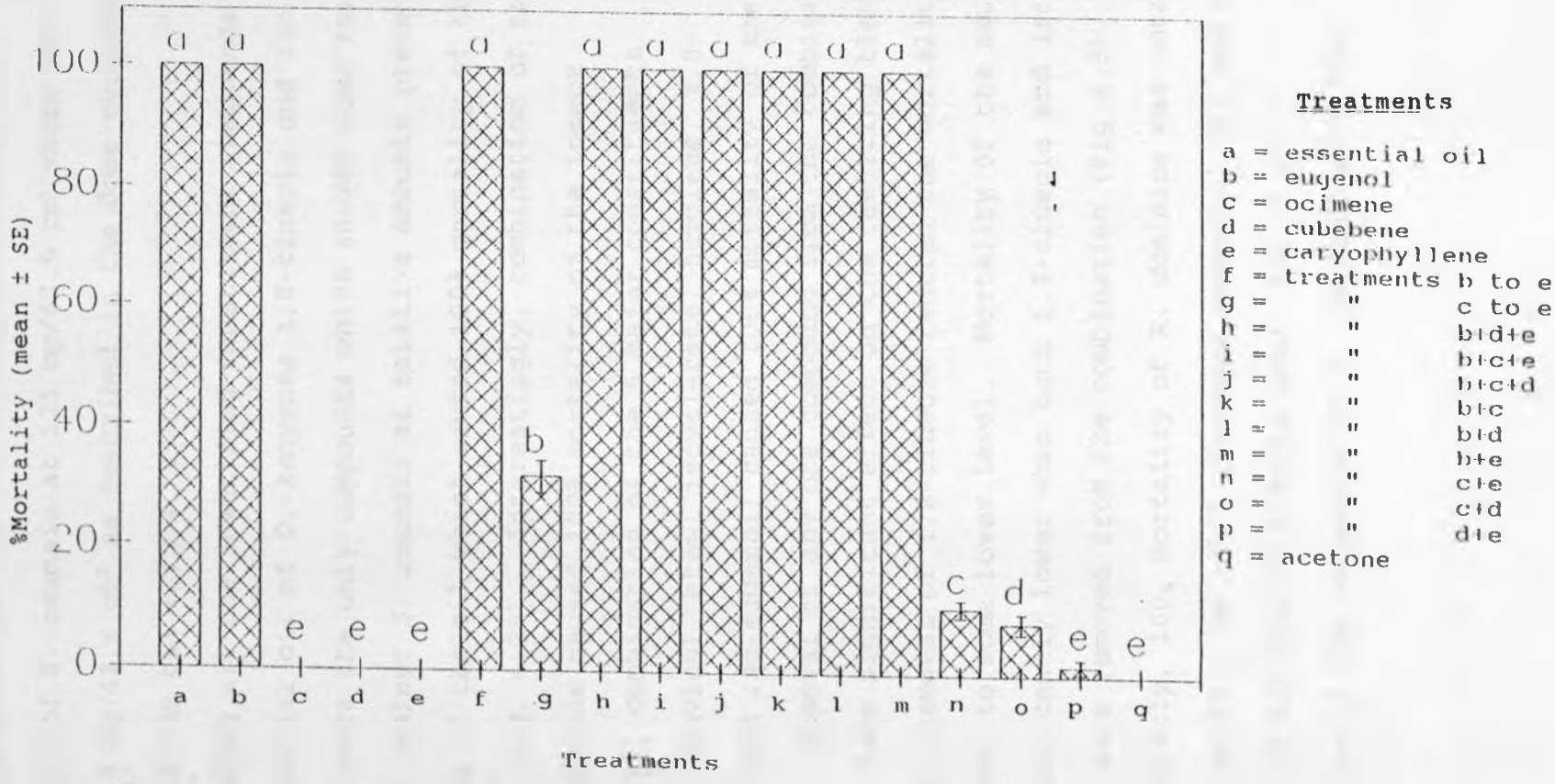


Fig. 4.28 Percent mortality of *R. dominica* due to different compounds and essential oil treatments of *O. suave*.
Histograms (average of 6 replicates) with the same letter are not significantly different from each other, SNK, $P < 0.05$.

4.7.2 Toxicity of *O. kenyense* essential oil

The essential oil of *O. kenyense* caused 100% mortality of S. zeamais at 125 mg/62.6 cm^2 where the LC_{50} was 53.5 mg/62.6 cm^2 as described in the dose response curve of the oil (Fig.4. 25).

Among all the identified constituent compounds of the essential oil of *O. kenyense* 1,8-cineole and *iso*-eugenol were the only compounds which showed some toxic effects against *S. zeamais* at relative amounts present in the dose of the oil which caused 100% mortality of the insect (Fig. 4.29). Interestingly, combination of the two compounds caused 100% mortality of the insect. Similarly, combination of the 5 major constituents (methyl chavicol, ethyl isovalerate, humulene, 1,8-cineole and *iso*-eugenol) caused 100% mortality of the insect. Removal of any one compound from the combination did not have significant effect on the resulting blend, although, removal of 1,8-cineole reduced the mortality of the insect to some lower level. Mortality of the adults was significantly lower when both 1,8-cineole and *iso*-eugenol were removed from the combination (Fig.4.9).

Similarly, 100% mortality of *R. dominica* was obtained with 40 mg/62.6 cm^2 of *O. kenyense* essential oil and the LC_{50} of the oil was 22.6 mg/62.6cm2 (Fig. 4.27).

None of the compounds of *O. kenyense* with the

exception of *iso*-eugenol caused any mortality of *R. dominica* individually at relative amounts present in the dose of the oil which was 100% lethal to the insect (Fig.4.30). The combination of the 5 major constituents in the same relative amount resulted in 100% mortality of the insect. Removal of any of the compounds from the combination did not have any significant effect on the toxicity of the resulting blend. Higher mortality rate of the insect was observed when both 1,8-cineole and *iso*-eugenol were present together with any one of the other 5 constituents than when they were not.

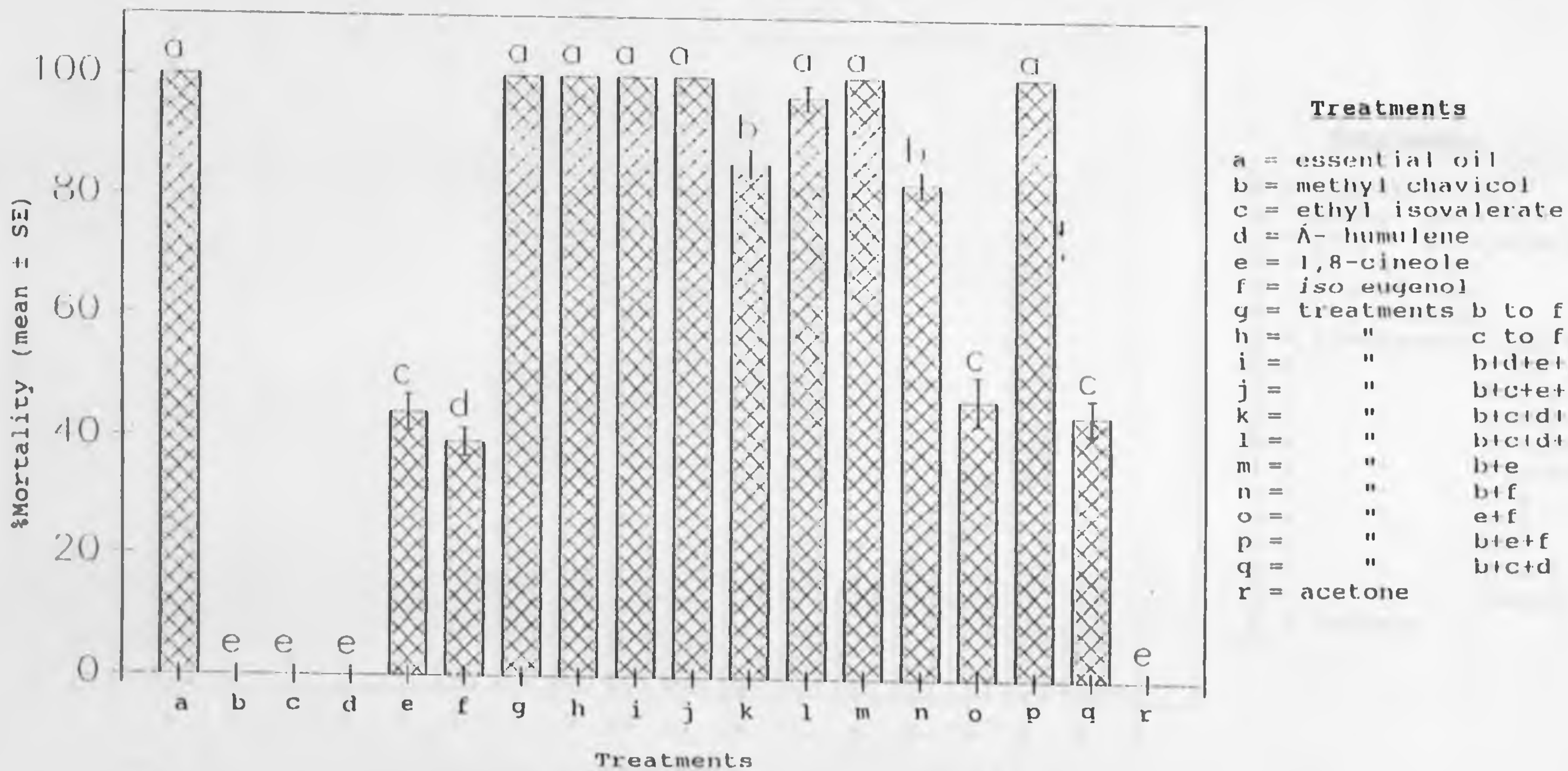
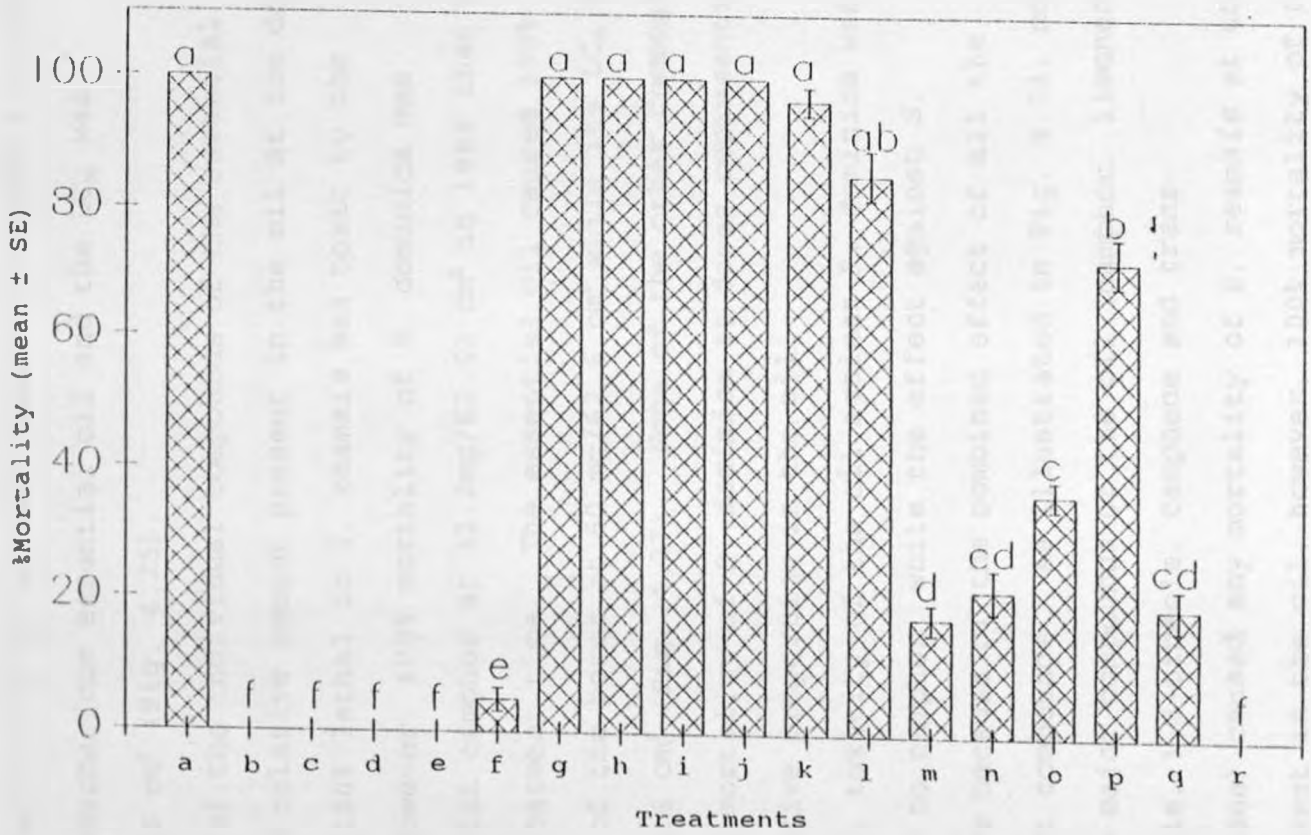


Fig. 4.29 Percent mortality of *S. zeamais* due to different compounds and essential oil treatments of *O. kenyense*.
Histograms (average of 6 replicates) with the same letter are not significantly different from each other, SNK, $P < 0.05$.



Treatments

a = essential oil
b = methyl chavicol
c = ethyl isovalerate
d = Á- humulene
e = 1,8-cineole
f = *iso* eugenol
g = treatments b to f
h = " c to f
i = " b+d+e+f
j = " b+c+e+f
k = " b+c+d+f
l = " b+c+d+e
m = " b+e
n = " e+f
o = " b+f
p = " b+e+f
q = " b+c+d
r = acetone

Fig. 4.30 Percent mortality of *R. dominica* due to different compounds and essential oil treatments of *O. kenyense*.
Histograms (average of 6 replicates) with the same letter are not significantly different from each other, SNK, $P < 0.05$.

4.7.3 Toxicity of *O. kilimandscharicum* essential oil

All adults of *S. zeamais* died at 90 mg/62.6 cm^2 of *O. kilimandscharicum* essential oil and the LC_{50} was 47.3mg/62.6 cm^2 (Fig. 4.25).

None of the individual compounds of the essential oil at the relative amount present in the oil at the dose which was 100% lethal to *S. zeamais* was toxic to the insect. However, 100% mortality of *R. dominica* was obtained with camphor at 32.2mg/62.63 cm^2 in less than 24 h after treatment time. The essential oil caused 100% mortality of the borer at 50 mg/62.6 cm^2 while its LC_{50} was 43.4 mg/62.6 cm^2 (Fig. 4.27). None of the other compounds caused any mortality of *R. dominica* at doses representing their relative proportion in the oil.

Thus, toxicity of the oil against *R. dominica* was mainly due to camphor, while the effect against *S. zeamais* was because of the combined effect of all the constituent compounds. As illustrated in Fig. 4.31, none of the six major compounds of the oil (camphor, limonene, 4-terpeneole, 1,8-cineole, camphene and *trans*-caryophyllene) caused any mortality of *S. zeamais* at the amount present in the oil; however, 100% mortality of the insect was obtained when all the compounds were mixed together. Removal of any of the compounds from the

mixture, except camphor, did not cause any significant effect on the toxicity of the resulting blends showing that the presence of this compound is essential for the toxicity of the blend. Combination of camphor and limonene caused lower mortality than the combination of camphor and 1,8-cineole. Significantly higher mortality rate of the insect was obtained when the three major compounds (camphor, limonene and 1,8-cineole) were combined together (Fig. 4.31). All other possible combinations of the compounds tested did not cause any mortality of the weevil.

Toxicity of the oil against *R. dominica* was mainly because of camphor. No mortality of the insect was obtained in the absence of camphor. Camphor alone or combined with other compounds caused 100% mortality of the borer. All the other compounds individually or in combination did not cause any mortality of the insect.

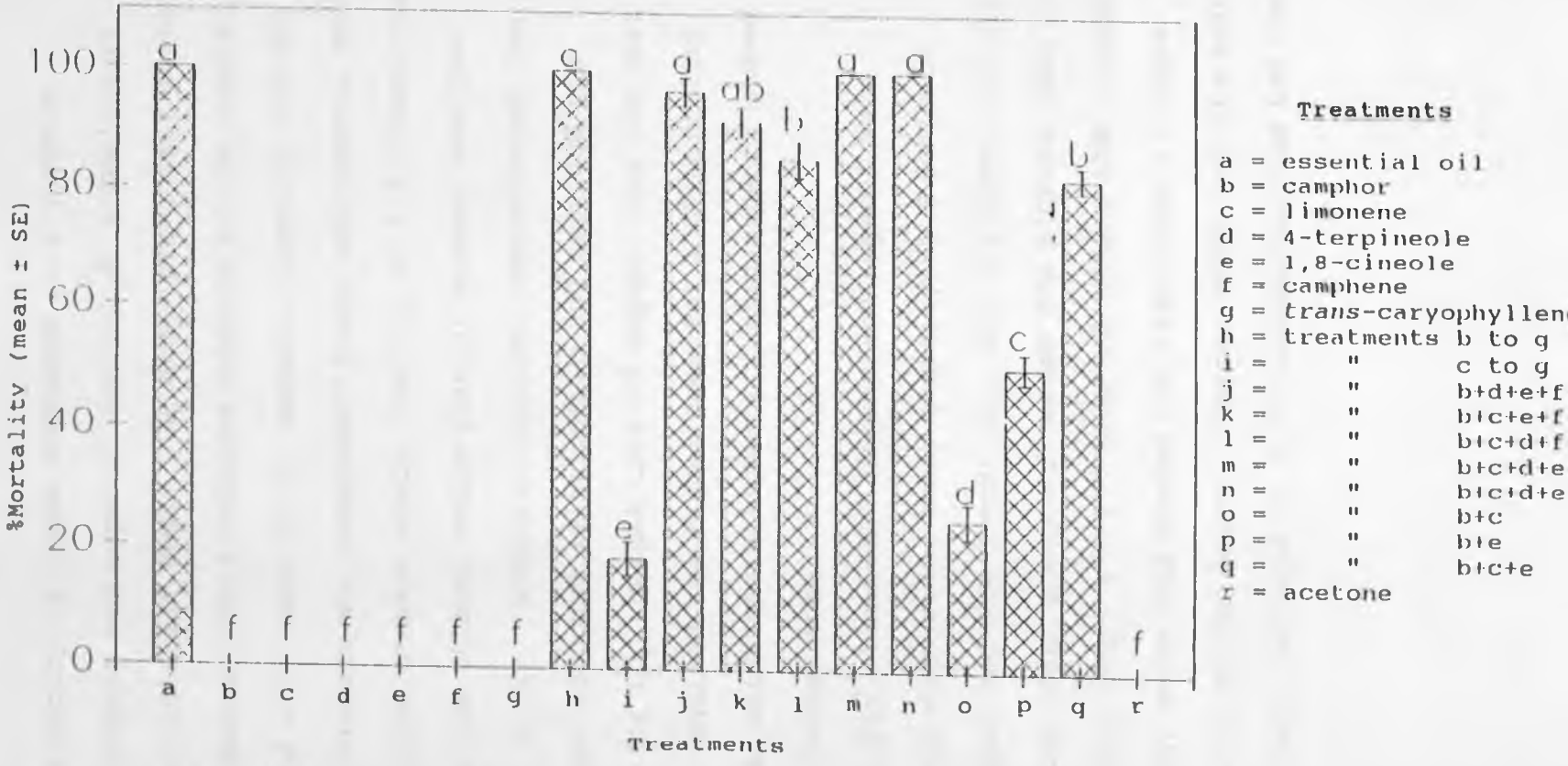


Fig. 4.31 Percent mortality of *S. zeamais* due to different compounds and essential oil treatments of *O. kilimandscharicum*.
Histograms (average of 6 replicates) with the same letter are not significantly different from each other, SNK, $P < 0.05$.

4.8 Toxicity comparison of different constituents of the essential oils

The toxicity of those compounds (1,8-cineole, linalool, camphor and eugenol) against *S. zeamais* were compared at different doses. At doses previously tested which represented their relative amounts in the quantity of essential oils toxic to *S. zeamais*, linalool was not toxic, 1,8-cineole was moderately toxic and eugenol most toxic . In the present study, the LC_{50} of 1,8-cineole was higher than the others, while that of eugenol was the least (Fig. 4.32). However, linalool demonstrated the steepest dose response relationship and required the least dose for LC_{50} whereas that of eugenol was the most gentle and required the higher dose for 100% mortality. 1,8-cineole, which had a higher LC_{50} than eugenol showed lower 100% lethal dose.

Similarly, linalool, camphor and eugenol were compared with respect to their toxicity to *R dominica*. Again, eugenol had the lowest LC_{50}, but its dose-toxicity curve was the least steep and needed the highest dose for 100% mortality (Fig. 4.33). Linalool showed the steepest dose-toxicity curve and needed the least dose to cause 100% mortality of the borer. Camphor which was the most important toxic compound in *O. kilimandscharicum* had the

least LC_{50}, but intermediate steepness in its dose-toxicity relations.

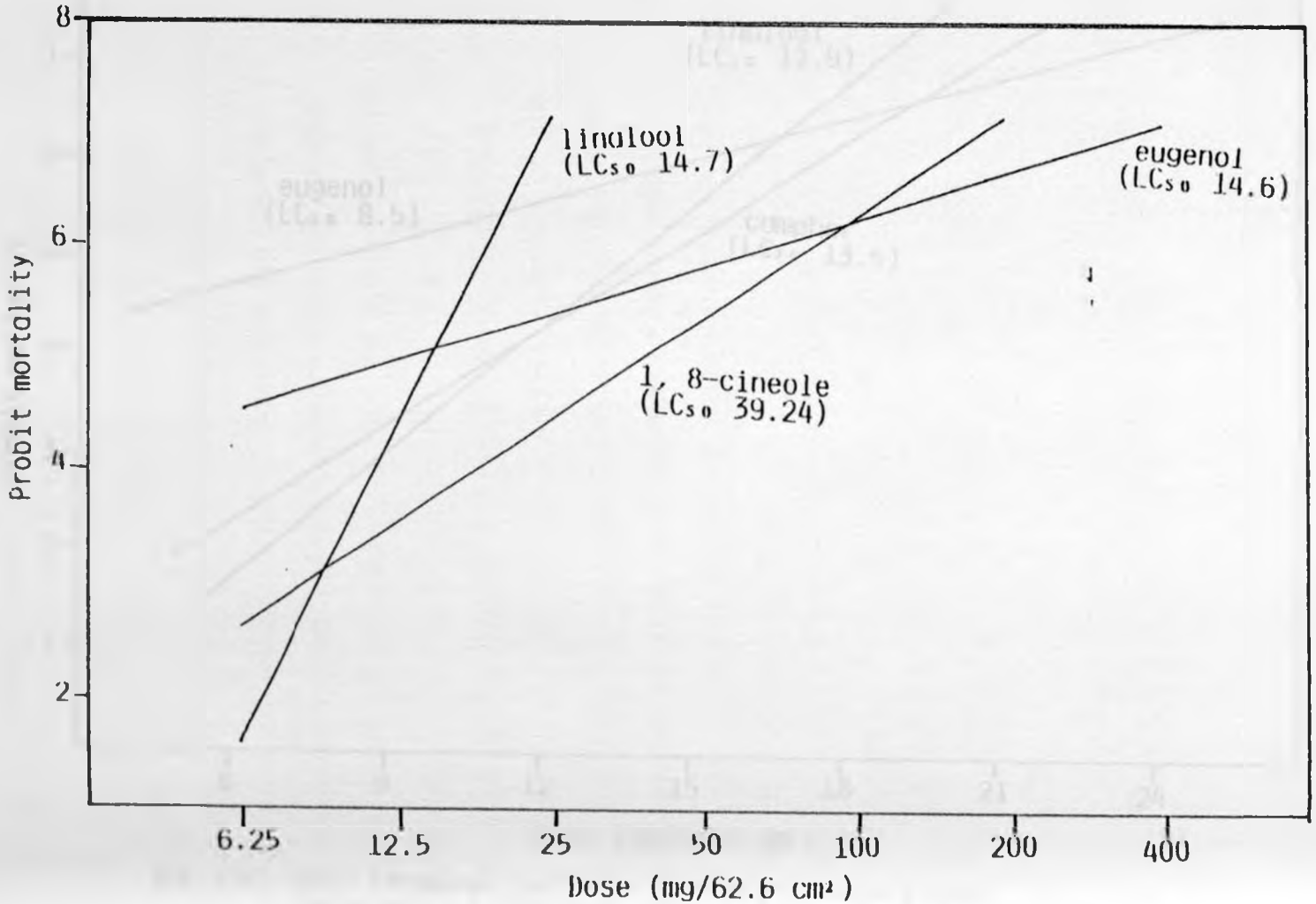


Fig. 4.32 Dose response curve of different constituent compounds of the essential oils against *S. zeamais*

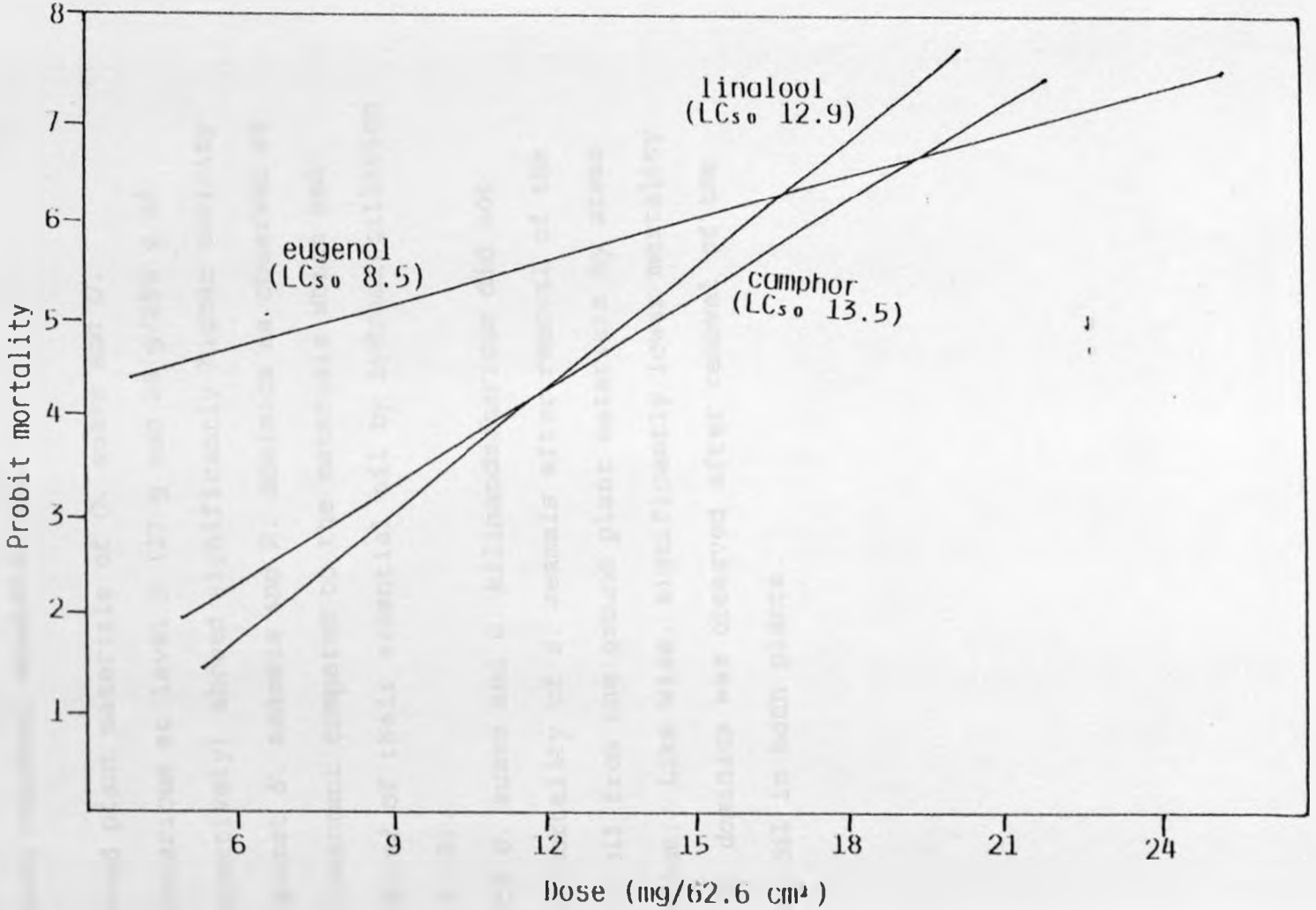


Fig. 4.33 Dose response curve of different constituent compounds of the essential oils against *R. dominica*

4.9 Toxicity of dried, ground plant materials with and without residual essential oil

Ground plant materials of *O. suave* and *O. kilimandscharicum* at level 3 (27 g and 25 g/250 g of seeds respectively) showed significantly higher toxicity effect against *S. zeamais* and *R. dominica* as observed 48 h after treatment compared to the materials which had been deprived of their essential oil by hydrodistillation (Table 4.18).

Both *O. suave* and *O. kilimandscharicum* did not cause any mortality of *S. zeamais* after removal of the essential oil from the ground plant materials by steam distillation. Like wise, significantly lower mortality rate of *R. dominica* was observed after removal of the essential oil in both plants.

Table 4.19 Toxicity of ground plant materials of *O. kilimandscharicum* (a) *O. suave*, (b) with and with out essential oil, against *S. zeamais* and *R. dominica*

Treatments	*S. zeamais* %mortality[1] *	*R. dominica* %mortality[1] *
(a) *O. kilimandscharicum*		
GrOkl	86.3 ± 5.52a	100.0 ± 0.00a
GrOkl,without oil	0.0 ± 0.00b	10.0 ± 3.00b
Control	0.0 ± 0.00b	0.0 ± 0.00c
(b) *O. suave*		
GrOs	11.3 ± 4.33a	32.5 ± 4.33a
GrOs,with out oil	0.0 ± 0.00b	6.3 ± 1.33b
Control	0.0 ± 0.00b	0.0 ± 0.00c

1. Average of 4 replications
*. In columns, treatment means with the same letter are not significantly different from each other, SNK, $P < 0.05$.
GrOkl, ground *O. kilimandscharicum*; GrOs, ground *O. suave*.

CHAPTER 5

Discussion

5.1 Responses of the test insects to different treatments of the three plants

In the present study, three *Ocimum* species in different forms were evaluated against *S. zeamais, R. dominica* and *S. cerealella*. The effects of the plant materials included their toxicity and repellency. *S. cerealella* was most susceptible to the toxic effect of all treatments of the three plants while, *S. zeamais* was the most resistant (Fig 4.1, 4.8, 4.15). On the other hand, *S. zeamais* was most repelled by the plants compared to the other two insects (Table 4.1, 4.3, 4.5). These differences may be due to the variation of the insects' responses to secondary plant metabolites.

Response of insects to toxic effects of secondary plant metabolites depends on their sex, size, age and physiological status (Busvine, 1971). Insect susceptibility to these metabolites also depends on their mode of administration, the chemical composition of the constituent compounds and their structural features. Thus, toxic and other physiological effects of one crude essential oil upon

a particular insect can be less noticeable upon an other (Busvine, 1971; Weaver et al., 1991; Roger et al.,1993). These kinds of differences in insect responses have led to a general theory of co-evolution between plant and insects (Feeny, 1976). Terpenoids and alkaloids both common classes of allelochemicals were absent in plants until about 200 million years ago. Their emergence appears to have coincided with the development of modern insect life and suggests one possible reason for their evolution (Smith, 1989). According to the co-evolutionary theory, plants evolved and began to utilize a number of novel metabolic pathways which produced compounds noxious to insects and other herbivores, with a consequent improvement in their competitiveness. However, the herbivores themselves evolved and began utilizing these metabolites, detoxifying them or simply avoiding them. The first two evolutionary responses led to the polyphagous mode of life, while the latter evolutionary response led to specialization on certain plants and to a mode of life that reflected this specialization. Thus, the susceptibility of the test insects, in the present study, to the repellent effects of the plants could be largely attributed to the species mode of life and their evolutionary line in addition to other factors. *S. zeamais* is mainly confined to stored grains and it would be anticipated

that it would be repelled most by non-host plant chemicals. *R. dominica* is originally from the wood-boring family (the Bostrichidae) and sometimes bores into the stems of bamboo hence it is padapted to a broad range of chemicals. *S. cerealella* adults do not feed on grains while only the larvae and pupae are confined to grain seeds (Cotton, 1963). On the other hand, susceptibility to the toxic effects of the plants' chemicals may be attributed to the size of the insects: *S. zeamais* is the largest and least susceptible. However, other factors such as the insect adaptive physiology and the mode of action of the compounds may also play a role.

5.2 Variation in the effects of different plants to different insects

In the present study, *O. kilimandscharicum* was found to be the most toxic of the plants to all the three storage insect pests. This was followed by *O. suave*. *O. kenyense* being the least toxic. In all treatments that did not cause significant mortality (fresh and dry materials), most of the adults of the insects survived until their oviposition time ((Fig 4.3, 4.10, 4.17). The surviving adults reproduced and the highest number of F1 progeny was found on seeds treated with fresh materials level 3 of all the plants with the exception of on seeds treated with *O.*

kilimandscharicum (level 3) and infested with *S. cerealella*. As expected high weight losses and number of damaged seeds were observed in treatments associated with large size of progeny (Fig. 4.4, 4.11, 4.18). These differences between the effects of plants may be attributed to the variation in the composition of secondary plant metabolites and mode of life of the insects.

Secondary plant chemicals attract or repel insects and influences their locomotor, ovipositional, feeding behavior, developmental and physiological processes as well as behavioral patterns (Beck and Reese, 1976). Metcalf and Metcalf (1992) have suggested that among the secondary plant metabolites, the already identified 3000 terpenoid compounds affect the lives of 500,000 or so species of insects in different ways. Toxicity is one of the various effects of plant terpenoids to insects (Rhodes, 1976; Metcalf and Metcalf, 1992) Thus variation in the toxic effects of some plants can be attributed to their essential oil composition, which in turn is affected by the growing conditions and interaction of the plants with pests and diseases (Hedin, 1976). Variation in the different effects of the plants can also be attributed to the method of application of the plants which is discussed in the following sections.

5.2.1 Variation in the toxic effect of the different treatments against the three insects

Among the different forms of treatments evaluated essential oils of *O. suave* and *O. kenyense* at levels 2 and 3 were toxic to *S. zeamais* and *R. dominica* while, ground materials of *O. suave* at levels 2 and 3 and that of *O. kenyense* at level 3 were toxic only to *R. dominica* (Fig. 4.1, 4.8). Essential oil and ground materials of *O. kilimandscharicum* at all levels of application showed toxicity to both *S. zeamais* and *R. dominica* (Fig. 4.15). All treatments of the three plants were toxic to *S. cerealella* (Fig. 4.2, 4.9, 4.16). Essential oil and ground materials of *O. kilimandscharicum* at level 3 were the most toxic inducing 100% mortality in all the three insects.

Toxicity of the ground plant material can be attributed to the residual essential oils of the plants as removal of these from the powders of *O. kilimandsharicum* and *O. suave* by hydrodistillation resulted in very low or no mortality of both *R. dominica* and *S. zeamais* (Table 4.18). The very low mortality of *R. dominica* may be attributed to the trace levels of the essential oils left in the free powder. The toxic effect of plant essential oils has been reported by a number of authors (Smith, 1965;

Ryan and Byrne, 1988; Singh et al., 1989; Coats et al., 1991; Shaaya et al., 1991; Weaver et al., 1991; Roger et al., 1993) who attributed their effect to different terpene constituents of the essential oils . Similarly, variation in the toxicity of the essential oils of *Ocimum* plants in the present study, can be attributed to their essential oil constituents. Low or lack of toxicity of the ground materials of *O. suave* and *O. kenyense* may be attributed to the loss of some toxic volatile constituents of the essential oils through volatilization during the drying period.

Essential oil and freshly milled dry plant materials of *O. kilimandscharicum* when initially added to the seeds were effective in protecting the maize and sorghum seeds. However, long-term protection by such treatments may be less reliable as toxic effects did not last for more than 4 days (Table 4.6). *O. kenyense* was least promising for practical use. In addition to low level of toxicity of its essential oil its effect also had a low persistence of only 4 days (Table 4.4). *O. suave* essential oil though less toxic than that of *O. kilimandscharicum* was more persistent(Table 4.2). This could be attributed to the tendency of its toxic constituents to vaporize and adhere to the surface of the treated medium as this has been shown to be a source of persistence of some

plant materials or essential oils (Weaver et al., 1991).

5.2.2 Effect of the three Ocimum plants on the reproduction of the three insects

Fresh and dry material of the three plants treated maize and sorghum seeds did not affect the survival of *S. zeamais* and *R. dominica*. *On the other hand, S. cerealella* was affected by fresh and dry materials of *O. kilimandscharicum* and *O. kenyense* (Fig. 4.3, 4.10, 4.17). However, the survived adults of all the insects laid their eggs during their normal oviposition times and reproduction was not significantly affected. Thus, none of the treatments had significant effect on the viability of laid eggs.

An interesting observation is the enhancement of reproduction of the insects in seeds treated with the highest dose of fresh plant materials. This is attributed to the high humidity maintained by the fresh leaves in the glass jars. About 80% of the fresh plant materials was water. This would be expected to evaporate during the course of the experiment and the resulting moisture being observed by the dry grain. Indeed, this was reflected in the germination of some of the seeds in these treatments.

High humidity in the presence of other optimum conditions (temperature, food) enhances reproduction

of insects (Cotton, 1963; Appert 1987). A similar effect was observed by Cotton (1963) who found that percent moisture content increment from 9 to 14% in wheat increased the number of F1 progeny produced by *S. oryzae* from 0 to 951 at 60^{O}F and from 0 to 13,551 at 80^{O}F in 5 months storage time. Similarly, spraying with pirimiphos methyl (actelic 25%) in water on 250 g of sorghum seeds at the rate of 8 ppm gave *R. dominica* F1 progeny of 839 compared to 32 produced in the untreated seeds (Bekele, 1989). This indicates that the insecticide at the dose used was not toxic to the insect and the water (carrier of the insecticide) increased moisture content of the sorghum seeds and so reproduction was enhanced.

5.2.3 **Effect of the three Ocimum plants on feeding activity of the three insects**

Maize seeds treated with the highest level of fresh materials (level 3) of *O. suave, O. kenyense* and *O. kilimandscharicum* were severely damaged due to feeding by *S. zeamais* (Fig. 4.5, 4.12, 4.19). In all these treatments large F1 progenies were also produced by the insects. Similar result were obtained for *S. cerealella* on maize seeds treated with the highest dose of *O. kenyense* (Fig.4.7). Under conditions favorable for storage insects, the extent of damage is related to the number of insects present (Cotton,

1963). This finding is also in agreement with what Bekele (1989) observed with sub-lethal dose of pirimiphos methyl on sorghum seeds where water was the carrier of the insecticide. Percent weight loss was increased to 43.3% in treated seeds compared to the 16% weight loss in the untreated seeds.

In the case of *R. dominica*, %weight loss was not as high as the number of damaged seeds in sorghum seeds treated with fresh *O. kenyense* and *O. suave* (Fig. 4.6, 4.13, 4.20). Similarly, %weight loss was not as high as number of damaged seeds in *O. suave* fresh level 3 treated maize seeds due to feeding by *S. cerealella* (Fig. 4.7). These results suggest that under these treatments, feeding by could have been periodically interrupted by chemical constituents of the materials, thus resulting in more seeds being damaged but corresponding to lower level of weight loss.

Some secondary plant metabolites may act both as insecticides and antifeedants as has been observed for rotenone against *T. castaneum* (Nawrot et al., 1989). Reinhold (1970) reported that some terpenoid such as rotenone and pyrethrins have antifeedant effect in addition to their insecticidal properties. Similarly, Sharma et al. (1991) tested seven different essential oils for their antifeedant activity against *Spodoptera litura* and found two these have antifeedant effect.

5.2.4 Repellency effect of the three Ocimum plants on the three storage insects

Our study showed that whereas *S. zeamais* was repelled in virtually all treatments involving different forms of the three plants, results with *R. dominica* and *S. cerealella* were variable. Interestingly, as pointed out earlier *S. zeamais* is highly specialized to stored products and mainly confined to stores and its sensitivity to volatile secondary compounds from exotic sources may be reflection of this specialized mode of life.

R. dominica was indifferent to fresh *O. suave* but was repelled by dried materials and its essential oil suggests that in the fresh material, the more volatile components probably lost during drying may neutralize the less volatile but repellent constituents. On the other hand, a reverse trend was observed with respect to *O. kenyense* which suggests that it is the more volatile fraction of this plant which is more repellent to the insect. All, except the fresh materials of *O. kilimandscharicum* showed varying degrees of repellency to the insect. However, the lowest dose of the fresh material was significantly repellent. Interestingly, the same trend was observed with fresh *O. kilimandscharicum* with

respect to *S. cerealella*. These puzzling results are difficult to explain, but may be due to different dose-response effects of the different volatile constituents some of which may be attractant and some repellent. A detailed investigation of the effects of the individual constituents and different blends of these would throw some light on the question.

S. cerealella was attracted to fresh *O. suave* but repelled by its essential oil and the highest dose of the dried material. Thus, the more volatile components of the plant are attractant to the insect while the less volatile constituents, enriched during drying, are repellent to the insect. On the other hand, only fresh *O. kenyense* was significantly repellent showing that the more volatile fraction of this *Ocimum* species is repellent to this insect. With respect to *O. kilimandscharicum*, the lowest dose of fresh plant was repellent as in the case of *R. dominica* and probably for the same reason. In addition, the essential oil and the highest dose of dried materials were also repellent suggesting that the less volatile fraction of this plant is repellent to *S.cerealella*. Interestingly, during the bioassays with seeds treated with essential oil and ground materials, none of the insects were attracted to the seeds presumably by residual volatile constituents. After 24 h the insects were found dead probably

through toxication by the less volatile constituents. Thus, in the volatile constituents of *O. kilimandscharicum* represent a very interesting composition with respect to *S. cerealella* with volatile attractants and less volatile allomonial compounds. Further research is needed to exploit such a composition of insect-active compounds.

The repellent effect of all treatments of *O. kilimandscharicum* and *O. suave* could be attributed to their major components camphor, 70% and eugenol, 60% respectively.

Camphor is a known moth repellent (The Mrk Index 1983). The repellent effect of eugenol against *S. zeamais* was clearly demonstrated by Hassanali et al. (1990). Interestingly, eugenol is attractive to Japanese beetle (Ladd, 1980; Ladd, et al., 1983) and corn root worm (Lance, 1988). This is in line with the suggestion that storage insects, unlike other phytophagous insects, did not have the chance to coevolve with secondary plant materials which would have allowed them to use allomonic secondary plant metabolites as kairomone (Kumar, 1984).

Small scale farmers in East Africa and Cameroon mix *O. suave* leaves with stored cereal products to protect them against infestation of storage insects (Hassanali et al., 1990; Kokwaro, 1976; Parh et al., 1990). The scientific basis for this practice of

could be attributed to the allomonal effects of the plants including their repellency.

5.3 Effect of the three Ocimum plants on germination maize and sorghum seeds

Essential oil of *O. suave* suppressed germination of both maize and sorghum seeds (Table 4.7, 4.6). Germination of maize and sorghum seeds treated with 150 and 125 g (level 3) of fresh plant materials of *O. kenyense* and *O. kilimandscharicum*, respectively, was significantly reduced (Table 4.10, 4.11, 4.12, 4.13). Other treatments did not have significant effect on the germination of the seeds. In the laboratory seeds treated with the latter two plant materials showed signs of germination while they were in the bottles. The effect of the fresh plant materials could be attributed to the high moisture content of the plant materials. The leaves, succulent stems and inflorescence of the two plants had more moisture (80%) compared to *O. suave*. This could have provided enough moisture to the extent of enhancing degradation of food reserves for the commencement of germination of the seeds. Such effect was not observed with fresh plant materials at levels 1 and 2 of both plants which indicates that the treatments did not have much moisture to the extent of enhancing germination. Some seeds started to germinate while

they were in the bottles (in store) and this was interrupted as the plant materials started to dry. Once the food reserves of seeds are mobilized for germination and germination does not occur because of interruption of favorable conditions they would not be useful for future germination. For this reason optimum germination of seeds may not be expected from seeds which have mobilized food reserves for germination (with no external signs of germination) during their storage time.

Essential oils and phenolic compounds are among the numerous compounds which inhibit germination of seeds while, compounds like potassium nitrate, thiourea, gebberellins (terpenoid) and kentia are germination promoters (Salisbury and Ross, 1986; Deviln and Witham, 1983). The effect of essential oil on germination seeds was also observed with essential oil of *Artmisia princeps* var *orientalis* where germination of tree seeds was suppressed with the essential oil (Yun et al., 1993). Cremlyn (1978) reported that phenolic compounds are powerful herbicides and therefore phytotoxic. In line with the above reports the effect of *O. suave* essential oil could be attributed to eugenol, which is the principal constituent of the essential oil and phenolic in structure. On the other hand, dry and ground plant materials of the same plant enhanced germination of

maize seeds. This effect might be due to less constituents, but this needs to be investigated in detail.

5.4 Toxicity of the three essential oils and their constituents

In filter paper disc bioassay, with respect to *S. zeamais* the oil *O. kilimandscharicum* was the most toxic followed by that of *O. kenyense* and than that of *O. suave*. This is reflected in the LC_{50} as well as the doses needed to induce 100% mortality of the insect (Fig.4.25). In case of *R. dominica*, LC_{50} of the oils was in the order of *O. suave* > *O. kenyense* > *O. kilmandscharicum*. However, because of the relative differences in the steepness of the dose-toxicity curves, the dose with respect to 100% mortality was *O. kenyense* . kilimandscharicum O. suave. Surprisingly, *O. kilimandscharicum* which was the most toxic against *R. dominica* in admixture treatment showed less relative toxicity in the filter paper bioassay (Fig.4.25). This may be due to differences resulting from physiological behavior of toxic constituents in different assays and suggests that care needs to be exercised in drawing conclusion from one type of bioassy. In this respect, Weaver et al. (1991) reported that in filter paper toxicity bioassay of

linalool, the compound appeared to be bound to the filter paper in the trials. They suggested that this could be a function of interaction between the hydroxy group of linalool and polar sites on the filter paper. Such interaction have to be stronger than the forces of volatilization and gaseous dissolution to be maintained. Since linalool acts as a fumigant (Weaver et al., 1991; Shaaya et al., 1991), reduced volatilization due to its interaction with the filter paper can reduce its toxicity effect and increase the dose needed to toxicate the insect. According to Weaver et al.(1991), most unprocessed food stuff (i.e. grains or beans) do not have polar surfaces so the dosage required for protection against post harvest damage may be considerably less. Like wise, the extent of susceptibility of the test insects to the various essential oils in the admixture and filter paper bioassays could have been determined by the surface interaction of the essential oils and the grains or the filter paper.

The composition of the essential oils would also affect their toxicity (Roger et al., 1993). Thus, eugenol the main constituent of oil of *O. suave*, was primarily responsible for the potency of this essential oil. It was toxic to *S. zeamais* and its absence from the combination of the 4 major constituent compounds of *O. suave* essential oil

significantly reduced the mortality of *S. zeamais* (FIg 4.26). Eugenol also caused 100% mortality of *R. dominica* at its natural proportion in the dose of essential oil which was 100% lethal to the insect, while none of the other identified compounds caused any mortality of the insect (Fig. 4.28). Toxicity of eugenol may be attributed to its phenolic structure (Cremlyn, 1978). The physiological activity of phenols is considerably greater than that of alcohols which are powerful insecticides, fungicides, bactericides and herbicides. However, other constituents may also contribute significantly to the toxicity of the blend. For example, linalool which is present in the essential oil of *O. suave* at a very low level had LC_{50} value against *S. zeamais* similar to that of eugenol (Fig. 4.32). The toxicity of linalool, and unsaturated alcohol, is also related to its chemical structure. Cremlyn (1978) has pointed out that unsaturated and cyclic alcohols have stronger insecticidal, fungicidal and bactericidal activity, unlike their saturated counterparts.

In the case of *O. kenyense* 1,8-cineole and *iso*-eugenol, which are the major constituents of the essential oil were toxic to *S. zeamais* at their natural relative proportion (Fig. 4.29). The other constituents of the oil did not show any toxicity effect against the insect. A blend of the two

compounds in the dose of the oil which caused 100% mortality of *S. zeamais* also caused 100% mortality of the insect. With respect to *R. dominica* none of the constituents of the *O. kenyense* essential oil except *iso*-eugenol caused any mortality of the insect at their natural proportions (Fig. 4.30). Higher mortality of the insect was obtained when both *iso*-eugenol and 1,8-cineole were present in a blend of the 5 major compounds of the essential oil. This is in agreement with the findings of Shaaya et al. (1991) and Shaaya Vladimir (1991) who screened 22 essential oils and their constituents and found that and ethers such as 1,8-cineole were toxic to *R. dominica* while the ether 1,8-cineole alone was toxic to *Tribolium castaneum*.

The toxicity of essential oil of *O. kilimandscharicum* to *S. zeamais* appears to be due to a combination of all its constituent compounds as no individual compound at its natural proportion caused any toxicity to the insect although, camphor, limonene and 1,8-cineole were the principal compounds responsible (Fig. 4.31). On the other hand, the toxicity of the essential oil against *R. dominica* was primarily due to camphor as no other compound caused any mortality of the insect at its natural proportion in the dose of essential oil which was 100% lethal to the insect. Ryan and Byrne (1988) have reported that

pulegone, (a ketone) linalool (an alcohol) and 1,8-cineole (an ether) were toxic to *T. castaneum* because of they were reversible competitive inhibiters of the enzyme acetyl choline estrase. They further noted that the compounds apparently occupyed the hydrophobic site of the enzyme's active center. Camphor being a ketone compound may similarly have a similar effect as pulegone. Camphor is present in the essential oil of *O. kilimandscharicum* at about 70%.

Interestingly, limonene which is available commercially as shampoo, dip and aerosol for the control of cat flea (*Ctenocephalides felis*) control (Hink, 1986) was not toxic to either *S. zeamais* or *R. dominica* in amounts that represented their relative proportions in the dose of the essential oil of *O. kilimandscharicum* which caused 100% mortality of the insect. The doses, used in our tests were 120 μg and 100 $\mu g/cm^2$ for *S. zeamais* and *R. dominica* respectively while the LC_{50} of the compound against the cat flea was 160 $\mu g/cm^2$ (Hink, 1986). If present in the essential oil in larger proportions the compound may have contributed significantly to its allomonal effects.

The essential oil composition of a given plant varies considerably depending on its geographical location (Roger et al., 1993). Essential oil of *O. kilimandscharicum* collected from Ngong (Nairobi)

contained lower camphor (40%) compared to those plants collected from Ugunja (Siaya district) which contained 70% camphor. This variation the chemical composition of the plant was reflected on the toxicity of the oil. Essential oil obtained from plants collected from Siaya district was more toxic to all the test insects compared to the essential oil obtained from the Ngong variety..

The camphor content of *O. kilimandscharicum* can be increased significantly by addition of nitrogen fertilizer as reported by Dahatonde and Joshi (1985) and Dahatonde (1986). This can lead to the production of more potent *O. Kilimandwscharicum* plant. According to Bestman, et al., (1989) the insecticidal activity of pyrethrins was enhanced by the addition of (-)-carvone (the main constituent of *Chrysanthemum balsamila* essential oil) against grain aphids. Similarly, there might be a possibility of reducing the volatility of the essential oil or camphor through appropriate formulation and therefore enhance their persistence.

5.5 Large scale evaluation of the effective plant material

Ground plant materials of *O. kilimandscharicum* were observed to be toxic to *R. dominica* and *S.*

zeamais at 2% and 10% levels of application, respectively. However, the toxic effect of these treatments were found not to be persistent, but the repellent effect of the treatments may be useful in protecting the seeds from infestation when the toxic effect ceases to act. Under small-scale rular conditions the use of ground plant materials of *O. kilimandscharicum* can be of dual advantage where such treatment is toxic as well as repellent to all insects tested. When the toxic effect of the plant ground plant material ceases to act the repellent effect may play the role of protecting the grains. Further investigation is required on the persistence of the repellent effect of the plant.

Some repellents directly or in the vicinity of crops interfere or mask the natural attractants of crops (Odebiyi, 1978). In line with this report the farmers practice of mixing seeds with some *Ocimum* plant species could have also played the same role. The repellent and toxic action of the *Ocimum* plants in protecting grains from the infestation of stored product insect pests increases the potential practical value of the plants. The farmers practices of mixing seeds with *Ocimum* plant materials can be worthwhile if combined with the ground plant materials treatments of *O. kilmandscharicum*. For example, treating seeds with the mixture of ground materials of both *O. suave* and

O. kilimandscharicum (at optimum level) could be more effective in controlling the storage insects. The ground materials of *O. kilimandscharicum* can kill the insects which are present in the store during the treatment time and further infestation can be protected by the ground plant materials of *O. suave* as the toxic effect of the latter is more persistent and the essential oil is more repellent. In this regard selection of plant materials requires prior consideration as the effectiveness of the plant materials vary from place to place.

In conjunction with the use of the *Ocimum* plant materials, periodical monitoring of the storage insects using grain probe traps can also be useful as a component of the control strategies of the storage insects.

CHAPTER 6

CONCLUSIONS

The study has established that:-

1. Essential oils of all the plants, and ground plant materials of *O. kilimandscharicum* were toxic to all the test insects used.
2. *S. cerealella* was susceptible to the toxicity of the *Ocimum* plant species in all treatments. Lower levels of essential oils and ground plant materials were enough to cause 100% mortality of the insect compared to the other two insects.
3. Toxicity of *O. kilimandscharicum* essential oil against *R. dominica* was mainly because of camphor while, toxicity of the oil against *S. zeamais* was mainly due to camphor, limonene and 1,8-cineole.
4. Toxicity of *O. suave* essential oil against *S. zeamais* and *R. dominica* was mainly due to eugenol.
5. Toxicity of *O. kenyense* essential oil against *S. zeamais* and *R. dominica* was attributed mainly to 1,8-cineole and *iso*-eugenol.
6. Toxicity of ground plant materials of *O. kilimandscharicum* and *O. suave* was due to the essential oils of the plants.

7. Toxicity of *O. suave* essential oil was more persistent compared to the essential oils of the other two plants.
8. Dry and ground plant materials and essential oils of the three plants were repellent to all insects. Fresh plant materials of the three plants were significantly repellent to *S. zeamais*. Fresh plant materials of *O. suave* was attractant to *S. cerealella*.
9. *O. suave* in all forms was the most repellent among the plants while, *S. zeamais* was the most susceptible to the repellent effect of the three plants among the insects.
10. Some of the treatments involving the three plants had no any direct effect on reproduction and feeding. However, the highest levels of fresh plant materials of the more succulent plants increased reproduction rate of the three insects attributed to increased humidity in the glass jars.
11. Essential oil of *O. suave* reduced germination of maize and sorghum seeds while, dry plant materials of the same plant enhanced germination of maize seeds. Other treatments of the same plant or all treatments of the other plants did not have any and significant effect on the germination of maize and sorghum seeds.

12. Ground plant material of *O. kilimandscharicum* can protect sorghum seeds from *R. dominica* at the rate of 2% and maize seeds from *S. zeamais* at the rate of 10% in bulk storage.
13. Pheromone baited grain probe trap is the best sampling method for sampling *R. dominica* in sorghum seeds while, grain probe was more efficient in sampling out *S. zeamais* in maize seeds compared to sieve sampling.
14. Fresh plant materials of *O. kenyense* affected germination of maize seeds while, fresh plant materials of *O. kilimadscharicum* affected germination of both maize and sorghum seeds at the highest levels of application by increasing humidity within the glass jars. If fresh plant materials of the two plants are required to be applied at high rates for grain protection it should be in a well ventilated container or should be placed on the top of the seeds.

During centuries of warfare between mankind and his insect enemies, chance observation or desperate experiment revealed that certain plants and minerals provided useful products which could ward off or even control the invading insects. The eventual realization of these ordinary chemical substances, some of them quite simple, indeed could serve broadly as ecological weapons

led directly to the development of the numerous synthetic insecticides we know today (Jacobson, 1989). In line with this, the plant materials and secondary compounds isolated from them, including essential oils deserve further investigation to help mankind identifiy cheep, redily available and environment friendly pest control agents.

CHAPTER 7

REFERENCES

Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. **J. Econ. Entomol. 18:265-67.**

Agnew, A. D. Q. (1974). Upland Kenya wild life flowers; a flora of the ferns and herbaceous flowering plants of upland Kenya. **Oxford university Press. 827 pp.**

Annonymous, (1966). International Seed testing Association: the germination test. **Seed Sci, Tech. 4: 99-131.**

Appert, J. (1987). The storage of food grains and seeds. **Macmilan Publisher. Hong Kong. 146 pp.**

Back, E. A. and Cotton, R. T. (1938). Stored grain pests. **U. S. Dept. Agr. Bull. 47 pp.**

Barak, A. V. and Burkholder, W. E. (1976). Trapping studies with Dermestid sex pheromones. **Environ. Ecol. 5(1): 111-114.**

Beck, S. D. and Reese, J. C. (1976). Insect-plant interaction: nutrition and metabolism: In biochemical interaction between plants and insects: **Recent Advances in Phytochemistry. 10: 168-213.**

Bekele, J. (1989). Residual toxicity of three plant essential oils against three storage insects. **University of Los Banos, M. Sc. thesis, Philippnes. 84 pp.**

Bell, W. J. and Carde, R. T. (1984). Chemical ecology of insects. **Chapman and Hall. 524 pp.**

Bestman, H. J.; Classen, B.; Vostrowsky, O.; Klingaut, F. and Stein, U. (1989). Synergistic activity of *levo* carvone and pyrethrin I in the essential oil of *Chrysanthemum balsamila* L. **Abst. Entomol. 20(1): 15.**

Birch, L. C. (1944). Two strains of *Calandra oryzae* (L.). **Aust. J. Exp. Biol. Med. Sci. 22: 231-255.**

Black, E. A. and Cotton, R. T. (1938). Stored grain pests . U. S. Dept. Agri. Farmers Bull. 1260, 354 pp.

Bowers, W. S. and Nishida, R. (1980). Juvocimenes: potent juvenile hormone mimics from sweet basil. **Science. 209: 1030-1032.**

Burkholder, W. E. Michael, M. (1985). Pheromones for monitoring and control of stored product insects. **Ann. Rev. Entomol. 30:257-272.**

Busvine, J. R. (1971). A critical reviev of the techniques for testing insecticides. **Commonwealth Agricultural Bureaux (CAB). 345 pp.**

Charles, D. J. and Simon, J. E. (1990). Comparison of extraction methods for the rapid determination of essential oil content and composition of basil. **J. Ame. Sco. Hort. 115(3): 458-462.**

Chaven, S. R., Shah, N. P. and Nikan, S. T. (1983). Individual and synergistic activity of some essential oils as mosquito larvicidal agents. **Bull. Haffkine Ints. 11, 18-21.**

Chogo, J.B. and Crank, G. (1981). Chemical composition and biological activity of the Tanzanian plant *Ocimum suave*. **J. Nat. Prod. 44(3): 308-311.**

Cline, L. D. (1972). Rearing the lesser grain borer on ground dog biscuits to obtain mature larvae and pupae. **Ann. Entomol. Soc. Amer. 66(3): 551-552.**

Coats, J. R., Karr, L. L. and Drewes, C. D. (1991). Toxicity and neuro toxic effects of monoteropenoids, pp. 305-306. In naturally occuring pest bioregulators. **ACS symposium series 449. Amer. Chem. Soc. Washington, D. C.**

Colin, M. E. (1990). Essential oils of Labiatae for controlling honey bee varoosis. **Abs. Entomol. 21(11): 9.**

Cotton, R. T. (1963). Pests of stored grain and grain products. **Burgess Publishing Company. 318 pp.**

Cremlyn, R. (1978). Pesticides; preparation and mode of action. **John Wiley and Sons. 239 pp.**

Dahatonde, B. N. (1986). Studies on the response of Kapur tulsi (*Ocimum kilimandscharicum*) to nitrogen and phosphorus fertilization. **Pakistan Res. J. 10(1): 19-22.**

Dahatonde, B. N. and Joshi, B. G. (1985). Studies on the response of nitrogen fertilization on the vegetative growth, camphor and oil yield from Kapur tulsi (*Ocimum kilimandscharicum*). **Punjabrao Kirishi Vidyapeeth Res. J. 9: 1.**

Devlin, R. M. and Witham, F. M. (1983). Plant physiology. Fourth ed. PWS Publishers, Wadsworth **Inc. 577 pp.**

Dichter, D. (1976). The stealthy thief. **Ceres, F.A.O. Rev. 9 (4): 51-55.**

Dube, S. Upadhyay, P. D. and Tripathi, S. C. (1989). Antifungal, physiochemical and insect repelling activity of the essential oil of *Ocimum basilicum*. **Canadian J. Bot. 67(7): 2085-2087.**

Dubey, N. K.and Kishore, N. (1987). Fungitoxity of some higher plants and synergistic activity of their essential oils. **Tropical Sci. 27(1): 23-27.**

Ekundayo, O. (1986). Essential oils: volatile constituents of the leaves of *Ocimum viride*. **Planta Medica, 3: 200-202.**

F. A. O. (1985). Prevention of post harvest food losses. **Training series No. 10. Italy, Rome. 122 pp.**

Feeny, P. P. (1991). Theories of plant chemical defense: A brief historical survey; In insects-plants. **Akademiai Kiado, Budapest. pp. 163.**

Finney, D. J. (1978). Statistical method in biological assay. 3rd edition. **Charles Griffin and Company Ltd. London. 508 pp.**

Fukami, H. and Nakajima, M. (1971). Rotenone and rotenoids. In naturally occuring insecticides. **Marcel Dekker, Inc. pp. 71-97.**

Fun, C. E., Svendsen, A. B. and Baerheim, S. A. (1990). Composition of the essential oils of *Ocimum basilicum* var. *canum* and *O. gratissimum* L. grown on Aruba. **Flavour Fragr. J. 5(3): 173-177.**

Gaydou, E. M., Faure, R., Bianchini, J. P., Lamaty, G., Racotonirainy, O. and Rabdriamiharisoa, R. (1990). Sesquiterpene composition of basil oil. **Chemorec. Abst. 18(2): 47.**

Geissman, T. A. and Crout, D. H. G. (1969). Organic chemistry of secondary plant metabolites. **Freeman, Cooper and Company. 592 pp.**

Georghiou, G. P. (1986). The magnitude of the resisitance problem. In pesticides resisitance, strategies and tactics for management. **National Academy Press, Washington D. C. pp. 14-43.**

Gomez, K. A. and Gomez, A. A. (1984). Statistical procedures for agricultural research. Second edition **John Wiley and sons, Inc. 680 pp.**

Grossman, J. (1993). Botanical pesticides in Africa. **The IPM Practitioner, 15(1): 1-9.**

Gunderson, G. A., Samuelien, J. H., Evans, C. K. and Brattsten, L. B. (1985). Effects of mint monoterpenes pulegone on *Spodoptera eridona* (Lepidoptera: Noctuidae). 14(6): 859-863.

Hall, D. W. (1970). Handling and storage of food grains in tropical and sub-tropical areas. **F. A. O. Development Paper No. 90. Rome. 350 pp.**

Halliday, W. R., McGovern, T. P. and McDonald, L. L. (1986). Cycloalkyl and cycloaryl esters of hydroxybenzeneacetic acid as repellent of adult *Tribolium confusum* in the laboratory. **J. Entomol. Sci. 21(4): 322-328.**

Halstead, D. G. H. (1963). External sex difference in stored products Coleoptera. **Bull. Entomol. Res. 54: 119-134.**

Hardborne, G. B. (1973). Phytochemical methods. **Chapman and Hall, London. 278 pp.**

Hassanali, A. and Lwande, W. (1989). Anti-pest secondary metabolites from African plants; In insecticides of plant origin . **Am. Chem. Soc.Symp. Ser. 387, 78-94**

Hassanali, A., Lwande, W., Sitayo, O., Moreka, L., Nokoe, S. and Chapya, A. (1990). Weevil repellent constituents of *Ocimum suave* leaves and *Eugenia caryophyllata* cloves used as grain protectant in parts of East Africa. **Discov. Inovat., 2(2): 91-95.**

Hedin, P. A. (1976). Use of natural products in pest control; developing research trends. In naturaly occuring pest bioregulators. **Amer. Chem. Soc. Washington DC. ACS symposyium series 449. pp.11.**

Hink, W. F. and Fee, B. J. (1986). Toxicity of D-limonene, the major component of citrus peel oil, to all life stages of the cat flea, *Ctenocephalides felis* (Siphanoptera: Pulicidae). **J. Med. Entomol. 23(4): 400-404.**

Hink, W. F., Liberati, T. A. and Collart, M. G. (1988). Toxicity of linalool to life stages of cat flea, Ctenocephalides felis (siphanoptera: Pulcidae), and its efficacy in carpet and on animals. **J. Med. Entomol. 25(1): 1-4.**

Howe, R. W. (1952). The biology of rice weevil, *Calandra oryzae* . **Ann. Appl. Bio, 39: 168-180.**

Iwuabe, M. O. E., Osisiogu, I. U. W. and Agbakwaru, E. O. P. (1981). Dennettia oil, a potential new insecticide; tests with adults and nymphs of *Periplaneta americana* and *Zonocerus variegatus*. **J. Econ. Entomol. 74(3):249-252.**

Jacobson, M. (1989). Botanical pesticides: past, present and future. In Insecticides of Plant Origin. ACS symposium series 38. **Ame. Chem. Soc. p. 1-10**

Jacobson, M. and Crosby, (1971). Naturally occuring insecticides. Marcel Dekker, Inc. New York.

Jadhav, K. B. and Jadhav, L. D. (1983). Use of some vegetable oils, plant extracts and synthetic products as protectants from pulse beetle, *Callosobruchus maculatus* Fabr in stored gram. **J. Food Sci. Tech. 21: 110-113.**

Jain, H. K. (1983). Neem in agriculture. **Indian Agricultural Research Institute, New Delhi. 61 pp.**

Janssen, A. M., Scheffer, J. J. C., Ntezurubanza, L., Baerheim, S. A. and Svendsen, A. B. (1989). Antimicrobial activities of some *Ocimum* species grown in Rwanda. **J. Ethnopharmacology. 26(1): 57-63.**

Jilani, G., Saxena, R. C. and Rueda, B. P. (1988). Repellent and growth inhibiting effects of turmeric and sweet flag oils and margosn-o on red flour beetle (Coleoptera, Tenebrionidae). **J. Econ. Entomol. 81(4): 1226-1230.**

Khorramsha, A. and Burkholder, W. E. (1981). Behavior of the lesser grain borer, *R. dominica* (Coleoptera: Bstrichidae): male produced aggregation pheromone attracts both sexes. **J. Chem. Ecol. 7(1): 33-38.**

Khosla, M. K., Sobti, S. N. and Atal, C. K. (1985). Genetic studies on the inheritance pattern of different essential oil constituents of *Ocimum* species. **Indian Perfumer. 29:151-160.**

Kokwaro, J. O. (1976). Medicinal plants of East Africa. **Litrature Bureau, Nairobi. 384 pp.**

Kumar, R. (1984). Insect pest control with special reference to African agriculture. **Arnold Publishers Litd. 298 pp.**

Kumar, R. and Okonronkwo, N. O. (1991). Effectiveness of plant oils against some Bostrichidae infesting cereals in storage. **Insect Sci.Applic. 12(2): 77-85.**

Ladd, T. L. Jr., Stinner, B. R. and Kueger, H. R. (1983). Eugenol, a new attractant for the northern root worm (Coleoptera: Chrysomelidae). **J. Econ. Entomol. 76: 1049-1051.**

Ladd, T.L. (1980). Japanese beetle: enhancement of lures by eugenol and caproic acid. **J. Econ. Entomol.73: 718-720.**

Lal, R. N., Sen, T. K. and Nigman, M.C. (1978). Gas chromatogrphy of the essential oil of *Ocimum sanctum* L. **Chemrec. Abst., 7(2):44.**

Lance, D. R. (1988). Potential of 8-mehtyl-2-decyl propanoate and plant derived volatiles for attracting corn root worm beetles (Coleoptera: Chrysomelidae) to toxic bait. **J. Econ. Entomol. 81(5): 1359-1362.**

Longstaff, B. C. (1981). Biology of the grain pest species of *Sitophilus* (Coleoptera: Curculionidae). A critical review. **Prot. Ecol. 2: 83-130.**

Maeshima, K., Hayashi, N., Murukani, T., Takahashi, F. and Komae, H. (1985). Identification of chemical oviposition stimulants from grain for *S. zeamais* Motschulky (Coleoptera: Curculionidae). **J. Chem. Eco. 11(1): 1-9.**

Malik, M. S., Rafique, M. and Ahmed, R. (1986). Essential oils of the species of Labiatae, studies on the essential oil of *Ocimum sanctum*. **Pakistan J. Sci. Ind. Res. 29(2): 111-112.**

Mandava, N. B. (1985). Naturally occuring pesticides and the pesticides crisis, 1945-1980, in hand book of natural pesticides. **C. R. C. Press Inc. p. 297-328.**

Mansour, F.; Ravid, U. and Putievsky, E. (1986). Studies of the effects of essential oils isolated from 14 species of Labiatae on the carmine spider mite, Tetranychus cinnabarinus. **Phytoparasitica. 14: 137-142.**

Marinibettolo, G. B. (1983). The role of natural products in plant - insect and plant - fungi interaction In natural products of inovative pests management, Whitehead, D. L. and Bowers, W. S. **Pergamon Press Ltd. pp. 1878-222.**

Matsui, M. and Yamamoto, I. (1971). Pyrethroids, in naturally occuring insecticides. **Marcel Dekker, Inc. pp. 3-70.**

McLafferty, F. W. and Stauffer, D. B. (1989). The Wiley/ NBS registery of mass spectral data. **John Wiley and sons Inc. vol. 1-7.**

Merck Index, (1983). An encyclopedia of chemicals, drugs and biologicals, tenth ed. **Merck and Co. Inc., Rahway, N. J., U. S. A., pp. 238.**

Metcalf, R. L. and Metcalf, E. R. (1992). Plant kairomones in insect ecology and control. **Routledge, Chapman and Hall inc. USA, 168 pp.**

Mills, R. B. (1965). Early germ feeding and larval development of the Angoumois Grain Moth. J. Econ. Entomol. 58(1):220-223.

Mills, R. W. and Wilbur, D. A. (1967). Radiographic studies of Angoumois Grain Moth development in wheat, corn and sorghum kernels. **J. Econ. Entomol. 60(3): 671-977.**

Mishra, D. N.; Mishra, A. K.; Tiwari, R. and Upahyaya, P. S. (1990). Toxicity of some essential oils against pulse beetle *Callosobruchus maculatus*. **Indian Perfume. 33(2). 137-141.**

Morales, M. R., Simon, J. E., Charles, D. J. and Herbs, J. (1993). Comparison of essential oil content and composition between field and green house grown genotypes of methyl cinnamate basil (*O. basilicum*). **Chemorec. Abst. 22(1): 59.**

Morris, R. T. and Boyd, R. N. (1992). Organic chemistry. Sixth edition **Prentice - Hall Inc. 1323 pp.**

Mukhejee, U.; Singh, A. K. and Hameed, S. F. (1990). Evaluation of neem (*Azadiracta indica* A. juss) products as wheat grain protectants against *Sitophylus oryzae* L. **Neem news letter. 7(1): 2-4.**

Mwangi, J. W., Guantai, A. N., Muriuki, G. and Lwande, W. (1988). Constituents of the essential oil of *Ocimum fischeri* (Guerke). **Kenyan J. Sci. (B), 9, 119-121.**

Mwangi, J. W., Lwande, W. and Hassanali, A. (1994). Composition of the essential oil of *Ocimum kenyense* Ayobangira. **Flavour Fragr. J. 9: 1-2.**

Nandi, R. P. and Chatterjee, S. K. (1986). Successful cultivation of some aromatic plants from hilly tracts of West Bengal. **Indian Perfumer. 30(2): 321-326.**

Nawrott, J., Harmstha, J, Kostova, I. and Ognyanov, I. (1989). Antifeedant activity of Rotenone and some derivatives towards selected insect storage pests. **Biochem. Syst. Ecol. 17 (1): 55-57.**

Nigist, A. and Abegaz, B. (1989). Constituents of the essential oils of three indigenous Ocimum species from Ethiopia. **Ethiop. J. Sci. 12(2): 111-123.**

Nykaenen, J. (1989). The effect of cultivation on the composition of basil oil. **Chemorec. Abst. 18(2): 47.**

Obeng-Ofori, D. (1990). Monitoring stored products. Ph. D. thesis, Sidney Sussex College, Cambridge. pp.

Odebiyi, J. A. (1978). Trends in pest management: use of attractants, repellents, antifeedants and hormones. In **I.C.I.P.E. /U.N.E.P. group training course lecture notes. I.C.I.P.E. Nairobi, Kenya. p. 1-2.**

Owaga, M. L. A., Hassanali, A. and McDowell, P. G. (1988). The role of 4-cresoland 3-n-propylphenol in the attraction of tsetse flies to buffalo urine. **Insect Sci. Applic. 9(1): 95-100.**

Parh, I. A., Forbuzo, B. C., Matheney, E. L. and Ayafor, J, F. (1990). Plants used for control of insect pests on

stored grain in Cameroon. **Biopeticides and Pest Management In The Developing World, Proceedings. pp. 83-90**

Paton, A. (1991). A synopsis of *Ocimum* L. (Labiatae) in Africa. **Kew Bull. 47(3): 403-435.**

Pavia, D. L., Lampman, G. M. and Kriz, G. S. (1988). Introduction to organic laboratory techniques, second edition **CBS College Publishing. 139 pp.**

Pinniger, D. P. and Chambers, J. (1988). The use of pheromones in stored products protection. **Chemorec. Abst. 16(3): 37.**

Prasad, G., Abhay, k., Singh, A. K., Bhattacharya, A. K., Kamla, S. and Sharma, V.D. (1986). Antimicrobial activity of essential oils of some *Ocimum* species and clove oil. **Fitoterapia. 57(6): 429-432.**

Raghavariah, G. and Jayaramaiah, M. (1989). Efficacy of some essential oils as disinfectant of silk worm rearing equipment. **Abst. Entomol. 20(6): 14.**

Reinhold, L. (1970). Progress in phytochemistry. Vol 2. **John Wiley & Sons Ltd. London. 512 pp.**

Rhodes, D. F. (1976). Towards a general theory of plant anti-herbivore chemistry;. In biochemical interaction between plants and insects: Recen Advances in Phytochemistry 10: 168-213.

Rice, E. L. (1983). Pest control with nature's chemicals allelo chemicals and pheromone in gardening and agriculture. **University of Oklahoma Press. pp. 224.**

Roger, C. R., Hamraoui, A., Haleman, M., Theron, E. and Pinel, R. (1993). Insecticidal effect of essential oils from mediterranean plants upon *Acanthoscelides obtectes* Say (coleoptera: Bruchidae), a pest of kideny bean (*Phaseolus vulgaris* L.). **J. Chem. Ecol. 19(6): 1233-1244.**

Ryan, M. F. and Byrne, O. (1988). Plant-insect coevolution and inhibition of Acetylcholinesterase. **J. Chem. Ecol. 14(10): 1965-1975.**

Salisbury, F. B. and Ross, C. W. (1986). Plant physiology. **Wadsworth Publishing Company, U. S. A. 540 pp.**

Santhoy, G. and Rejesus, B. M. (1975). The development rate, body weight and reproduction capacity of *Sitophylus zeamais* Motsch. reared on three natural hosts. **Phil. Enomol. 2(5): 311-321.**

Scudamore, K. A. and Buckle, A. E. (1986). The Occurence and significance of molds and mycotoxines in cereals and animal feed stuff in the United Kingdome. In store products pest control, **Monograph. 37: 33-42.**

Seck, D., Lognay, G., Haubruge, E., Wathelet, J. P., Marlier, M., Gaspar, C. and Severin, M. (1993). Biological activity of the shrub *Bosica senegalensis* (Pers.) (Capparacae) on stored grain insects. **J. Chem. Entomol. 19(2): 377-390.**

Shaaya, E. and Vladimir P. (1991). Toxicity of plant oils and their major constituents against stored product insects. **J. Agr. Food Chem. 27: 629-638.**

Shaaya, E., Ravid, U., Poster, N., Juven, B., Zisman, U. and Pissarev, V. (1991). Fumigant toxicity of essential oils against four major stored products insects. **J. Chem. Eco. 17(3): 499-504.**

Sharma, R. N.; Deshpande, S. G. and Joseph, M. (1991). Evaluation of natural essential oils as antifeedants against Spodoptera litura (Fab.). **Indian Perfumer 34(3): 196-198.**

Simmons, P. and Ellington, G. W. (1953). Life history of Angoumois Grain Moth in Meryland. **U. S. Dept. Agri. Tech. Bull. 351 pp.**

Singh, D. Siddiqui, M. S. and Sharma, S. (1989). Reproduction retardant and fumigant properties in essential oils against rice weevil (Coleoptera: Curculionidae) in stored wheat. **J. Econ. Entomol. 82(3): 727-733.**

Smith, M (1989). Plant resistance to insects; a fundamental approach. **John Wiley ans Sons. 286 pp.**

Smith, R. H. (1965). Effect of monoterpene vapors on the western pine beetle. **J. Econ. Entomol. 58(3): 509-511.**

Sobti, S. N., Pushpangadan, P., Thapa, R. K., Aggarwal, S. G., Vashist, V. N. and Atal, C. K. (1978). Chemical and genetic investigation in essential oils of some *Ocimum* species, their F1 hybrids and synthesized allopolyploids. **J. Nat. Pro. 491):50-55.**

Stemley, P. G. and Wibur, D. A. (1966). Color characteristics for sexing live adult lesser grain borer. **J. Econ. Entomol. 59: 760-761.**

Teteny, P., Nterzerubanza, L., Ayobangira, F., Hethelyi, E. and Puyvelde, L. V. (1986). Essential oil variations of *Ocimum suave* in Rwanda. **Herba Hungarica, 25(2): 27-42.**

Tilton, E. W. and Schroeder, H. W. (1963). Some effects of infrared irradiation on the mortality of immature insects in kernels of rough rice. **J. Econ. Entomol. 56: 727-730.**

Tilton, E. W., Burkholder, W. E. and Cogburn, R. R. (1966). Effect of gamma radiation on *Rhizoperta dominica, Sitophylus oryzae, Tribolium castaneum* and *Lasioderma serricorne*. **J. Econ. Entoml. 59: 1363-1368.**

Tipping, P. W., Mikalajczak, K. L., Rudriguez, J. G. Poneleit, C. G. and Legg, D. E. (1987). Effects of whole corn kernels and extracts on the behavior of maize weevil (Coleoptera: Curculionidae). **Entomol. Soc. Amer. 80(5): 1010-1013.**

Trematerra, P. and Girgenti, P. (1989). Influence of pheromone and food attractants on trapping of *Sitophylus oryzae* (L.), (coleoptera, Curculionidae): a new trap. **J. Appl. Entomol.108: 12-20.**

Trivedi, K. C., Gupta, R. S. and Verma, S. (1981). Performance of different *Ocimum* species under agroclimatic conditions of Malwa region of Madya Pradesh. **Indian Perfumer. 25: 84-88.**

Walgenbach, C. A. and Burkholder, W. E. (1986). Factors affecting the response of maize weevil, *Sitophylus zeamais* (Coleoptera:Curculionidae), to its aggregation pheromone. **Environ. Entomol. 15: 733-738.**

Walgenbach, C. A., Burkholder, W. E. Curitus, M. J. and Khan, Z. A. (1987). Laboratory trapping studies with *Stophilus zeamais* (coleoptera: Curculionidae). **J. Econ. Entomol. 80: 763-767.**

Weaver, D. K., Dunkel, F. V., Ntezurubanza, L., Jackson, L. L. and Stock, D. T. (1991). The efficacy of linalool, a major component of freshly-milled *Ocimum canum* Sims (Lamiacae), for protection against post harvest damage by certain stored product coleoptera. **J. Stored Prod. Res. 27(4): 213-220.**

Whittaker, R. H. and Feeny, P. P. (1971). Allelochemics: chemical interaction between species. **Science. 191: 575-770.**

Williams, D. H., Stone, M. J., Hauk, P. R. and Rahman, S. K. (1989). Why are secondary metabolites (natural products) biosynthesized? **J. Nat. Prod. 52(6): 1189-1208.**

Yaro, N. D., Krysan, J. L. and Ladd, T. L. (1987). *Diabrotica cristata* (Coleoptera: Chrysomelidae), attraction to eugenol and related compounds compared with *D. barberi* and *D. virgifera virgifera*. **Envirn. Entomol. 16: 126-128.**

Yun, K. W., Kil, B. and Han, D. M. (1993). Phytotoxic and antimicrobial activity of volatile constituents of *Artemisia princeps* var. *orientalis*. **J. Chem. Eco. 19(11): 2757-2766.**

Yun-Tai, Q. (1984). Eugenol-related attractants for the northern corn root worm (Coleoptera: Chrysomelidae). **J. Econ. Entomol.77: 339-341.**

Yun-Tai, Q. and Burkholder, N. E. (1981). Protection of Stored wheat from granary weevil by vegetable oil. **J. Econ. Entomol. 74(5): 502-505.**