

COMPOSITION AND BIOACTIVITY OF ESSENTIAL OILS OF *Lantana camara* L.,
Tephrosia vogelii HOOK AND *Ocimum americanum* L. AGAINST MAJOR
COLEOPTERAN PESTS OF STORED FOOD GRAINS

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A thesis submitted to the Graduate School in fulfilment of the requirements for the Degree
of Doctor of Philosophy in Crop Protection of Egerton University



EGERTON UNIVERSITY

NOVEMBER 2008

2009/76720 X

DECLARATION AND RECOMMENDATION

Declaration

I declare that this thesis is my original work and has not been presented in this or any other University for any degree.

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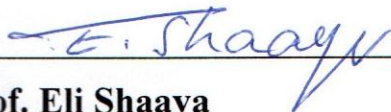
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DEDICATION

To my late half-sib brother, Benson Oloo Ogendo, who a quarter of a century ago, foresaw “a Professor” in his younger brother; a vision that has immensely motivated, shaped and inspired the young man’s academic path.

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ABSTRACT

Food security and post-harvest handling are greatly undermined by insect pests which cause about 20% post-harvest losses of food grains in subsistence agriculture. Despite the enormous potential for pest control, the indigenous plant-based products have remained largely unexploited. In an attempt to address the insect pest menace in grain storage, a scientific study was conducted to evaluate the contact toxicity (instant and residual), fumigant toxicity (space and grain), repellence (instant and residual) and reproduction inhibition of essential oils extracted from aerial parts of three indigenous plants, *Ocimum americanum* L., *Lantana camara* L. and *Tephrosia vogelii* Hook, and selected isolated constituents against adult *Sitophilus oryzae* L., *Rhyzopertha dominica* F., *Tribolium castaneum* (Herbst) and *Callosobruchus chinensis* L. insects. The study also evaluated the intra-species and inter-plant variations in composition of essential oils and possible phytotoxic effects of selected essential oils on treated seed grains. In the contact toxicity, repellence and reproduction inhibition studies, essential oils and a constituent, eugenol, were each evaluated at four rates (0.5, 1.0, 1.5 and 2.0 $\mu\text{L/g}$ grain) with 4-6 replicates per concentration. In the same bioassays, soya oil (10 $\mu\text{L/g}$ grain) and Actellic 5EC (1.0 $\mu\text{L/g}$ grain) were included as positive controls with Acetone (0.5 $\mu\text{L/g}$ grain) and untreated grains as negative controls. In the space and grain fumigation studies, four (0, 1, 5 and 10 $\mu\text{L/L}$ air) and five (0, 30, 50, 70 and 100 $\mu\text{L/L}$ air) rates, respectively, with 4-6 replicates per essential oil concentration or constituent were tested against adult stages of three or four test insects. All the laboratory bioassays, arranged in a completely randomized design (CRD), were conducted in a room maintained at $30\pm 2^\circ\text{C}$, $68\pm 2\%$ RH and 12D: 12D (total darkness). Except for residual (contact and repellence) studies and chemical compositional analysis, bioactivity of test essential oils and their constituents against adult stages of four coleopteran pests were significantly influenced ($P<0.0001$ - 0.0484) by intra-species and inter-plant variations in chemical composition, concentration applied, time after application, insect species and corresponding factor interactions. With a few exceptions, test essential oils demonstrated clear temporal dose-dependent contact toxicity, repellence and reproduction inhibition against adult stages of test insects and phytotoxicity on treated wheat seeds. Results of space fumigation showed that, irrespective of plant part assayed, *O. americanum*, *L. camara* and *T. vogelii* essential oils produced LC_{50} values of 0.38- 34.68, 0.77- 64.10 and 0.44- 43.50 $\mu\text{L/L}$ air, respectively, against adult stages of the four test insects. In the grain fumigation studies with *O. americanum* leaf essential oil, 50 $\mu\text{L/L}$, 7 days exposure and 120 h post-fumigation time were enough to obtain 95.4-100% kill of adult *S. oryzae* and *R. dominica* and 65.5% mortality of *T. castaneum*, which was comparable to methyl bromide's recommended dose of 30-50 g/M^3 grain and that of highly active Labiate sp. Oil, ZP51[®] (50 $\mu\text{L/L}$). At the highest concentration (0.20% v/w) and 120 days of storage, *O. americanum* and *T. vogelii* fruit essential oils caused 25.0- 40.0 and 33.0- 51.0% inhibition of wheat seed germination with corresponding EC_{50} values of 6.22- 0.37 and 2.10- 0.19% v/w, respectively. Results of GC-MS analysis showed marked intra-species and inter-plant variations in which eugenol (49.2%) and germacrene D (19.22%) were dominant in leaves of *O. americanum* and *T. vogelii* whereas E-caryophyllene (15.5%) in the fruits of *L. camara* implying the test plants were eugenol, germacrene D and E-caryophyllene chemotypes, respectively. The potential of essential oils and their volatile constituents as alternative contact insecticides, fumigants and protectants of durable agricultural products and scientific implications are discussed.

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LIST OF ACRONYMS

BIPM	Bio-intensive pest management
CRD	Completely randomized design
DEET	N,N-diethyl- <i>m</i> -toluamide
EC	Emulsifiable concentrate
EC ₅₀	Effective concentration that causes 50% inhibition
FL	Fiducial limits for LC ₅₀ values
GC-MS	Gas chromatography-mass spectrometry
GDP	Gross domestic product
IPM	Integrated pest management
LC ₅₀	Lethal concentration that kills 50% of test insects
LT ₅₀	Lethal time that kills 50% of test insects
PCM	Pest control method
PR	Percent repellence

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LIST OF ACRONYMS

BIPM	Bio-intensive pest management
CRD	Completely randomized design
DEET	N,N-diethyl- <i>m</i> -toluamide
EC	Emulsifiable concentrate
EC ₅₀	Effective concentration that causes 50% inhibition
FL	Fiducial limits for LC ₅₀ values
GC-MS	Gas chromatography-mass spectrometry
GDP	Gross domestic product
IPM	Integrated pest management
LC ₅₀	Lethal concentration that kills 50% of test insects
LT ₅₀	Lethal time that kills 50% of test insects
PCM	Pest control method
PR	Percent repellence

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background

Agriculture is a major contributor in the Kenyan economy accounting for 23.8 and 75% of the gross domestic product (GDP) and employment respectively (WFB, 2008). Seventy five percent of food grain production in Kenya is typically carried out by small scale producers who usually cultivate less than 2.0 hectares using limited technology. Although tremendous increase in food production has been recorded over the past four decades, the rate of human population growth (2.5-3.0% per annum) has outstripped the gross increase (1.0% per annum) in production (Sallam, 1999; ECA, 2002; WFB, 2008). The food situation is made more insecure by the revelations that per capita food production index and per capita cropland have equally been declining (ECA, 2002). The depressed per capita food production coupled with inequalities in income and food distribution has increased the number of food-insecure and malnourished people in recent years. Agricultural growth in the tropics is constrained by, among others, continuous rapid population growth, low productivity of rainfed agriculture, low status of agro-processing industry, poor post harvest systems, environmental degradation and weak science and technological advancement (ECA, 2002; Kimenyi, 2002). Moreover, seasonal climatic variability and change dictate the food supply. Thus, food security and nutrition status depend not only on production, but also on the safe storage, value addition and marketing (Richter, 1988; Ogendo *et al.*, 2003a, 2004a).

Subsistence grain production is essential for the growing population in sub-Sahara Africa (SSA). Grain storage is the most important and critical post harvest operation (Sallam, 1999) with the duration being dependent upon market demand, size of production and the farmer's consumption needs (Wanjekeche, 1997; Sallam, 1999; Ogendo *et al.*, 2003a). Insect pests pose the greatest challenge to the provision of quality food to mankind in the 21st century. Insects cause substantial quantitative and qualitative pre- and post-harvest losses varying in magnitude from <10 to 100% (Saxena *et al.*, 1990; Allen *et al.*, 1996). In Kenya, insect pests cause 10-60% losses of stored cereal and legume grains depending on the insect species, storage duration and

form (shelled or on cobs), pest control practices and whether the grain is stored in central stores or on-farm (Schulten, 1982; Sallam, 1999; Ogendo *et al.*, 2003a). The gelechiid moths and coleopteran beetles are pests of stored cereal and legume grains with *Sitophilus* spp., *Sitotroga cerealella* Olivier, *Prostephanus truncatus* Horn, *Rhyzopertha dominica* F., *Tribolium castaneum* Herbst, *Acanthoscelides obtectus* Say and *Callosobruchus* spp. causing substantial losses (Schulten, 1982; Haines, 1991; NRI, 1997; Sallam, 1999; Sing, 1997; Ngatia, 2000; Fields *et al.*, 2001; Ogendo *et al.*, 2003a).

Synthetic insecticides, as contacts or fumigants, have been the recommended methods of pest control in grain storage worldwide (Shaaya and Kostyukovsky, 1998; Fields *et al.*, 2001). However, in resource-poor farming environments in the tropics adoption rate and use for pest control in grain storage has remained remarkably low owing to the subsistence nature of agriculture, high rural poverty, prohibitive costs, toxicity concerns and erratic supply of synthetic pesticides (NRI, 1997; Sing, 1997; Ogendo *et al.*, 2004a). Recent studies revealed that most resource-poor farmers in western Kenya use traditional methods of storage pest control that have served mankind for generations (Ogendo *et al.*, 2003a). In view of their potential as alternative ecosocio-friendly insect control agents, there has been growing interest for scientific rationalization of the bioactivity and basis of protective action of plant products (Obeng-Ofori *et al.*, 1995; Shaaya and Kostyukovsky, 1998; Fields *et al.*, 2001). Based on the findings of a recent needs-driven farm survey and preliminary laboratory studies (Ogendo *et al.*, 2003a; b), an in-depth study was conducted to monitor and evaluate the bioactivity of essential oils of three indigenous Kenyan plants, *Lantana camara* L., *Tephrosia vogelii* Hook and *Ocimum americanum* L. against four major insect pests of stored food grains, namely, the rice weevil, *Sitophilus oryzae* L., the lesser grain borer, *Rhyzopertha dominica* F., the rust-red flour beetle, *Tribolium castaneum* Herbst and the cowpea beetle, *Callosobruchus chinensis* L. The results of this study are expected to stir further scientific exploits geared towards biosafety assessments, the packaging of the new technology for the target user domains and stimulating local micro enterprise investment in grain storage protection.

1.2 Statement of the Problem

Recent studies revealed that most subsistence farmers in western Kenya never apply any pest control measures against field insect pests of staple food crops. Moreover, over 60% of the population still live below absolute poverty characterised by declining per capita land and crop productivity, soaring food-insecurity and widening gap between the rich and the poor (Ogendo *et al.*, 2003a).

Literature surveys indicate that, of the causal agents of the cyclic food deficits, insect pests pose the greatest threat to increased productivity, storage and handling of food grains. Post-harvest insect pests have been identified to cause qualitative and quantitative losses of between 10 to 60% depending on the insect species, duration of storage, pest control methods (PCM) and grain storage practices (Hell *et al.*, 2000; Ogendo *et al.*, 2003a). The gelechiid moth (*Sitotroga cerealella* Olivier), curculionid (*Sitophilus* spp), bostrichid (*Prostephanus truncatus* Horn, *Rhyzopertha dominica* Fabricius), tenebrionid (*Tribolium castaneum* Herbst) and bruchids (*Acanthoscelides obtectus* Say, *Callosobruchus* spp) are the most destructive storage insect pests in the tropics (Haines, 1991; Ogendo *et al.*, 2003a, 2004a).

The PCMs have been largely inadequate since the use of synthetic insecticides is yet to be fully integrated in subsistence agriculture due to their high costs, potential toxicity and incompatibility with existing socio-economic environment (Saxena *et al.*, 1990; Ogendo *et al.*, 2003a, 2004a). Since more than 70% of the subsistence farmers protect their stored food grains using indigenous PCMs (powders, extracts, ashes, etc), scientific rationalization is needed for enhanced insect pest control. The use of locally available botanical pesticides holds good promise for cost-effective, environment-friendly and sustainable stored-product insect pest management in subsistence agriculture (Rahim, 1998; Ogendo *et al.*, 2004b). Neem (*Azadirachta indica*), hot chilli pepper (*Piper* sp.) and *Ocimum* spp. products have been used for generations throughout Africa, Asia and the Americas (Obeng-Ofori *et al.*, 1995; Edelson *et al.*, 2002). However, modes of action of most botanicals have not been explained and are difficult to recommend for use due to their unauthenticated application regimes and bioactivity against storage insect pests, non-target organisms and environment. Hence, the study was conducted with the following objectives:

1.3 Objectives

1.3.1 General Objective

The study, broadly, aimed at the scientific rationalization of essential oils obtained from three indigenous Kenyan plants as alternatives to synthetic pesticides in grain protection.

1.3.2 Specific Objectives

The specific objectives of this study were to evaluate the:

1. Intra- species and inter-plant variations in chemical composition of essential oils obtained from *L. camara* L., *T. vogelii* Hook and *O. americanum* L. Benth.
2. Contact and fumigant toxicity of essential oils obtained from leaves, succulent stems and fruits of *L. camara* L., *T. vogelii* Hook and *O. americanum* L. Benth on adult *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis*.
3. Reproduction inhibitory effects of essential oils obtained from leaves, succulent stems and fruits of *L. camara* L., *T. vogelii* Hook and *O. americanum* L. Benth on adult *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis*.
4. Repellent effects of essential oils obtained from leaves, succulent stems and fruits of *L. camara* L., *T. vogelii* Hook and *O. americanum* L. Benth against adult *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis*.
5. Residual toxic, repellent and reproduction inhibitory effects of essential oils obtained from leaves, succulent stems and fruits of *L. camara* L., *T. vogelii* Hook and *O. americanum* L. Benth on adult *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis* and the viability of treated seed grains.
6. Toxic and repellent effects of selected isolated major chemical constituents of *L. camara* L., *T. vogelii* Hook and *O. americanum* L. Benth essential oils against adult *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis*.

1.4 Hypotheses (H₀)

1. There are no intra-species and inter-plant variations in chemical composition of essential oils from *O. americanum*, *L. camara* and *T. vogelii*.
2. The essential oils from leaves, succulent stems and fruits of *L. camara* L., *T. vogelii* Hook and *O. americanum* L. Benth have no contact and fumigant toxicities against adult *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis*.

3. The essential oils from leaves, succulent stems and fruits of *L. camara* L., *T. vogelii* Hook and *O. americanum* L. Benth have no effects on reproduction of *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis*.
4. The essential oils from leaves, succulent stems and fruits of *L. camara* L., *T. vogelii* Hook and *O. americanum* L. Benth have no repellent effects against adult *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis*.
5. The essential oils from leaves, succulent stems and fruits of *L. camara* L., *T. vogelii* Hook and *O. americanum* L. Benth have no residual toxic, repellent and reproduction inhibitory effects on adult *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis* insects and viability of treated seed grains.
6. The isolated major essential oil constituents have no toxic and repellent effects on adult *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis*.

1.5 Justification of the study

Food security in sub-Sahara Africa is greatly undermined by insect pests which cause, on average, 20% post-harvest grain losses produced by subsistence farmers in Kenya. This magnitude of loss impacts negatively on the gross domestic product (GDP) and the national budget since this category of farmers contribute about 75% of the national staple grain production. Hence, reduction of post-harvest food losses is a significant contribution towards increased food security in the developing countries. Although synthetic pesticides have been promoted in Kenya for over four decades, subsistence farmers are yet to fully integrate their use in grain storage systems. Recent local studies revealed continued use of traditional methods to preserve durable agricultural products. These revelations are consistent with existing recommendations in organic agriculture that recognize micro-organisms, microbial products, mineral-bearing rocks and plant and animal derivatives as sources of pesticides. It emerges from this premise that simple natural anti-pest products can be developed from existing indigenous knowledge base through targeted scientific rationalization, validation and packaging.

Despite the enormous potential that has existed for generations, the indigenous bio-intensive pest management (BIPM) practices have received little local research intervention and resource commitments and hence are largely unexploited. The exploitation of indigenous plant resources,

as biopesticides, is expected to eliminate complex mechanisms of pesticides distribution, contribute to enhanced local industrial initiatives arising from domestication and cultivation of botanical plants and provide a cost-effective insect pest management in subsistence agriculture. The use of rationalized botanical pesticides in grain storage is an eco-friendly option with a high sustainability index once the plants are proven efficacious, domesticated and propagated using good agronomic practices.

1.6 Scope and limitations of the study

Studies using domesticated plants were not carried out due to a technical oversight at the project planning phase. It later emerged that agronomic characterisation of growth stages must be understood and documented. Also on the basis of research agenda covered, factoring different stages of sampling was impractical owing to resource limitations.

Unsexed adult insects were used in all bioactivity studies owing to difficulties in sexing of individual insects and the voluminous nature of research agenda. The toxic, repellent and reproduction inhibitory effects of essential oils and major constituents against four coleopteran pests of stored products were studied. However, due to prohibitive cost of standards, only selected individual compounds, rather than blend effects were studied. Residual contact toxicity, seed germination and repellence studies were studied over a 4-month period using those essential oils that had proven active during the immediate contact studies. In all contact (instant and residual) toxicity studies, untreated, acetone-treated, Actellic 5EC (0.10% v/w) and crude soya oil (1.0% v/w)-treated grains were included as controls. However, Phosphine (phostoxin tablets) as a control fumigant could not be included among the treatments owing to technical limitations.

CHAPTER TWO

LITERATURE REVIEW

2.1 Insect pest control methods in grain storage

Food grains constitute an important component in the diets of people in the tropics and usually stored to provide food and seed reserve (Niber, 1994). Literature available indicates that stored grains and their products are subject to attack by many insect species especially in subsistence agriculture (Liu and Ho, 1999; Ogendo *et al.*, 2003a). In the developing countries, such as Kenya, the control of stored-product insect pests is either through contact insecticides (synthetic or plant-based dusts), fumigants or repellents. The control of these insects relies heavily on the use of synthetic insecticides and fumigants which are largely incompatible with subsistence agriculture owing to their health hazard to warm-blooded animals, pest resistance, high costs of application and lethal effects against non-target beneficial organisms (Shaaya and Kostjukovsky, 1998; Liu and Ho, 1999; Kéïta *et al.*, 2000; Ogendo *et al.*, 2003a). Besides being expensive, synthetic insecticides pose potential risks to users owing to lack of adequate technical knowledge and skills related to their safe use (Saxena *et al.*, 1990; Ogendo *et al.*, 2003a). In Kenya, less than 10% of the subsistence farmers use synthetic insecticides to protect their stored food grains (Ogendo *et al.*, 2003a).

Although fumigation remains one of the most effective methods for the protection of stored food grains against insect infestations, only one fumigant, phosphine is still recommended for use. This follows the phasing out of methyl bromide which is a major contributor to ozone depletion (Shaaya and Kostjukovsky, 1998; Carlos, 2004). The loss of methyl bromide and possible restriction of phosphine owing to fears of insects developing resistance and its carcinogenic potential, in addition to rising popularity of organic produce lines, has created interest in non-chemical alternatives for future of insect control.

Plant derivatives (ashes, powders and extracts), mineral powders and sand were commonly used in the tropics for insect control before the advent of synthetic pesticides (Bajwa and Schaefer, 1997; Kéïta *et al.*, 2000). It is important to note that some of the earliest insecticides- nicotine, pyrethrins and rotenone, were derived from plants. However, the commercial exploitation of

pyrethrins through synthetic options has rendered these insecticides largely uneconomical in subsistence agriculture. Although collection and utilization of natural products may be expensive in terms of time and labour, these resources are often more available than hard cash (Bajwa and Schaefer, 1997). Effective control of insect pests of stored grains with minimal use of insecticides requires an IPM approach combining sanitation, monitoring, preventive and curative (control) measures. Therefore, there is renewed scientific effort aimed at seeking cost-effective and environment friendly alternative stored-product insect control strategies based on indigenous knowledge and practices of the target users.

2.2 Use of plant-based essential oils for insect pest control in grain storage

Aromatic plants contain volatile compounds, mainly essential oils, known to possess insecticidal properties (Shaaya and Kostjukovsky, 1998; Huang *et al.*, 2000). Essential oils are naturally occurring terpenic mixtures whose bioactivity against specific pests and pathogenic micro organisms have recently received research attention (Dafetera *et al.*, 2003; Ketoh *et al.*, 2005). Most essential oil constituents are monoterpenoids which are secondary plant metabolites with little metabolic importance. They are believed to be allelopathic agents or irritants that protect plants from predation by insects and infestation by parasites (Huang *et al.*, 2000).

The toxic, repellent, reproduction inhibition and antifeedant effects of a large number of essential oils and their chemical constituents have been evaluated against insect pests of several stored products. Essential oils obtained from *Elletaria cardmomum* (L.) Maton., *Lavandula hybrida*, *Rosmarinus officinalis*, *Mentha microphylla*, *M. viridis* and *Eucalyptus globulus* showed insecticidal activity against *Sitophilus* spp., *Callosobruchus* spp., *Tribolium castaneum* Herbst, *Lasioderma serricorne* and *Acanthoscelides obtectus* Say (Huang *et al.*, 2000; Papachristos and Stamopoulos, 2002a,b). Pungitore *et al.* (2005) reported that five triterpenoids from *Junellia aspera* (Gillies ex. Hook) (Verbenaceae) exhibited feeding deterrence activity and were highly toxic to adults of the rice weevil, *Sitophilus oryzae* L. Several studies involving terpenoids, such as linalool, cineole, *p*-cymene, camphor, terpineol, α -pinene and eugenol showed significant toxicity against insect pests of a number of stored products and inhibited reproduction (Regnault-Roger and Hamroui, 1995; Lee *et al.*, 2003; Park *et al.*, 2003; Sundufu and Shoushan, 2004).

2.2.1 Essential oil from *Lantana camara* L. and insect control

Lantana camara L. (Verbenaceae) is an invasive roadside perennial plant introduced originally from South America (Knight and Walter, 2003; Neuwinger, 2004). There are two main varieties, one with orange-yellow flowers and another with red flowers. Aerial parts give a strong odour essential oil with a 0.2% yield and have general medicinal and cosmetic values (Rasoanaivo and De La Gorce, 2004).

Dweck (1994) reported that leaves contain an essential oil and a quinine-like alkaloid, Lantanine, which was later, renamed Lantadene A and B. The essential oil is rich in caryophyllene, aldehydes and alcohol which together account for the antiseptic action of the plant. Although medicinal, the green unripe fruits and leaves of red sage *L. camara* L. contain a phototoxic triterpene derivative, lantadene-A, which has poisoning effect in livestock. It causes photosensitization through cholestasis preventing elimination of phylloerythrin through the bile (Knight and Walter, 2003). However, the orange-yellow flowered sage commonly found in Kenya is grazed by livestock especially during periods of pasture scarcity with no documented cases of poisoning.

Scientific attempts to rationalize the use of essential oils from *L. camara* L. for insect pest control just gained momentum during the last two decades. Literature survey indicates that the chemical composition and insecticidal activity of this plant varies with geographical location, tissue or plant part sampled, dosage and insect pest species. Hiremath *et al.* (1997) reported that Indian *L. camara aculeate* (Verbenaceae) leaves yielded 16-19% methanol extracts which produced less than 40% mortality against *Nilaparvata lugens*. Studies with 0.02-0.04% of essential oils extracted from *L. camara* leaves produced 90-100% mortality against the 4th instar larvae of three mosquito species (Adebayo *et al.*, 1999). The oils at 0.02% were more toxic, causing 100% mortality in 24 h, to *Culex pipiens fatigans* and *Aedes aegypti* than *Anopheles gambiae* (Adebayo *et al.*, 1999). Recent local studies using crude ground powders showed that *L. camara* has strong insecticidal and repellent effects against the maize grain weevil, *Sitophilus zeamais* Motschulsky (Ogendo *et al.*, 2003b).

Analytical information available on the chemical composition of essential oils from *L. camara* reveals an amazing variation on the basis of geographical location and tissue or plant part sampled. In their GC-FID and GC-MS analysis of essential oils of leaves and flowers of *L. camara* from Cameroon and Madagascar, Ngassoum *et al.* (1999) found that the oils were characterised by a high percentage of sesquiterpenes with major constituents being α -curcumene (25%), β -caryophyllene (13%), caryophyllene epoxide (7%) (Cameroon); davanone (15%) and β -caryophyllene (12%) (Madagascar). The monoterpene percentages were lower in the two essential oils and were represented by Sabinene (1-9%), α -pinene (2-4%), 1,8-cineole (1-3%) and linalool (1-3%) (Ngassoum *et al.*, 1999). Similarly, Jose and Thoppil (2000) observed that essential oil of *L. camara* contained beta-caryophyllene, a sesquiterpene hydrocarbon and geranyl acetate, an oxygenated monoterpene as major constituents. Other constituents included terpinyl acetate, bornyl acetate, 1,8-cineole (all oxygenated monoterpenes) and D-limonene (monoterpene). In related investigations, steam distilled and GC/GC-MS analysed volatile oils from leaves of *L. camara* were characterised by a high percentage of sesquiterpenes with main components being germacrene D (15.85%), β -caryophyllene (12.35%), α -humulene (9.31%) and germacrene-B (6.19%). An oxygenated monoterpene, 1,8-cineole (4.61%), was also present (Sundufu and Shoushan, 2004). Terpenes, which are the main constituents of essential oils play an important role in insect communication systems, offering prospects of opportunities for manipulation, hence, their great potential as insect pest control agents (Ngassoum *et al.*, 1999). Little local research efforts have been directed towards a scientifically rationalised exploitation of *L. camara*, a plant that has widespread distribution, for the management of insect pests of stored staple food grains in the tropics. Understanding the variation in chemical composition (leaves, stems, fruits and flowers) and the bioactivity of specific chemical constituents against the major insect pests of stored cereal and legume grains requires immediate scientific attention. This provides principal mechanisms relating to insecticidal, repellent, anti-feeding, reproduction inhibition and related effects of essential oils extracted from the plant against specific stored-product insect pests and use the chemical composition and bioactivity of *L. camara* in Kenya to make comparisons with what is documented for other regions around the world.

2.2.2 Essential oil from *Tephrosia vogelii* Hook and insect control

Fish poison bean, *Tephrosia vogelii* Hook (Fabaceae), is a semi-perennial, shrubby plant indigenous to Africa and grows best on depleted light soils where it is a valuable fallow-improvement species particularly for the control of *Striga hermonthica* (Mathias, 1997). It is also among the ten plants most used for poison fishing in Africa and fish eaten without problems (Berger, 1994; Ogendo, 2000; Neuwinger, 2004).

The commercial exploitation of *T. vogelii* for rotenone production in the United States of America (USA) dates back to 1960s when it was regarded as a more promising plant than *Derris* and *Lonchocarpus* spp which were the main sources of the pesticide. In addition to its insecticidal properties, ethnobotanical surveys indicate that *T. vogelii* can be used for treatment of helminthiasis, mange, tumor, Newcastle disease, intestinal parasitosis and washing of animal body to kill ectoparasites such as ticks and mites (Kambewa *et al.*, 1997; Chifundera, 1998). Preliminary field studies in Zambia showed that crude aqueous leaf extracts (10% w/v) of *T. vogelii* produced promising acaricidal effect comparable to synthetic chemicals by protecting cattle from tick infestation for up to 10 days (Berger, 1994). In the same study aqueous fresh leaf and crushed pods extracts (15% w/v) reduced the infestation of maize stem borers, maize streak virus and termites, possibly through antifeedant effects. Kambewa *et al.* (1997) reported that aqueous extracts of *T. vogelii* caused 100% mortality in adult ticks and fleas after 24 and 12 hr, respectively. Mathias (1997), using a 16% w/v aqueous extracts plus soap as a wetting agent, reported significant insecticidal activity against American Bollworm in cotton.

In recent years, research attention has been directed towards studying the bioactivity of crude extracts/slurries, powders and essential oils of *T. vogelii* against the major insect pests of stored food grains. Studies conducted in Congo showed that *Tephrosia* leaf powder admixed with groundnut seeds at a ratio of 1:40 gave a 98.8% mortality of the Groundnut borer, *Caryedon serratus* Olivier, after 13 days (Delobel and Malonga, 1987). Similarly in Kenya, crude powders from a mixture of leaves, succulent stems and pods caused 100% adult insect mortality and 85-93% repellence against the maize grain weevil, *S. zeamais* after 21 days and 4 h, respectively (Ogendo *et al.*, 2003b). Boeke *et al.* (2004a and b) reported that powders and slurries obtained from *T. vogelii* showed significant repellent and reproduction inhibition effects against the cowpea beetle, *C. maculatus*.

Like many plants in the Leguminosae family, *T. vogelii*, contains organic chemicals in sufficient quantity to be economically and commercially exploited as a phytochemical in the pesticide industry. The toxic principle in this plant is based on the presence of rotenoids known to be mitochondrial chain inhibitors, inhibiting cellular respiration in almost every living organism including insects and mammals. These compounds block the enzymes glutamate- and succinate dehydrogenase and thus H^+ transport (Neuwinger, 2004). Yenesew *et al.* (2003) reported that two rotenoids, diguelin and tephrosin, isolated from seeds of *Millettia dura* Dunn (Leguminosae) had larvicidal effects, with LC_{50} values of 1.6 and 1.4 $\mu g\ ml^{-1}$ at 24 h, respectively, against second-instar larvae of mosquito, *Aedes aegypti* L. (Diptera: Culicidae). Gas chromatography (GC) analysis of *T. vogelii* crude extract contained 1.20% rotenone while the liquid concentrate from the same extract and leaves had 3.0 and 0.70% rotenone, respectively. Although not quantified, presence of rotenone and other rotenoid compounds, such as tephrosine, have been notably reported in *T. vogelii*, *T. candida* and other species and that the compounds have insecticidal, piscidal and repellent properties (Delobel and Malonga, 1987; Boeke *et al.*, 2004a, b; Niassy *et al.*, 2004). Just like *L. camara*, little local research efforts have been directed towards a scientifically rationalized exploitation of *T. vogelii*, a plant that is widely used for poison fishing in Africa and for the management of insect pests of stored staple food grains in the tropics. Understanding the variation in chemical composition (leaves, stems, fruits and flowers) and the bioactivity of specific chemical constituents against the major insect pests of stored cereal and legume grains requires immediate scientific attention. This provides useful information on the mechanisms relating to insecticidal, repellent, anti-feedant, reproduction inhibition and related effects of essential oils extracted from the plant against specific stored-product insect pests and use the chemical composition and bioactivity of *T. vogelii* in Kenya to make comparisons with what is documented for other geographical regions around the world.

2.2.3 Essential oil from *Ocimum americanum* L. Benth and insect control

Sweet basil, *Ocimum americanum* L Benth (syn. *O. canum* Sims.; Lamiaceae / Labiatae) is a lemon-scented bushy, semi-woody herb and one of the 160 species occurring in the warmer parts of the tropics up to 2900 metres above sea level (masl). Regional ethnobotanical surveys have shown that the plant is predominantly present in low to medium altitude areas of East Africa including the forest and woodland clearings (Berger, 1994; Golob *et al.*, 1999; Ogendo *et al.*,

2003b). The *Ocimum* species are reportedly of medicinal and insecticidal value and are widely used by the small scale farmers (Bekele, 1994). In West Africa, it is used to treat fevers, dysentery, to relieve toothache and as flavouring (Golob *et al.*, 1999). In related studies, Djibo *et al.* (2004) reported that the yields of the essential oils of the air-dried leaves of *O. americanum* from different locations varied from 1 and 2% v/w.

Chemical analyses of the essential oils from *O. americanum* have revealed that the yields and the principal compounds, like other species, are dependent upon the chemical race, climate, soil, geographical location (origin), plant part or tissue sampled, maturity, time of harvest and extraction method (Hegarty *et al.*, 2001; Vasconcelos-Silva *et al.*, 2003; Djibo *et al.*, 2004; Katzer, 2004). Studies in Ethiopia reported the presence of linalool (35.7%), methyl cinnamate (34.3%), estragole (8.9%), 1,8-cineole (6.8%), carvacrol (6.4%), and alpha-humulene (1.2%) as the major essential oil constituents of *O. americanum* (ALNAP, 2001). Golob *et al.* (1999) reported that the composition of essential oil varied according to origin of *O. canum* (Syn. *O. americanum*). For example, in East Africa, the species contains 16-25% camphor whereas methyl cinnamate and methyl chavicol (estragol) are predominant (up to 75%) in Central and West Africa, respectively. Working with *O. americanum* L. grown in north-eastern Brazil, Vasconcelos-Silva *et al.* (2003) reported that the essential oils obtained by microwave and steam distillations from several parts of *O. americanum* had (E)-methyl cinnamate as major constituent of leaves (82.7%) and inflorescence (87.1-97.5%) whereas eugenol constituted 88.2% of the root essential oil. These results reveal promising novel chemotypes whose potential bioactivities must be ascertained and adapted for region-specific pest control options.

Several *Ocimum* species such as *O. suave*, *O. basilicum* and *O. kilimandscharicum* have been reportedly used in various forms as pesticides. Their leaves and seeds are rich in essential oils which are toxic, repellent or have growth inhibitory effects against many field and storage insect pests. *O. americanum* is among the six most commonly known repellent plants in western Kenya particularly through direct burning (Seyoum *et al.*, 2003) and that use of potted plants or burning of *O. americanum* (Labiatae) and *L. camara* (Verbenaceae) repelled an average of 39.7 and 32.4% of the mosquitoes, respectively (Seyoum *et al.*, 2003). Field studies have shown that

fresh *O. canum* significantly repelled (63.6%) mosquitoes in Guinea Bissau (Pålsson and Jansson, 1999).

Phyto-toxicity studies with various formulations from *Ocimum* species have shown significant potencies against field and storage insect pests. For instance, Umerie *et al.* (1998) reported that leaf extract aerosol and mosquito coil from *O. basilicum* had potencies of 93 and 95%, respectively, against mosquitoes and that the efficacy of formulation was dependent upon duration of fumigation. Chemical analyses of essential oils from *O. suave* identified eugenol as the principal compound with strong repellent effects against mosquitos and inhibitory effects on growth of many fungi (Chogo and Crank, 1981; Garg and Siddiqui, 1992). Similarly, another terpenoid, linalool, found in dried leaves of hoary basil; *O. canum* was reportedly the source of toxic effects against the bruchid *Zabrotes subfasciatus* and other storage pests (Weaver *et al.*, 1991). Locally, extracts from *O. suave* have shown promising results in bioassays on maize weevils, *Sitophilus zeamais*, and the plant holds promise for future botanical pesticide evaluations (Hassanali and Lwande, 1989). Just like *L. camara* and *T. vogelii*, little local research efforts have been directed towards a scientifically rationalized exploitation of *O. americanum*, a plant that is widely used as mosquito repellent in western Kenya and for the management of insect pests of stored staple food grains in the tropics. Understanding the variation in chemical composition (leaves, stems, fruits and flowers) and the bioactivity of specific chemical constituents against the major insect pests of stored cereal and legume grains requires immediate scientific attention. It is hoped that this will provide useful information on the mechanisms relating to insecticidal, repellent, anti-feeding, reproduction inhibition and related effects of essential oils extracted from the plant against specific insect pests of stored food grains and use the chemical composition and bioactivity of *O. americanum* in Kenya to make comparisons with what is documented for other geographical regions around the world.

2.3 Major insect pests of stored food grains in sub-Sahara Africa

The preservation, storage and postharvest-handling of staple food (cereal and legume) grains is of immense socio-economic importance in the developing countries where the grains often constitute $\geq 90\%$ of the dietary carbohydrates and proteins for human consumption and also used for animal rearing (Baba, 1994; Ferdu *et al.*, 2001). The adoption of improved high yielding

cereal and legume grains, known to be more susceptible, has exposed the subsistence farmers to increased insect pest attack causing quantitative and / or qualitative post-harvest losses varying in magnitude from 10 to $\geq 60\%$ depending on grain type, insect pest species, duration of storage and PCM used (Golob *et al.*, 1996; Shaaya *et al.*, 1997; Lee *et al.*, 2003; Ogendo *et al.*, 2003a; Obeng-Ofori and Amiteye, 2005). The situation is made worrisome by the climatic conditions in the tropics which favours the development and proliferation of storage pests and fungal diseases (Obeng-Ofori and Amiteye, 2005) with annual post-harvest losses resulting from insect and microbial damage and other factors estimated to be 10-25% of global food production (Pungitore *et al.*, 2005).

The grain weevils (*Sitophilus* spp), angoumois grain moth (*Sitotroga cerealella* Olivier), bostrichid beetles (*Prostephanus truncatus* Horn and *Rhyzopertha dominica* F.), bean bruchid (*Acanthoscelides obtectus* Say) and cowpea beetle (*Callosobruchus maculatus* F.) have been identified as the major primary insect pests of stored cereal and legume grains in the tropics (Raja *et al.*, 2001; Lee *et al.*, 2003; Ogendo *et al.*, 2003a; Gomez, 2004; Obeng-Ofori and Amiteye, 2005). The rust-red flour beetle (*Tribolium castaneum* Herbst), the saw-toothed grain beetle (*Oryzaephilus surinamensis* L.), *Cryptolestes* sp., and *Trogoderma granarium* Everts are the major secondary insect pests of food grains in sub-Saharan Africa (Haines, 1991; Ferdu *et al.*, 2001; Boeke *et al.*, 2004a; Lee *et al.*, 2003). On the basis of seasonal occurrence, distribution and destruction (loss) caused, the grain weevils (*Sitophilus* spp.), bostrichid beetles (*P. truncatus* and *R. dominica*), angoumois grain moth (*Sitotroga cerealella*), bruchids (*A. obtectus* and *C. maculatus*) and the rust-red flour beetle (*T. castaneum*) continue to attract research attention owing to their immense economic importance (Shaaya *et al.*, 1997; Lee *et al.*, 2003; Ogendo *et al.*, 2003a; Obeng-Ofori and Amiteye, 2005; Pungitore *et al.*, 2005).

2.4 Biology of selected insect pests of stored food grains

2.4.1 Rice Weevil (*Sitophilus oryzae* L)

The rice weevil, *Sitophilus oryzae* L., is a curculionid beetle found in all warm-temperate and tropical climates of the world (Haines, 1991). It is a primary insect pest of economic importance to stored cereal grains preferably wheat. *Sitophilus oryzae* L. closely resembles the maize weevil, *S. zeamais* Motschulsky, and can only be distinguished from the latter by examination of the

genitalia and the four red dots on the upper wings of *S. oryzae*. The male aedeagus in *S. oryzae* is smooth and convex as opposed to a central ridge between the two longitudinal depressions in *S. zaimais* (Haines, 1991).

S. oryzae thrives best at warm temperatures of about 28°C and in grain in which the moisture content is not less than 13% (NRI, 2001). The adults are 2.5-4.5 mm in size, long-lived (several months to one year) and are characterized by a narrow snout-like forward extension of the head which carries mouthparts. The adult flies actively and often infests cereals pre-harvest in the field. The adult female lays up to 150 eggs throughout adult life, at temperatures of 15-35°C (optimum: 25°C) and at grain moisture contents of over 10%, in tiny holes in the grain and then seals the cavity with a plug of waxy secretion (egg-plug). The incubation period of the egg is 4-6 days (Haines, 1991; NRI, 2001). The larval (25-30 days) and pupal (4-5 days) stages of development take place entirely within a single grain. The larval feeding (Haines, 1991) hollows out the inside of an infested grain with the newly developed adult chewing its way out leaving a characteristic emergence hole roughly circular in shape. Adults also feed on whole cereal grains leaving large irregularly shaped cavities. Total developmental period (egg-adult) ranges from 33-41 days under optimal conditions to over 110 days in unfavourable conditions (Haines, 1991).

2.4.2 Lesser grain borer (*Rhyzopertha dominica* F.)

This is a wood-boring insect in the family Bostrichidae. It is a serious primary pest of a wide range of cereal grains (mostly wheat and paddy rice) and dried cassava roots in the tropical, subtropical and warm temperate zones (Haines, 1991; NRI, 2001). In Kenya, the pest is predominant in the drier parts of Eastern, Central and Rift Valley Provinces. The wood-boring activities of *R. dominica* in stores can weaken timber and dunnage and provide hiding places for residual pest populations. The adult *R. dominica* are small in size (≈2-3 mm), cylindrical body and head is ventral to the prothorax (deflected) (Haines, 1991) and held beneath the prothorax making it obscure when the insect is viewed from above (NRI, 2001). The pronotum has a rasp-like teeth or hooks and antennae are straight and have a loose 3-4 segmented club and the tarsi are five-segmented. The apex of the wing cases is rounded and gently sloping downwards as opposed to an apex that is flattened and truncated in the larger grain borer, *P. truncatus* Horn (NRI, 2001).

The adult females preferentially lay their eggs in crevices or on rough surfaces of a seed. The number of eggs laid by an adult female is temperature dependent with means of 244 and 418 per female at 25°C and 34°C, respectively (Haines, 1991). The pre-adult stages of *R. dominica* develop within cereal grains but larvae may bore out of one grain and into another (NRI, 2001). After pupation the newly-developed adult escapes from the grain by chewing its way out but continues to bore through grains. When *R. dominica* is reared on a diet of whole wheat under optimum conditions (Temp: 34°C and 70% r.h), the larvae undergo 3-5 moults (14 days) and develop to pupae (3 days) (Haines, 1991).

R. dominica are adapted to rather higher temperatures and lower moisture contents than *Sitophilus* spp. and are therefore a dominant pest in areas which are hot, dry or both (NRI, 2001). In such areas, *R. dominica* is frequently associated with sorghum but the larger grain borer is almost exclusively associated with maize and dried cassava chips.

2.4.3 Rust-red flour beetle (*Tribolium castaneum* L.)

This is a red-brown tenebrionid beetle (2.5-4.5 mm in length), parallel-sided and partially dorso-ventrally flattened. It is found throughout all tropical, subtropical and warm temperate areas of the world (Haines, 1991). The males possess a hairy puncture on the ventral surface of the anterior femur. It is a secondary pest of a range of commodities especially cereals but also groundnuts, spices, coffee, cocoa, dried fruit and occasionally pulses (NRI, 2001). *T. castaneum* also feeds on animal tissues including immobile stages of living insects (larvae and pupae) and are cannibalistic under conditions of over crowding. The eggs and pupae are often cannibalised by the adults, the males showing preference for pupae and females for eggs.

The females lay their rather sticky eggs loosely among their food throughout adult lives (NRI, 2001). The number of eggs laid depends upon temperature with averages of 2.5 and 11 eggs each day at 25°C and 32.5°C, respectively (Haines, 1991). Under optimum conditions (35°C and 75% RH), larvae emerge from eggs approximately 3 days after oviposition on a diet of wheat feed. The larvae moult 7 or 8 times (within 13 days) to reach pupa stage that lasts 4.5 days (Haines, 1991). Development (egg-adult) can be very quick (20-30 days) leading to rapid population growth. Heavy infestations can produce unpleasant odours and flavours in commodities due to

production of quinines from the abdominal and thoracic defence glands of the adults (NRI, 2001). Adults can live for about six months. *Tribolium castaneum* is a colonizing species that successfully disperses by flying from the surfaces of infested sacks, especially when infestations are heavy, to neighbouring storage facilities and surroundings (Haines, 1991). This is further facilitated by the wide range of diets on which it can live and by its success as a competitor resulting from its high rate of increase and its predatory habits.

2.4.4 Cowpea beetle (*Callosobruchus chinensis* L.)

Callosobruchus chinensis L. is a bruchid beetle that is an important primary pest of pulses in tropical and subtropical climates (Haines, 1991; Semple *et al.*, 1992). Males have pectinate to strongly pectinate antennae whilst in females they are subserrate. It has an apical tooth on the inner carina of the hind femur which is long, narrow, parallel sided and blunt at the apex. Adults are generally 2.5-3.5 mm in length with typical white markings on the scutellum. Species of *Callosobruchus* are important primary pests of a number of legume grains including cowpea, pigeon pea, chickpea, adzuki beans, peas, grams and occasionally soya beans (NRI, 2001). The adult beetles, which do not feed on stored products, are very short-lived (≤ 12 days under optimum conditions) and the females lay many eggs up to 115 per adult at optimum temperature of 30-35°C.

The females lay their eggs which are firmly glued to the surface of infested seed with smooth-seeded varieties being more suitable for oviposition than the rough-seeded. Upon hatching, the larva bites through the base of the egg, the testa of the seed and into the cotyledons (Haines, 1991). The larva feeds entirely within a single seed, excavating a chamber within the cotyledons as it grows. Under optimum conditions (32°C and 90% rh), the development (egg-adult) period of *C. chinensis* is very short (21 days) as compared to 36 days at 25°C and 70% rh (Haines, 1991; Semple *et al.*, 1992).

CHAPTER THREE

GENERAL MATERIALS AND METHODS

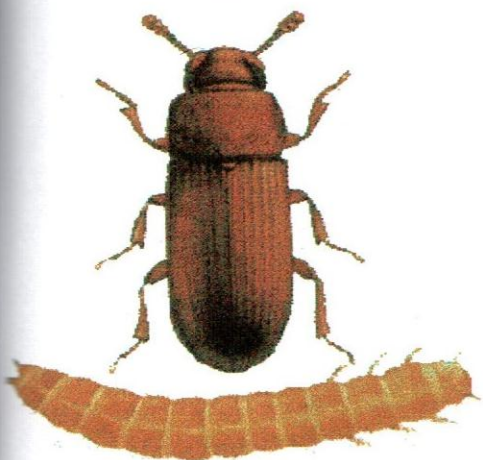
3.1 Mass rearing of test insects

Laboratory cultures of the rice weevil (*Sitophilus oryzae* L.), rust-red flour beetle (*Tribolium castaneum* (Herbst)), lesser grain borer (*Rhyzopertha dominica* F.) and cowpea beetle (*Callosobruchus chinensis* L.) (Plate 3.1) maintained at $30\pm 2^{\circ}\text{C}$, $68\pm 2\%$ RH and 12D: 12D (total darkness) were used. Adult *S. oryzae* and *R. dominica* were reared on whole soft wheat grains whereas *T. castaneum* and *C. chinensis* were reared on standard wholemeal wheat flour plus 5% brewer's yeast (19:1) and whole chickpea grains, respectively (Haines, 1991; Kostyukovsky *et al.*, 2002a; Liu *et al.*, 2007). Ages of test insects were determined according to Papachristos and Stamopoulos (2002a) with modifications. To obtain desired age cultures, 300-400 adults (5-10 day old *S. oryzae* and *R. dominica*; 0-3 days for *C. chinensis* and 30-60 days for *T. castaneum*) of each species were allowed to breed separately for two weeks, except for *C. chinensis* which were allowed 24-48 h, in respective rearing media in 2-litre glass jars (Rajendran and Muralidharan, 2005). Thereafter the adults were sifted from the cultures. All emerging *S. oryzae* and *R. dominica* adults were collected into labelled rearing glass jars every five days (0-5d) and *C. chinensis* daily (0-1d). Series of cultures of respective insect species were maintained continuously during the study period.

3.2 Collection and preparation of test plants

Three indigenous Kenyan plants, *Ocimum americanum* L. Benth (Basil: Lamiaceae), *Lantana camara* L. (Wild sage: Verbenaceae) and *Tephrosia vogelii* Hook (Fish poison bean: Fabaceae) were selected as test botanical plants based on their availability and locally perceived insecticidal properties (Ogendo *et al.*, 2003a; Plate 3.2). Fresh leaves, succulent stems and fruits were separately collected in labelled bags from Oyugis ($0^{\circ}30'S$, $34^{\circ}43'E$) and Kabondo ($0^{\circ}27'S$, $34^{\circ}52'E$) in Kasipul Division of Rachuonyo District, Kenya and transported to Egerton University within 24 hours. The samples were shade-dried in well ventilated room at ambient temperatures ($18-28^{\circ}\text{C}$) for 2 weeks and further oven-dried at 35°C for 48 hours (Asawalam *et al.*, 2006). Dry samples were then ground into fine powder using an electric laboratory hammer mill, weighed and stored in a cool place prior to being used for extraction of crude essential oils.

(a)



(b)



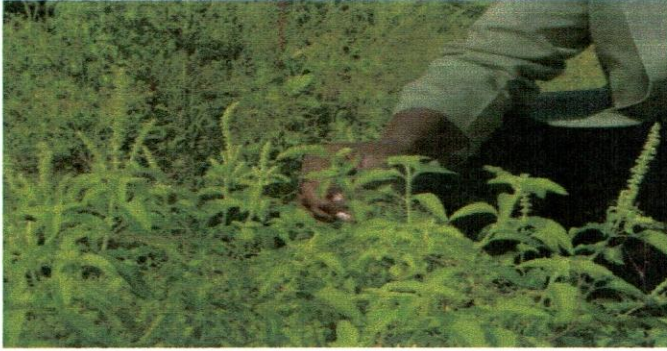
(c)



(d)



Plate 3.1: (a) Adult and larval stages of rust-red flour beetle (*Tribolium castaneum* (Herbst)) (b) adult rice weevil (*Sitophilus oryzae* L.), (c) adult lesser grain borer (*Rhyzopertha dominica* F.) and (d) adult cowpea beetle (*Callosobruchus chinensis* F.).



(a)



(b)



(c)

Plate 3.2: Test plants: (a) *Ocimum americanum* L. (syn. *O. canum*; Lamiaceae), (b) *Lantana camara* L. (Verbenaceae) and *Tephrosia vogelii* Hook (Fabaceae).

The identity of plants was confirmed at the Department of Biological Sciences and live herbaria kept at the Botanical Gardens, Egerton University.

Ocimum americanum L. [syn. *O. canum*; Lamiaceae; see Plate 3.2 (a) above], is a lemon-scented bushy, semi-woody perennial herb that is widely distributed in Africa and Asia (Vieira *et al.*, 2003). It is commonly found in low to medium altitude areas of East Africa including the forest and woodland clearings and used in folklore medicine (Ogendo *et al.*, 2003a) and a source of aroma compounds and essential oils containing bioactive constituents with insect repellent, insecticidal, nematicidal and antimicrobial activity (Vieira *et al.*, 2003; Cavalcanti *et al.*, 2004; Labra *et al.*, 2004). Studies with *O. americanum* (Syn. *O. canum*) essential oils in west and central Africa revealed five distinct chemotypes: citral, (E)-methyl cinnamate, camphor, methyl chavicol and linalool/camphor (Kyle *et al.*, 2002; Tchoumboungang, 2006). However, in Cameroon essential oils of *O. americanum* were dominated by 1, 8-cineole (41.3%) and sabinene (33.8%) among other monoterpene hydrocarbons with different structures (Amvam-Zollo *et al.*, 1998; Tchoumboungang, 2006).

Lantana camara L. [Verbenaceae; see Plate 3.2(b) above] is an invasive roadside perennial plant introduced originally from South America. There are two main varieties, one with orange-yellow flowers and another with red flowers. Although medicinal, the green unripe fruits and leaves of red sage *L. camara* L. contain a phototoxic triterpene derivative, lantadene-A, which has poisoning effect in livestock. However, the orange-yellow flowered sage commonly found in Kenya is grazed by livestock especially during periods of pasture scarcity with no documented cases of poisoning (Knight and Walter, 2003). Extracts and powders of *L. camara* have been reported to possess insecticidal, repellent, nematicidal and antimicrobial effects (Ogendo *et al.*, 2003b; Boughalleb *et al.*, 2005; Qamar *et al.*, 2005; Koona *et al.*, 2007a).

Fish poison bean, *Tephrosia vogelii* Hook [Fabaceae; see Plate 3.2 (c) above], is a semi-perennial, shrubby plant indigenous to Africa and grows best on depleted soils where it is a valuable fallow-improvement species particularly for the control of *Striga hermonthica*. It is also among the ten plants most used for poison fishing in Africa and fish eaten without undesirable health problems (Ibrahim *et al.*, 2000; Ogendo *et al.*, 2003a). In addition to its medicinal value,

T. vogelii derivatives have acaricidal, insecticidal, anti-feeding, repellent and molluscicidal effects against ectoparasites, mollusks, pre- and post-harvest insect pests (Marston *et al.*, 1984; Morris, 1999; Ibrahim *et al.*, 2000; Hilje *et al.*, 2003; Koon, 2005). Despite the extensive research endeavours to exploit its pesticidal potential, there is scanty documented information on the chemical composition of *T. vogelii* essential oils. Studies conducted in Brazil showed that the major components of the leaf and stem oils were geijerene (39.0- 50.3%) and pregeijerene (13.8- 21.0%) (Arriaga *et al.*, 2005).

3.3 Data Analysis

The actual and corrected percent adult insect mortalities in contact and fumigant toxicity studies were computed according to Asawalam *et al.* (2006) and Abbott (1925) in equations 1 and 2 [Section 4.2 (a) below]. All data on corrected percent adult mortality, F_1 progeny counts, percent repellence (PR values), percent germination and inhibition data were first homogenized using appropriate arcsine transformations before being subjected to a multi-factorial analysis of variance (ANOVA) and treatment means separated by Tukey's studentized (HSD) test (Mead *et al.*, 1994; Talukder and Howse, 1995; Papachristos and Stamopoulos, 2002a; Batish *et al.*, 2004; Ketoh *et al.*, 2005; Asawalam *et al.*, 2006; Ogendo *et al.*, 2008).

Data obtained from various concentration-response bioassays (contact and fumigant toxicity and germination inhibition) were further log-transformed before being subjected to probit regression analysis using EPA Probit Analysis Program version 1.4 and LC_{50} / EC_{50} values and corresponding 95% fiducial limits obtained from derived regression equations (Finney, 1971; Mead *et al.*, 1994; Mao *et al.*, 2004; Omolo *et al.*, 2005; Wheeler *et al.*, 2007). The LC_{50} values in a column were considered significantly different when 95% fiducial limits did not overlap.

CHAPTER FOUR

ANALYSIS OF *Ocimum americanum* L., *Lantana camara* L. AND *Tephrosia vogelii* HOOK ESSENTIAL OILS' CHEMICAL COMPOSITIONS

Abstract

Hydro-distilled essential oils from *O. americanum* L, *L. camara* L. and *T. vogelii* Hook were analyzed by GC and GC-MS to investigate intra-species and inter-plant variations in chemical composition. Fifty six, 57 and 69 constituents were identified accounting for 81.8-90.3, 60.7-81.9 and 87.5-94.1% of essential oils of *O. americanum*, *T. vogelii* and *L. camara*, respectively. The *O. americanum* L essential oils had a high percentage of monoterpenes which accounted for 27.57-79.96% of essential oil composition compared to 22.28-51.86% for the sesquiterpenes. Intra-species comparisons revealed that *O. americanum* leaf oil was high in eugenol (49.22%) and (Z)- β -ocimene (9.96%), the stem oil had high percentage of caryophyllene oxide (7.35%) whereas the fruit oil was high in elemicin (15.19%), δ -bisabolene (13.12%) and 1,8-cineole (6.63%). The *T. vogelii* essential oils were dominated by sesquiterpenes which accounted for 69.0-90.57% of oil composition compared to 9.56-19.99% for the monoterpenes. Intra-species comparisons have revealed that *T. vogelii* leaf oils had a high percentage of both sesquiterpenes and monoterpenes (seven and two percent, respectively), fruit oil was high in (E)-nerolidol (16.10%) whereas the essential oils from stems were high in farnesol isomer (5.20%), caryophyllene oxide (3.82%) and germacrene-D-4-ol (2.41%). The *L. camara* oils were dominated by sesquiterpenes which accounted for 47.71-72.24% of essential oil compared to 28.01-61.65% for monoterpenes identified. Intra-species comparisons have revealed that *L. camara* leaf oil was high in monoterpenes whereas stem and fruit oils were dominated by sesquiterpenes. The *O. americanum*, *T. vogelii* and *L. camara* plants were eugenol, germacrene D and caryophyllene chemotypes, respectively. The observed variability in chemical composition calls for a strict chemical profiling and mapping of all chemotypes and targeted bioactivity studies against major insect pests of stored durable agricultural products and adapted for user-specific pest management options.

4.1 Introduction

The new scientific push for alternative insecticides is accelerated by the high cost-benefit ratio, residues on treated grains, development of pest resistance and serious health concerns posed to non-target organisms by synthetic pesticides (Lamiri *et al.*, 2001; Ogendo *et al.*, 2003a; Talukder, 2006). In addition to their flavouring, medicinal and cosmetic values, aromatic plants present potentially high level efficiency in pre- and post-harvest crop protection (Lamiri *et al.*, 2001) and increasingly used as ingredients in foods, drinks, toiletries, medicine and cosmetics (Sacchetti *et al.*, 2005). Additionally, essential oils and their constituents are increasingly gaining attention from crop protectionists owing to their relative safety status, wide acceptance by consumers and potential multi-purpose functional use. Literature survey has revealed that the yields and the principal compounds of essential oils are dependent upon the chemical race (chemotype), climate, soil, geographical location (origin), plant part or tissue sampled, maturity, time of harvest and extraction method (Oyedemi *et al.*, 2003; Vieira *et al.*, 2003; Vasconcelos-Silva *et al.*, 2004; Huguette, 2005; Freire *et al.*, 2006).

Studies on essential oils of *L. camara* aerial parts in the Asian and African countries have revealed significant variability in chemical composition based on chemotype, flower colour, origin, time of harvest and plant part or tissue sampled. In studies conducted in Madagascar, Randrianalijaona (2006) reported that pink-flower type contained mainly davone (23.5%), β -caryophyllene (11.7%), sabinene (10.4%), linalool (5.9%) and α -humulene (4.7%) whereas the main constituents of yellow-flower type were β -caryophyllene (29.8%), β -bisabolene (15.3%), sabinene (13.3%), γ -cadinene (3.7%) and α -humulene (3.1%). Other findings revealed that essential oils of leaves and flowers of *L. camara* from Cameroon were characterized by a high percentage of sesquiterpenes with the major constituents as α -curcumene (25%), β -caryophyllene (13%), and caryophyllene epoxide (7%) while the main constituents of the oil from Madagascar were davanone (15%) and β -caryophyllene (12%). The monoterpenes composition of the two essential oils were lower and represented by sabinene (1-9%), α -pinene (2-4%), 1,8-cineole (1-3%) and linalool (1-3%) (Ngassoum *et al.*, 1999). Oyedemi *et al.* (2003) reported that the principal components of *L. camara* leaf oil from Nigeria were β -caryophyllene (24.6%), α -humulene (19.5%), sabinene (8.8%), germacrene D (5.7%) and cubebol (5.7%). In

similar studies, Sundufu and Shoushan (2004) showed that volatile oils from the leaves and flowers of *L. camara* in south China were characterized by a high percentage of sesquiterpenes with main components being germacrene D (15.85%), β -caryophyllene (12.35%), α -humulene (9.31%), germacrene B (6.19%) and an oxygenated monoterpene, 1,8-cineole (4.61%).

Literature indicates that relative amounts of essential oil components could be used to ascertain the identity of various chemotypes of aromatic plants from different geographic locations. Despite their enormous folklore values and documented preservation potential, the chemical composition of essential oils obtained from different parts of *O. americanum*, *L. camara* and *T. vogelii* are yet to be profiled. This study evaluated the intra-species and inter-plant variations in chemical composition of essential oils extracted from aerial parts of *O. americanum* L. Benth, *L. camara* L. and *T. vogelii* Hook. In an attempt to replace synthetic pesticides with less expensive, biodegradable and locally available pesticidal plant derivatives, there is need for isolation, identification and possible synthesis of active compounds. If pursued to logical conclusion, suitable compounds or their analogues can be availed by local industries to subsistence farmers and organic farming systems in the developing countries where the technology is highly needed.

4.2 Materials and Methods

4.2.1 Extraction, analysis and identification of essential oils

Essential oils were extracted from weighed dry powders of test plant materials, obtained as described in section 3.2 above, by hydro-distillation using Clevenger apparatus (Negahban *et al.*, 2006), dried over anhydrous sodium sulphate and re-refrigerated at 4°C. The analysis, identification and determination of relative amounts of essential oil constituents were done on an Agilent GC-MSD apparatus equipped with an Rtx-5SIL MS ("Restek") (30 m x 0.25 mm i.d. x 0.25 μ m) fused-silica capillary column. The injector was kept at 250°C and the transfer line at 280°C. Helium at constant pressure (13 psi) was used as a carrier gas with retention time locking. The column was maintained at 50°C for 2 min and then programmed to 260°C at 5°C/min and kept for 10 min at 260°C. The MS was operated in the EI mode at 70 eV, in a range of 42 to 350 m/z.

The identification was performed by comparing their relative retention indices and mass spectra with those of authentic samples or with those found in the literature (Adams, 1995) and supplemented with, Wiley, NIST 98 and QuadLib 1607 GC-MS libraries. The relative proportion of the essential oil constituent was computed in each case from the GC-MS peak areas and identified constituents summarized according to their retention times. The standards used for identification process and bioactivity studies were obtained from Sigma-Aldrich Israel Ltd.

4.3 Results and Discussion

Results demonstrated the existence of clear intra-species and inter-plant variability in the chemical composition of *O. americanum*, *L. camara* and *T. vogelii* essential oils (Tables 4.1-4.3). On the basis of plant part sampled, 56, 57 and 69 constituents accounting for 81.8-90.3, 60.7-81.9 and 87.5-94.1% of essential oils of *O. americanum*, *T. vogelii* and *L. camara*, respectively, were identified. Results showed that *O. americanum*, *T. vogelii* and *L. camara* essential oils were eugenol (49.2% in leaves), germacrene D (19.2% in leaves) and E-caryophyllene (15.5% in fruits) chemotypes, respectively (Fig. 4.1).

Intra-species variations in qualitative and quantitative chemical composition of essential oils obtained from the aerial parts of *O. americanum* were observed (Fig. 4.1; Table 4.1). A total of 56 constituents accounting for 90.33, 81.83 and 84.69% of the leaf, stem and fruit essential oils, respectively, were identified. Five major monoterpenes, eugenol (12.59-49.22%), (Z)- β -ocimene (2.89-9.96%), 1,8-cineole (3.39-6.63%), linalool (1.42-2.39%) and β -pinene (0.51-1.05%), accounted for 23.67- 64.58% compared to six major sesquiterpenes (21.71-46.28%) (Fig.4.1). The six major sesquiterpenes were elemicin (8.19-15.19%), δ -bisabolene (3.81-13.12%), unknown $C_{15}H_{24}$ (5.49-7.02%), caryophyllene oxide (0.32-7.35%), E-caryophyllene (2.87-4.87%) and germacrene D (3.02-4.53%). Comparative analysis showed that leaf oil had high percentage of eugenol and (Z) - β -ocimene, stem oil was high in caryophyllene oxide and fruit oil had a high percentage of 1,8-cineole, δ -bisabolene and elemicin.

Although these results conform to expected variations on the basis of chemotype, origin, plant part sampled and extraction method, the chemical composition contrast sharply with documented information from Africa and Latin America.

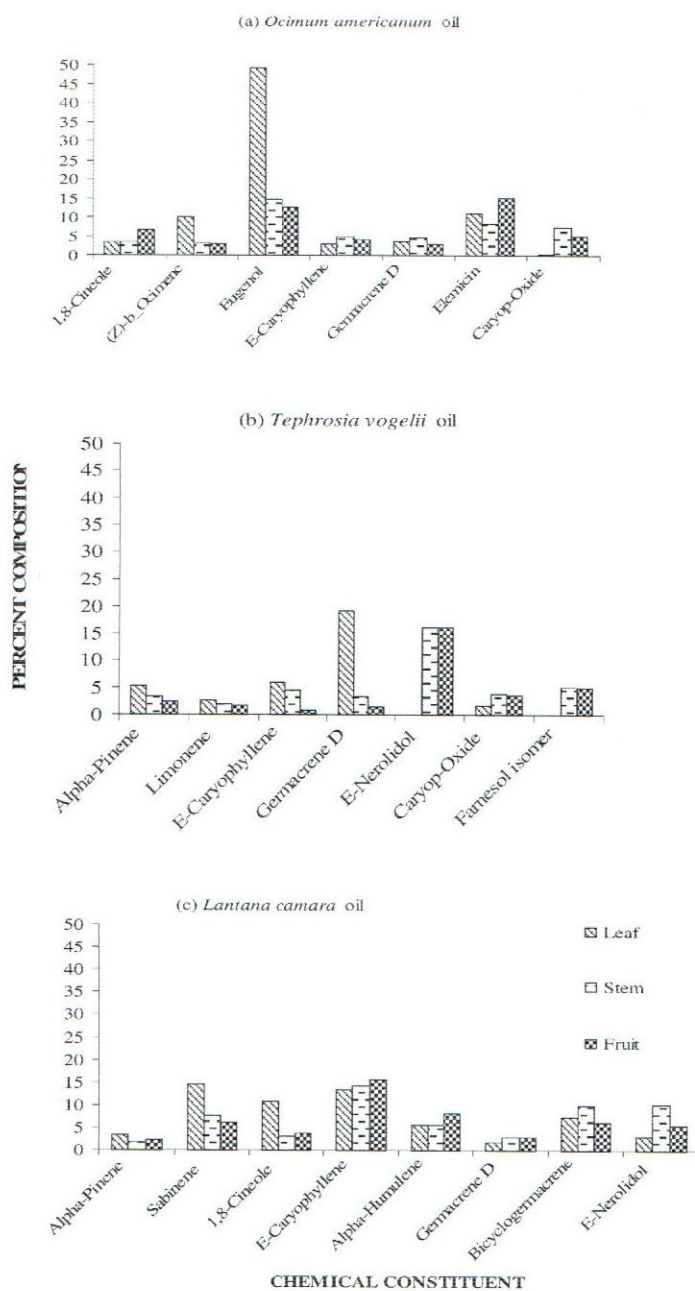


Fig.4.1: Major constituents of essential oils obtained from aerial parts of (a) *Ocimum americanum* L., (b) *Tephrosia vogelii* Hook and (c) *Lantana camara* L

The *O. americanum* oils showed the species as a eugenol chemotype which is outside the five chemotypes: citral, camphor, methylcinnamate, epicamphor and eucalyptol identified by Kyle *et al.* (2002). Golob *et al.* (1999) reported that the composition of essential oil varied according to origin of *O. canum* (Syn. *O. americanum*) and that, in East Africa, the species contained 16-25% camphor whereas methyl cinnamate and methyl chavicol (estragol) were predominant (up to 75%) in Central and West Africa, respectively. Studies in Ethiopia reported the presence of 1,8-cineole (6.8%), linalool (35.7%), estragole (8.9%), carvacrol (6.4%), methyl cinnamate (34.3%) and alpha-humulene (1.2%) as the major essential oil constituents of *O. americanum* (ALNAP, 2001). However, this chemical composition closely resembles the Brazilian chemotype whose essential oils had (E)-methyl cinnamate as major constituent of leaves (82.7%) and inflorescence (87.1- 97.5%) whereas eugenol constituted 88.2% of the root essential oil (Vasconcelos-Silva *et al.*, 2003). These results have revealed a major departure from known chemotypes in the region. As earlier observed by Tchoumboungang (2006), the existence of high variability in chemical composition of *Ocimum* species calls for a clear botanical identification (chemical profiling) and knowledge of the origin (mapping) of all chemotypes in Kenya. In addition, each chemotype identified be studied for bioactivity against major insect pests of stored durable agricultural products and promising chemotype(s) be adapted for user-specific pest management options.

Marked intra-species variations in qualitative and quantitative chemical composition of essential oils obtained from the aerial parts of *T. vogelii* were observed (Table 4.2). A total of 57 constituents accounting for 81.87, 60.69 and 65.87% of the leaf, stem and fruit essential oils, respectively, were identified. Irrespective of plant part sampled, the *T. vogelii* essential oils were dominated by sesquiterpenes with the 12 major sesquiterpene compounds accounting for 31.03- 57.39% of oil composition. The 12 major sesquiterpenes identified, in order of decreasing amounts, were germacrene D (1.50-19.18%), (E)-nerolidol (16.05- 16.10%), (E,Z)-Farnesol (11.22%), germacrene B (8.61%), E-caryophyllene (0.94- 6.06%), Farnesol isomer (4.88- 5.20%), caryophyllene oxide (1.64- 3.82%), bicyclogermacrene (1.61- 3.22%), germacrene-D-4-ol (1.63- 2.41%), β -elemene (0.61-2.39%), α -humulene (0.41- 2.01%) and β -selinene (1.04- 1.43%) (Fig.4.1). Monoterpenes, on the other hand, were the minor constituents with the six components (accounting for 4.83- 10.54% oil composition) present in fairly good amounts were α -pinene (2.30-5.45%), limonene (1.74- 2.67%), myrcene (0.24- 0.71%), (E)-beta ocimene

(0.20- 0.69%), β -pinene (0.25- 0.64%) and (Z)- β -ocimene (0.10-0.41%). Marked qualitative variations in chemical composition of essential oils were observed with the leaf oil having a high percentage of 9 constituents, namely germacrene D, farnesol, germacrene B, E-caryophyllene, α -pinene, bicyclogermacrene, limonene, β -elemene and α -humulene. The fruit oil was high in (E)-nerolidol only whereas essential oil obtained from succulent stems had a high percentage of caryophyllene oxide, germacrene-D-4-ol and farnesol isomer. The existence of intra-species variation calls for documentation of chemotypes (if any), geographical distribution (mapping) in Kenya and biological potential for stored-product insect pest control in specific user domains.

The qualitative and quantitative chemical composition of *L. camara* essential oils were influenced by plant part sampled. A total of 69 constituents which constituted 87.47, 92.74 and 94.10% of the leaf, stem and fruit oils, respectively, were identified. The essential oils were characterized by a high percentage of sesquiterpenes in which six major components constituting 38.60-56.46% compared to 15.94-38.10% for seven major monoterpene compounds (Table 4.3). The six principal sesquiterpenes identified were E-caryophyllene (13.35- 15.52%), bicyclogermacrene (6.25- 10.05%), unknown sesquiterpene oxide (9.03- 9.69%), α -humulene (5.73- 8.25%), (E)-nerolidol (3.16- 10.10%) and caryophyllene oxide (1.07- 2.85%). Seven major monoterpenes, sabinene (6.12- 14.53%), 1,8-cineole (3.11- 10.81%), α -pinene (1.88- 3.35%), terpinen-4-ol (1.31- 2.67%), camphor (1.13- 2.30%), β -pinene (1.26- 2.22%) and δ -2-carene (1.13- 2.22%). Leaf essential oils had a high percentage of monoterpenes whereas the stem (E-nerolidol, sesqui.oxide and bicyclogermacrene) and fruit (E-caryophyllene, α -humulene and caryophyllene oxide) oils were dominated by sesquiterpenes. These results are generally in agreement with several studies in Africa and Asia in which volatile oils of aerial parts of *L. camara* were characterized by a high percentage of sesquiterpenes (Ngassoum *et al.*, 1999; Oyedeji *et al.*, 2003; Sundufu and Shoushan, 2004; Randrianalijaona, 2006). Literature survey of documented information on the essential oil composition of the aerial parts of *L. camara* revealed existence of intra-species variability based on the geographical location (origin) and plant part assayed. Essential oils of leaves and flowers of *L. camara* from Cameroon contained α -curcumene (25%), β -caryophyllene (13%) and caryophyllene epoxide (7%) as major components whereas a Madagascan chemotype had davanone (15%) and β -caryophyllene (12%) as main components (Ngassoum *et al.*, 1999). The *L. camara* leaf essential oil used in this study

contained 24.6, 19.5% and 5.7% E-caryophyllene, α -humulene and germacrene D, respectively, compositions lower than a Nigerian chemotype but superior to the yellow-orange type in Madagascar (Oyediji *et al.*, 2003; Randrianalijaona, 2006). The study has demonstrated the existence of marked intra-species and inter-plant variations in chemical composition of essential oils obtained from *O. americanum*, *T. vogelii* and *L. camara*. These results form the main scientific principles for the differential bioactivities against the different stored-product insects.

Table 4.1: Chemical composition of the essential oils of *Ocimum americanum* L.

Chemical compound	Retention Time	% Composition			Chemical compound	Retention Time	% Composition		
		Leaf	Stem	Fruit			Leaf	Stem	Fruit
1 α -Thujene	5.38	-	Trace	0.03	29 Benzaldehyde	17.45	-	0.01	Trace
2 α -Pinene	5.56	0.20	0.55	0.40	30 Eugenol	17.61	49.22	14.57	12.59
3 Camphene	5.96	-	0.05	Trace	31 Octanal	17.76	-	0.06	0.06
4 Sabinene	6.55	0.16	0.17	0.28	32 α -Copaene	18.10	1.04	2.04	2.18
5 β -Pinene	6.68	0.51	1.05	0.97	33 β -Bourbonene	18.32	0.56	0.88	1.16
6 1-Octen-3-ol	6.75	-	0.04	0.03	34 β -Cubebene	-	-	0.68	0.69
7 6-methyl-5-Hepten-2-one	6.85	-	0.31	0.54	35 β -Elemene	18.45	-	0.18	0.12
8 Myrcene	6.98	0.42	0.28	0.40	36 Methyl eugenol	-	-	0.12	0.16
9 3-Octanol	7.20	-	0.02	Trace	37 Octanol	19.17	-	0.21	0.34
10 α -Phellandrene	7.44	-	0.02	Trace	38 E-Caryophyllene	19.28	2.87	4.87	4.23
11 α -Terpinene	7.74	-	0.05	Trace	39 β -Copaene	19.53	-	0.29	0.36
12 para-Cymene	7.95	-	0.17	0.22	40 α -cis-Bergamotene	19.61	-	0.05	0.06
13 Limonene	8.09	-	0.22	0.25	41 (Z)-b-Farnesene	20.12	0.83	2.33	1.59
14 1,8-Cineole	8.17	3.47	3.39	6.63	42 α -Humulene	20.22	0.25	0.46	0.38
15 (Z)- β -Ocimene	8.31	9.96	3.22	2.89	43 Germacrene D	20.90	3.66	4.53	3.02
16 (E)- β -Ocimene	8.57	-	0.20	0.40	44 Unknown (C₁₅H₂₄)	-	-	7.02	5.49
17 Linalool	8.58	1.42	1.44	2.39	45 (E,E)- α -Farnesene	21.58	3.81	11.0	13.12
18 γ -Terpinene	8.90	-	0.03	0.09	46 δ-Bisabolene	21.85	0.24	0.56	0.50
19 6-methyl-3,5-Heptadien-one	-	-	0.09	0.14	47 δ -Cadinene	-	-	0.16	0.15
20 1,3,8-para-Menthatriene	-	-	0.03	Trace	48 β -Sesquiphellandrene	22.56	11.05	8.19	15.19
21 Allo-Ocimene	10.97	0.34	0.05	0.05	49 Elemicin	22.89	-	0.30	0.21
22 Camphor	11.51	-	0.05	Trace	50 (E)-Nerolidol	23.32	-	0.17	Trace
23 Pinocarvone	12.53	-	0.05	Trace	51 Spathulenol	23.45	0.32	7.35	5.23
24 Terpinen-4-ol	12.86	-	0.06	0.49	52 Caryophyllene oxide	-	-	0.32	0.21
25 Methyl salicylate	12.96	-	0.16	0.17	53 Salvia-4(14)-en-1-one	-	-	0.60	Trace
26 α -Terpineol	-	-	0.54	0.42	54 Viridiflorol	-	-	0.55	0.40
27 Geraniol	-	-	1.42	Trace	55 Humulene epoxide 2	-	-	0.29	0.22
28 α -Cubebene	17.26	-	0.08	Trace	56 Cryptomerione	-	-	81.83	84.69
					Total % constituents identified				
					90.33				

Table 4.2: Chemical composition of the essential oils of *Tephrosia vogelii* Hook.

Chemical compound	RT	% Composition			Chemical compound	RT	% Composition		
		Leaf	Stem	Fruit			Leaf	Stem	Fruit
1 α -Thujene	5.38	0.23	0.10	0.06	31 (Z)- α -Bergamotene	19.09	0.73	0.09	0.12
2 α -Pinene	5.47	5.45	3.46	2.30	32 E-Caryophyllene	19.29	6.06	4.45	0.94
3 Sabinene	6.55	0.42	0.22	0.19	33 γ -Elenene	19.53	1.20	0.33	-
4 β -Pinene	6.68	0.64	0.31	0.25	34 (E)- α -Bergamotene	19.62	0.98	0.17	0.21
5 Myrcene	6.98	0.71	0.50	0.24	35 Sesquiasabinene	19.81	0.30	-	0.36
6 para-cymene	7.95	-	0.07	0.07	36 Geranyl acetone	19.99	0.55	-	0.11
7 Limonene	8.09	2.67	1.90	1.74	37 (Z)-b-Farnesene	20.14	0.91	-	-
8 β - Phellandrene	8.17	-	0.13	-	38 α -Humulene	20.23	2.01	1.71	0.41
9 1,8-Cineole	8.28	-	0.07	0.12	39 Allo-Aromadendrene	20.35	-	0.10	-
10 (Z)- β -Ocimene	8.58	0.41	0.33	0.10	40 Germacrene D	20.96	19.18	3.49	1.50
11 (E)- β -Ocimene	8.90	0.66	0.69	0.20	41 β -Selinene	21.12	1.43	1.04	1.39
12 <i>trans</i> -Sabinene hydrate	10.14	-	0.17	-	42 Bicyclogermacrene	21.29	3.22	1.44	1.61
13 γ -Terpinene	11.51	-	0.03	0.01	43 β -Bisabolene	21.57	1.36	-	-
14 Linalool	12.27	0.35	0.22	0.07	44 10-epi-cubebol + d-cadinene	21.81	3.39	-	-
15 α -Campholenal	12.53	-	-	0.07	45 γ -Cadinene	21.83	-	0.45	-
16 <i>trans</i> -Pinocarveol	12.86	-	0.67	0.06	46 δ -Cadinene	21.83	-	1.37	0.91
17 Camphor	12.97	-	0.07	-	47 (E)- Nerolidol	22.89	-	16.05	16.10
18 Borneol	12.97	-	0.25	-	48 Spathulenol	23.32	-	-	4.89
19 Terpinen-4-ol	12.97	-	0.25	0.12	49 Germacrene B	23.32	8.61	-	-
20 Verbenone	12.97	-	-	0.27	50 Germacrene-D-4-ol	23.33	1.63	2.41	-
21 <i>Trans</i> -Carveol	12.97	-	-	0.07	51 Caryophyllene oxide	23.46	1.64	3.82	3.59
22 Carvone	12.86	-	-	0.16	52 Unknown (M.w. 220)	-	-	3.08	18.55
23 Methyl salicylate	12.86	-	0.04	-	Selin-11-en-4- α -ol	25.20	0.26	0.87	-
24 <i>trans</i> -Verbenol	11.51?	0.22	-	0.57	α -Cadinol	-	-	2.85	-
25 α -Terpineol	12.97	0.63	0.23	-	Neo-Intermedeol	25.29	-	0.44	-
26 δ -Elemene	16.95	0.66	-	-	56 (E, Z)- Farnesol	26.61	11.22	-	-
27 α -Copaene	18.08	1.52	0.63	0.61	57 Farnesol isomer	-	-	5.20	4.88
28 β -Bourbonene	18.30	0.23	-	0.03	Total % constituents identified	-	81.87	60.69	65.87
29 β -Cubebene	18.46	-	0.05	0.16					
30 β -Elemene	18.46	2.39	1.19	0.61					

Table 4.3: Chemical composition of the essential oils of *Lantana camara* L.

Chemical compound		RT	% Composition		Chemical compound	RT	% Composition		Fruit
			Leaf	Stem			Leaf	Stem	
1	α -Thujene	5.38	0.53	0.25	36	d-Elementene	16.94	0.15	0.10
2	α -Pinene	5.55	3.35	1.88	37	α -Cubebene	17.26	0.02	0.08
3	Camphene	5.96	1.40	0.84	38	Eugenol	17.45	0.11	0.03
4	Sabinene	6.56	14.53	7.59	40	α -Copaene	18.07	0.43	1.52
5	β -Pinene	6.68	2.22	1.40	41	β -Bourbonene	18.28	0.04	0.18
6	1-Octen-3-ol	6.75	0.19	0.16	42	Unknown-sesqui,hydro	18.39	0.26	0.49
7	6-Methyl-5-Hepten-2-one	6.85	-	-	43	β -Cubebene	18.45	0.77	-
8	Myrcene	6.98	1.24	0.88	44	β -Elenene	19.09	1.05	1.25
9	3-Octanol	7.20	0.13	0.32	45	(Z)- α -Bergamotene	19.29	13.35	14.19
10	α -Phellandrene	7.44	0.27	0.18	46	E-Caryophyllene	19.53	0.62	1.21
11	d-2-Carene	7.51	2.22	1.27	47	β -Copaene	19.61	-	0.09
12	α -Terpinene	7.74	0.14	0.20	48	(E)- α -Bergamotene	19.81	-	-
13	para- Cymene	7.95	0.54	0.39	49	Sesquiasabinene	19.77	-	0.11
14	Limonene	8.09	1.48	0.96	50	Aromadendrene	20.11	-	1.17
15	1,8-Cineole	8.17	10.81	3.11	51	β - Bisabolene	20.23	5.73	8.25
16	β -(Z)-Ocimene	8.27	0.64	0.52	52	α -Humulene	20.35	0.24	0.51
17	β -(E)-Ocimene	8.57	0.92	1.00	53	Allo-Aromadendrene	20.72	0.30	0.67
18	γ -Terpinene	8.90	0.36	0.42	54	γ - Muurolene	20.88	1.75	2.99
19	cis-Sabinene hydrate	9.26	1.53	-	55	Germacrene D	21.15	0.14	0.27
20	Terpinolene	9.70	0.34	0.39	56	γ - Amorphene	21.29	7.48	6.26
21	trans-Sabinene hydrate	10.14	0.94	0.47	57	Bicyclogermacrene	21.83	0.07	0.13
22	2-Methyl butyl-2-methyl butyrate		0.10	-	58	γ -Cadinene	21.83	0.58	0.93
23	Linalool	10.86	-	0.80	59	d- Cadinene	21.91	-	0.19
24	cis-para-Menth-2-en-1-ol	11.38	0.06	0.05	60	cis- Calamenene	22.53	0.15	0.26
25	trans-para-Menth-2-en-1-ol	11.51	2.30	1.13	61	Elemicin	22.59	2.26	0.79
26	Camphor	12.22	-	0.15	62	trans- Davanone	22.76	-	0.72
27	d- Terpineol	12.27	0.56	1.25	63	Davanone	22.89	3.16	5.65
28	Borneol	12.53	1.31	1.80	64	(E)-Nerolidol	23.32	2.30	2.35
29	Terpinen-4-ol	12.72	-	-	65	Spathulenol	23.44	1.34	2.85
30	para-Cymen-8-ol	12.86	0.16	0.07	66	Caryophyllene oxide	24.88	0.34	0.53
31	Methyl salicylate	12.96	1.02	0.76	67	trans-Cadinol	-	-	9.03
32	α - Terpineol	14.19	-	-	68	Unknown- sesqui,oxide	25.29	-	0.17
33	Neral	15.04	-	-	69	Neo-Intermedeol	-	-	94.10
34	Geraniol	16.84	-	0.17	Total % constituents identified				
35	Bicycloelemene		-				87.47	92.74	

CHAPTER FIVE

CONTACT TOXICITY AND REPRODUCTION INHIBITORY EFFECTS OF ESSENTIAL OILS OF *O. americanum*, *L. camara* AND *T. vogelii* AGAINST FOUR STORED-PRODUCT INSECT PESTS

Abstract

A study was conducted to evaluate the instant and residual contact toxicity and reproduction inhibitory effects of essential oils extracted from aerial parts of *O. americanum*, *L. camara* and *T. vogelii* against adult stages of *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis* insects. Each test essential oil was evaluated at five rates (0, 0.05, 0.10, 0.15 and 0.20% v/w) and Actellic 5EC (0.1% v/w) and crude soya oil (1.0% v/w) included as positive controls. Each treatment was replicated 4 times against all four test insects. Results showed that instant contact toxicity and reproduction inhibition significantly ($P < 0.0001$) depended upon intra-species and inter-plant variations in chemical composition, essential oil concentration, contact duration, insect species and corresponding factor interactions. Irrespective of plant and plant part assayed, *T. castaneum* and *C. chinensis* were the most tolerant and susceptible insect species, respectively, against all the essential oils tested. In the instant contact toxicity studies, at the highest concentration (0.20% v/w), *O. americanum*, *L. camara* and *T. vogelii* oils caused 13- 96, 5-100 and 3- 83% kill, respectively, against the adult stages of the four test insects. In the instant reproduction inhibition studies, the highest concentration (0.20% v/w), *O. americanum*, *L. camara* and *T. vogelii* oils caused 28- 61, 35- 60 and 42- 68% reductions, respectively, in adult F_1 progeny counts of the four insect pests of stored food grains. On the other hand, results showed that residual contact efficacy and reproduction inhibitory effects of *O. americanum* and *T. vogelii* essential oils against test storage insects were significantly ($P < 0.0001$) influenced by storage duration, duration of contact with treated grains, essential oil concentration and corresponding factor interactions. At the highest concentration (0.20% v/w) and 90-120 days of grain storage, *O. americanum* oils caused 18- 21, 15- 22, 14- 18 and 58- 75% mortalities of adult *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis*, respectively. At the same concentration and 60-120 days, *T. vogelii* oils caused 31- 47, 18- 23, 3-5 and 37- 53% mortality of adult *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis*, respectively. Temporal comparisons of data from same

O. americanum essential oil treatments revealed 79-81, 13-40, 14-34 and 0-22% reductions in contact efficacy against adult *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis*, respectively, after 90-120 days. Similarly, data from same *T. vogelii* essential oils showed 7-38, 13-34, 0 and 36-57% reductions in contact efficacy against the same insects, respectively, after 60-120 d. The magnitude of depressed contact toxicity was clearly manifested in increased LC₅₀ values with storage duration. Temporal comparisons revealed 19-32 and 42-54% reductions in reproduction inhibitory activity of *O. americanum* and *T. vogelii* leaf essential oils, respectively, on *C. chinensis*. These results are discussed in the context of scientific implications for biological control of insect pests of stored food grains.

5.1 Introduction

In recent years scientists have directed their efforts towards the development of effective, biodegradable and eco-friendly alternatives to synthetic pesticides. Plant-derived materials with insecticidal properties have been used traditionally for generations throughout the world and remain the major control principle in subsistence agriculture (Belmain *et al.*, 2001; Ogendo *et al.*, 2003a). Botanicals have many advantages over synthetic pesticides because they are accessed locally by farmers, inexpensive, readily biodegradable, less toxic to mammals and less prone to resistance by insect species (Belmain *et al.*, 2001; Asawalam *et al.*, 2006; Rahman and Talukder, 2006). Higher plants are an excellent source of biologically active natural products known to be insecticidal (Kéita *et al.*, 2001; Clemente *et al.*, 2003).

Plants in the Fabaceae family such as the fish poison bean, *T. vogelii*, have been used for poison fishing in Africa for generations and have their toxicity principle based on the presence of rotenoids known to be mitochondrial chain inhibitors (Berger, 1994; Neuwinger, 2004). In past studies, crude powders (2.5-5.0% w/w) of *T. vogelii* caused 98-100% mortality of adult *Caryedon serratus* and *Sitophilus zeamais* insects after 13-21 days (Delobel and Malonga, 1987; Ogendo *et al.*, 2003b). Additionally, powders and slurries obtained from *T. vogelii* have been reported to have significant reproduction inhibition effects against *C. maculatus* whereas aqueous extracts (10-15% w/v) have been reported to have acaricidal and insecticidal effects in the field (Berger, 1994; Kambewa *et al.*, 1997; Chifundera, 1998; Boeke *et al.*, 2004 a, b). Plants in the Lamiaceae family such as *Ocimum* species reportedly have medicinal and insecticidal

values and are widely used in tropical agriculture (Bekele, 1994; Golob *et al.*, 1999). In her review article, Regnault-Roger (1997) reported that *O. basilicum* essential oil has been shown to have strong insecticidal activity against major coleopteran pests of stored grains. In separate instant contact toxicity studies, *O. basilicum* and *O. canum* extracts and kaolin aromatized with the two essential oils, respectively, had low to high toxicity against *T. castaneum* (20% kill after 30 days) and *C. maculatus* (99% kill after 24 h) (Kéita *et al.*, 2000; Clemente *et al.*, 2003). In one of the long-term contact toxicity studies with powders aromatized with *O. basilicum* and *O. gratissimum* essential oils, Kéita *et al.* (2001) reported LC₅₀ values of 65 and 116 µl/g, respectively, after 48 h against adult *C. maculatus* insects and the same aromatized powders provided complete protection for over 3 months. The other test plant, *Lantana camara* (Verbenaceae), is an invasive roadside perennial plant known to have general medicinal, cosmetic and forage values (Knight and Walter, 2003; Rasoanaivo and De La Gorce, 2004). In local studies with crude powders, *L. camara* (5.0% w/w) caused 85% mortality of adult *S. zeamais* insects after 21 days of contact (Ogendo *et al.*, 2003b). Impregnating storage bags (10% w/v) and admixing grains (4% w/w) with *L. camara* powder significantly reduced seed damage 4-6 fold after 6 months of storage (Koono *et al.*, 2007a). In the only instant contact toxicity data obtained, *L. camara* essential oil had an LC₅₀ value of 0.16% v/w after 24 h against adult *S. zeamais* insects (Bouda *et al.*, 2001). Essential oils of botanical origin and their major constituents are among the best known substances to have attracted research attention in recent years as potential alternatives to contact synthetic insecticides for control of storage insect pests. Little or no local research endeavours have been directed towards intra-species and inter-plant bioactivity studies against major insect pests of stored food commodities. The objective of the current study was therefore to evaluate the instant and residual contact toxicity and reproduction inhibitory effects of essential oils extracted from aerial parts of three indigenous aromatic African plants, *O. americanum*, *L. camara* and *T. vogelii* against adult stages of *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis*.

5.2 Materials and Methods

5.2.1 Mass rearing of test insects

The adult stages of *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis* insects used for contact toxicity and reproduction inhibition (F₁ progeny) studies were obtained from the insect cultures reared as described in Chapter 3 above.

4.2.2 Contact toxicity on adult insects and F₁ progeny

(a) Instant contact toxicity on adult insects and F₁ progeny

The essential oils samples extracted from leaves, succulent stems and fruits of *O. americanum*, *L. camara* and *T. vogelii*, as described in earlier in Chapter 4, were evaluated for contact toxicity against adults and resultant F₁ progeny of *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis* (Shaaya *et al.*, 1997; Awasalam *et al.*, 2006). The oils were applied to 10-g grain samples in 100-ml glass jars at five rates (0, 0.05, 0.10, 0.15 and 0.20% v/w) dissolved in acetone AR (99.8% GC) and shaken thoroughly to ensure even distribution over the grain surface. The negative controls consisted of starved insects (no food), untreated and acetone (0.05% v/w) treated grains whereas Actellic 5EC at 0.1%v/w and crude soya oil at 1.0%v/w were the positive controls. The treated grains were kept for 1 hour (h) to allow acetone to evaporate completely prior to being artificially infested with 20 (N_T) unsexed adults of test insects. The treatment jars were covered with towel paper held in place with rubber bands and kept in a room maintained at 30±2°C, 68±2% RH and 12D: 12D (total darkness). The laboratory bioassay was arranged in a completely randomized design (CRD) with four replicates per treatment. The number of dead insects (N_D) in each jar was counted and recorded at 2, 4, 6, 8 and 10 days (d) after treatment to estimate the adult insect mortality. All adult insects (live and dead) were then removed and insects that emerged at 35-63 d (*S. oryzae*, *R. dominica* and *T. castaneum*) and 21-40 d (*C. chinensis*) after treatment were used in the estimation of F₁ progeny production. No adult F₁ progeny counts were recorded after 40 d for *C. chinensis* and 63 d for the other test insects to avoid overlap of insect generations (Asawalam *et al.*, 2006).

(b) Residual contact toxicity on adult insects and F₁ progeny

Selected essential oil samples as described in Section 4.2.2 (a) above were further evaluated for long-term residual contact toxicity against adults and resultant F₁ progeny of *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis* insects (Shaaya *et al.*, 1997; Awasalam *et al.*, 2006). The essential oils, dissolved in acetone AR (99.8% GC), were applied to 50-g wheat (or 100 g chickpea) grain samples in special self-sealing polythene bags (20 cm x 25 cm; 2 L capacity) at five rates (0, 0.05, 0.10, 0.15 and 0.20% v/w). The negative controls consisted of untreated and acetone (0.05% v/w) treated grains whereas Actellic 5EC (0.1% v/w) and crude soya oil (1.0% v/w) were the positive controls. The treated grains were kept in fume chamber for 24 h to allow

vacuum to evaporate. Thereafter the polythene bags were sealed and treated grain samples transferred into a room maintained at $30\pm 2^{\circ}\text{C}$, $68\pm 2\%$ RH and 12D: 12D (total darkness) for long-term storage. The laboratory bioassay was arranged in a CRD with four replicates per treatment. Unbiased grain sub-samples (10 g wheat and 20 g chickpea) were then drawn from each experimental unit containing treated grains at 30, 60, 90 and 120 d after treatment. Into each sub-sample in 100-ml jar, 20 unsexed adult test insects (N_T) were introduced and the number of dead (N_D) insects in each jar was recorded 2, 4, 6, 8 and 10 d after onset of experiment to estimate the adult insect mortality. All adult insects were then removed and insects that emerged 35-63 d (*S. oryzae*, *R. dominica* and *T. castaneum*) and 21-40 d (*C. chinensis*) after treatment were used in the estimation of F_1 progeny production. No adult F_1 progeny counts were conducted after 40 d for *C. chinensis* and 63 d for the other test insects to avoid overlap of insect generations (Asawalam *et al.*, 2006). Actual and corrected percent mortalities in all contact toxicity studies were computed according to Asawalam *et al.* (2006) and Abbott (1925), respectively, in equations 1 and 2:

$$\text{Actual Mortality(\%)} = \left\{ \frac{N_D}{N_T} \right\} \times 100 \quad \text{Equation 1}$$

$$P_T = \frac{(P_O - P_C)}{(100 - P_C)} \times 100 \quad \text{Equation 2}$$

Where P_T , P_O and P_C represent the Corrected, Observed and Control percent mortalities, respectively. Data analysis and interpretation are as described in Chapter 3.

5.3 Results and Discussion

5.3.1 Instant contact toxicity on adult insects and F_1 progeny

Results showed that the contact toxicity of essential oils were significantly ($P < 0.0001$) influenced by both intra-species and inter-plant variations, contact duration, concentration, insect species and corresponding factor interactions except plant part by contact duration (*T. castaneum*) and plant species by plant part (*C. chinensis*) interactions that were insignificant. The two positive controls, Actellic 5EC (0.1% v/w) and crude soya oil (1.0% v/w) were highly toxic against all test insects with 100 and 91.7% kill, respectively, achieved after 48 h of contact with treated grains (Table 5.1). In the insect starvation studies, except for *C. chinensis* in which there were insufficient data points, LT_{50} values of 2.67, 3.75 and 12.44 days were obtained for *S.*

oryzae, *R. dominica* and *T. castaneum*, respectively (Fig. 5.1). *S. oryzae* was the most susceptible to starvation followed by *R. dominica* and *T. castaneum*, respectively. On the basis of these observations, the results were interpreted in the context of their potential as feeding deterrents and hence starvation-related insect mortalities.

The results showed the existence of marked dose- and contact duration-dependent intra-species variations in the efficacy of essential oils obtained from the aerial parts of *O. americanum* against adult *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis*. In all the bioassays the LC₅₀ values decreased with contact duration a manifestation of increased adult insect mortality. Against *S. oryzae*, the essential oil extracted from the fruits (LC₅₀ value: 0.01% v/w) showed highest toxicity followed by leaf (LC₅₀ value: 0.22% v/w) and stem (LC₅₀ value: 1.68% v/w) essential oils, respectively (Table 5.2). In the inter-insect species comparisons, except for *C. chinensis* which was not tested due to lack of test insects, bioassay with essential oil extracted from fruits showed that *S. oryzae* (LC₅₀ value: 0.01% v/w) was the most susceptible followed by *R. dominica* (LC₅₀ value: 0.78% v/w) and *T. castaneum* (LC₅₀ value: 0.87% v/w), respectively. However, essential oil extracted from the succulent stems was most efficacious against *R. dominica* (LC₅₀ value: 0.25% v/w) followed by *T. castaneum* (LC₅₀ value: 0.90% v/w) and *S. oryzae* (LC₅₀ value: 1.68% v/w), respectively. In the leaf essential oil studies in which only *S. oryzae* and *C. chinensis* were tested due to lack of other test insects, strong contact toxicity was observed with 49 and 74% kill, respectively, at 0.20% v/w, 10 and 4 days after treatment (Table 4.3). Overall, *T. castaneum* (stem and fruit oils) and *C. chinensis* (leaf oil only) were the most tolerant and susceptible insects, respectively, with 13-21 and 74% kill, at the highest concentration (0.20% v/w). The other two species, *S. oryzae* (leaf, stem and fruit oils) and *R. dominica* (stem and fruit oils) achieved 22-96 and 26-37% mortality, respectively, at the highest concentration (0.20% v/w) (Table 5.3). These results have revealed the existence of plant part by insect species interaction effects on the adult mortality of the four test insects. The overall ranking of test insects, in order of increasing susceptibility to *O. americanum* essential oils was as follows; *T. castaneum*, *R. dominica*, *S. oryzae* and *C. chinensis*. Similarly, the intra-species toxicities were ranked, in decreasing order, as fruit > leaf > stem essential oils.

Table 5.1: Mean percent mortality of adult *S. oryzae*, *R. dominica*, *C. chinensis* and *T. castaneum* in negative and positive control-treated grains

Control treatment (μ l/g grain)	N	PERCENT ADULT MORTALITY (MEAN $\pm$ SE; n=4)				
		DURATION OF CONTACT (DAYS)				
		2	4	6	8	10
<u><i>S. oryzae</i></u>						
Acetone 0.05	20	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
Insects only (starved)	20	31.5 $\pm$ 6.3	70.0 $\pm$ 3.4	98.4 $\pm$ 1.2	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
Actellic 5EC 0.10	20	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
4) Soya oil 10.00	20	91.7 $\pm$ 3.3	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
LSD _{0.05}		13.7	7.4	2.7	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
<u><i>R. dominica</i></u>						
Acetone 0.05	20	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
Insects only (starved)	20	16.7 $\pm$ 3.8	36.7 $\pm$ 3.3	83.4 $\pm$ 3.9	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
Actellic 5EC 0.10	20	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
Soya oil 10.00	20	96.7 $\pm$ 3.3	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
LSD _{0.05}		8.2	7.2	8.5	0.0	0.0
<u><i>T. castaneum</i></u>						
Acetone 0.05	20	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
Insects only (starved)	20	0.0 $\pm$ 0.0	3.3 $\pm$ 1.3	10.0 $\pm$ 2.9	26.7 $\pm$ 4.8	33.3 $\pm$ 5.3
Actellic 5EC 0.10	20	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
Soya oil 10.00	20	94.2 $\pm$ 4.3	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
LSD _{0.05}		10.3	5.0	6.3	10.5	11.6
<u><i>C. chinensis</i></u>						
Acetone 0.05	20	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	-	-	-
Insects only (starved)	20	16.7 $\pm$ 3.3	23.3 $\pm$ 3.3	-	-	-
Actellic 5EC 0.10	20	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	-	-	-
Soya oil 10.00	20	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	-	-	-
LSD _{0.05}		7.2	7.2	-	-	-

For each insect, any two means in a column with overlapping SE values are not different at $\alpha = 0.05$ when separated by LSD and Tukey's HSD test.

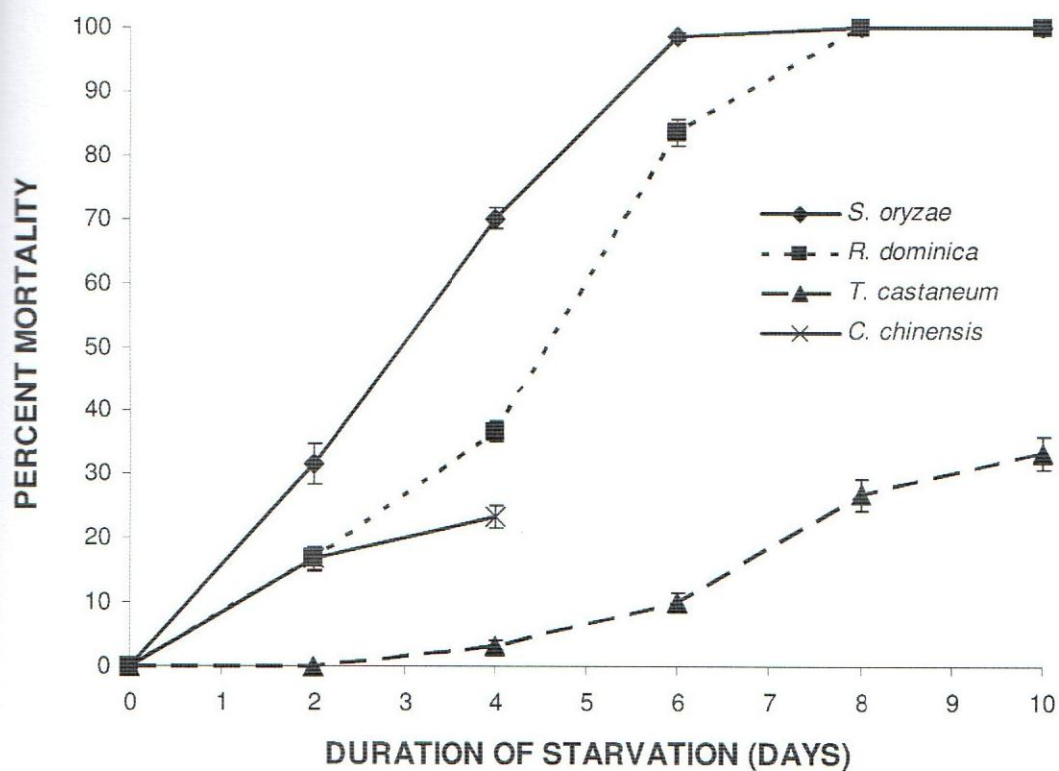


Fig. 5.1: Mean percent mortality ($\pm$ SE; $n=4$) of four coleopteran storage pests subjected to starvation during the contact toxicity studies

Table 5.2: End-point LC₅₀ values of *O. americanum*, *L. camara* and *T. vogelii* essential oils against four storage insects in contact toxicity studies¹.

Source of essential oil/ Test insect	Intra-species and inter-plant variability in LC ₅₀ values (% v/w) ^a against various test insects							
	N	LEAF		STEM		FRUIT		
		LC ₅₀	95% FDL ^a	LC ₅₀	95% FDL	LC ₅₀	95% FDL	
<u><i>Ocimum americanum</i>:</u>								
<i>S. oryzae</i>	20	0.22	(0.15, 0.76)	1.68	(0.51, 25.8)	0.01	(0.0, 0.008)	
<i>R. dominica</i>	20	-	-	0.25	(0.20, 0.37)	0.78	(0.38, 12.4)	
<i>T. castaneum</i>	20	-	-	0.90	(0.43, 23.6)	0.87	(0.41, 14.6)	
<i>C. chinensis</i>	20	0.03	(0.01, 0.06)	-	-	-	-	
<u><i>Lantana camara</i>:</u>								
<i>S. oryzae</i>	20	1.02	(0.45, 12.2)	22.0	(11.6, 121)	-	-	
<i>R. dominica</i>	20	1.28	(0.49, 5.84)	0.02	(0.0, 0.06)	-	-	
<i>T. castaneum</i>	20	4.50	(0.73, 10.0)	0.52	(0.31, 35.9)	-	-	
<i>C. chinensis</i>	20	0.16	(0.12, 0.31)	0.12	(0.10, 0.17)	-	-	
<u><i>Tephrosia vogelii</i>:</u>								
<i>S. oryzae</i>	20	0.83	(0.41, 10.9)	2.15	(0.59, 44.9)	0.23	(0.16, 0.73)	
<i>R. dominica</i>	20	1.33	(0.47, 53.9)	0.36	(0.25, 0.88)	1.64	(0.48, 57.1)	
<i>T. castaneum</i>	20	22.7	(10.9, 95.8)	20.7	(10.4, 12.1)	212.7	(99.5, 375.2)	
<i>C. chinensis</i>	20	0.03	(0.01, 0.05)	0.06	(0.03, 0.10)	-	-	

¹Twenty unsexed adult test insects, in four replicates, were in contact with treated grains for 4 days (*C. chinensis*) and 10 days (*S. oryzae*, *R. dominica* and *T. castaneum*).

^aLC₅₀ values were considered significantly different when 95% fiducial limits did not overlap.

No data on *O. americanum* leaf (*R. dominica*, *T. castaneum*) and *L. camara* (all test insects) essential oils due to lack of test insects and essential oils, respectively.

Table 5.3: Mean cumulative percent mortality of adult stages of four stored-product insects in grains treated with *Ocimum americanum* essential oils

Test insect/ Conc. (% v/w)	N	Cumulative percent adult mortality (mean±SE; n=4) ^a		
		PLANT PART ASSAYED		
		LEAF	STEM	FRUIT
<i>S. oryzae</i>:				
0.00	20	0.0±0.0	0.0±0.0	0.0±0.0
0.05	20	28.1±3.4	9.1±2.6	85.9±2.4
0.10	20	36.9±6.8	11.7±3.0	87.3±2.5
0.15	20	43.4±2.3	15.2±3.5	92.4±4.4
0.20	20	49.2±4.6	22.1±1.1	96.2±2.4
LSD _{0.05}		13.3	7.6	9.6
LC ₅₀ value ^b		0.22	1.68	0.01
95%FL		(0.15, 0.76)	(0.51, 5.77)	(0.00, 0.01)
<i>R. dominica</i>:				
0.00	20	-	0.0±0.0	0.0±0.0
0.05	20	-	4.6±1.5	8.6±3.5
0.10	20	-	23.9±1.3	12.4±3.1
0.15	20	-	35.3±3.1	18.6±4.2
0.20	20	-	37.2±1.8	25.7±3.3
LSD _{0.05}			6.7	9.2
LC ₅₀ value ^b			0.25	0.78
95%FL			(0.20, 0.37)	(0.38, 12.4)
<i>T. castaneum</i>:				
0.00	20	-	0.0±0.0	0.0±0.0
0.05	20	-	1.3±0.7	4.5±2.2
0.10	20	-	5.0±0.9	10.0±4.9
0.15	20	-	5.0±0.9	13.3±2.5
0.20	20	-	13.3±1.7	20.8±3.7
LSD _{0.05}			3.7	9.6
LC ₅₀ value ^b			0.90	0.87
95%FL			(0.43, 23.6)	(0.41, 14.6)
<i>C. chinensis</i>:				
0.00	20	0.0±0.0	-	-
0.05	20	59.1±9.0	-	-
0.10	20	59.1±4.5	-	-
0.15	20	68.2±5.2	-	-
0.20	20	74.2±1.5	-	-
LSD _{0.05}		17.6	-	-
LC ₅₀ value ^b		0.03	-	-
95%FL		(0.01, 0.06)	-	-

^a Cumulative mortality data recorded after 4 days for *C. chinensis* and 10 days for *S. oryzae*, *R. dominica* and *T. castaneum*. ^b LC₅₀ values were considered significantly different when 95% fiducial limits (FL) did not overlap.

No mortality data for leaf (*R. dominica*, *T. castaneum*) and stem and fruit (*C. chinensis*) essentials owing to lack of test insects

For each insect, any two means in a column with overlapping SE values are not different at $\alpha = 0.05$ when separated by LSD and Tukey's HSD test.

Results showed that cumulative counts and corresponding percent reductions in adult F_1 progeny were significantly ($P<0.0001$) affected by the intra-species and inter-plant variations, contact time, concentration, insect species and corresponding factor interactions except plant species by concentration (*S. oryzae*), plant part by concentration (*R. dominica*), plant species by contact time (*T. castaneum*) interaction effects and concentration (*T. castaneum*). The two positive controls, Actellic 5EC (0.1% v/w) and crude soya oil (1.0% v/w), caused 100% reductions whereas the untreated control produced the highest number of adult F_1 progeny (Tables 5.4-5.6).

Results showed existence of clearly marked dose-dependent intra-specific variations in the reproduction inhibitory effects of essential oils obtained from the aerial parts of *O. americanum* on adult F_1 progeny counts of *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis* insects. The actual adult F_1 progeny counts were both insect species- and dose-dependent. However, the magnitude of reproduction inhibition, measured in terms of percentage reduction in adult F_1 progeny, was clearly dependent upon the plant part from which the essential oil was extracted. The leaf essential oil (0.05-0.20% v/w) caused 33- 48 and 11- 41% reductions in the adult F_1 progeny counts of *S. oryzae* and *C. chinensis*, respectively (Table 5.4). Within the same dose range, essential oil extracted from succulent stems caused 20-58 and 19- 61% reductions against *S. oryzae* and *R. dominica*, respectively, whereas the fruit essential oil achieved 34- 43 and 9- 28% reductions of *R. dominica* and *C. chinensis*, respectively. Although certain aspects of contact toxicity studies were constrained by either lack of test insects or essential oils, the overall efficacy ranking, in order of decreasing reproduction inhibitory effects, against individual test insects was stem, leaf and fruit essential oils, respectively. These differential inhibitory effects could be explained by the observed differential contact toxicities of the essential oils against test insects due to existence of intra-species variations and to a lesser extent, starvation-induced.

Concentration- and contact duration-dependent intra-species variations in contact efficacy of essential oils obtained from the aerial parts of *L. camara* against adult *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis* insects were observed. The LC_{50} values decreased with contact duration a manifestation of increased adult insect mortality (see Table 5.2 above; Table 5.5).

Table 5.4: Mean cumulative counts and percent reduction in F_1 adults of *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis* in grains treated with graded levels of essential oils obtained from aerial parts of *O. americanum* L.^a

Test insect/ Conc. (% v/w)	N	Mean Cumulative counts and percent reduction in F_1 adult insect counts (Mean $\pm$ SE; n=4)					
		PLANT PART ASSAYED					
		LEAF		STEM		FRUIT	
		F_1 count ^b	%Reduction	F_1 count	%Reduction	F_1 count	%Reduction
<i>S. oryzae</i>:							
0.00	20	40.0 $\pm$ 7.0	0.0 $\pm$ 0.0	60.0 $\pm$ 8.1	0.0 $\pm$ 0.0	-	-
0.05	20	26.0 $\pm$ 7.1	32.6 $\pm$ 7.9	48.8 $\pm$ 8.8	20.4 $\pm$ 4.4	-	-
0.10	20	24.7 $\pm$ 3.2	34.7 $\pm$ 7.7	38.3 $\pm$ 6.6	36.4 $\pm$ 4.9	-	-
0.15	20	20.3 $\pm$ 1.5	46.0 $\pm$ 5.0	31.3 $\pm$ 8.0	50.1 $\pm$ 8.2	-	-
0.20	20	19.5 $\pm$ 2.7	47.6 $\pm$ 4.6	26.4 $\pm$ 6.8	58.1 $\pm$ 7.9	-	-
LSD _{0.05}		14.0	15.6	17.4	16.2	-	-
<i>R. dominica</i>:							
0.00	20	-	-	33.0 $\pm$ 4.2	0.0 $\pm$ 0.0	17.2 $\pm$ 4.7	0.0 $\pm$ 0.0
0.05	20	-	-	26.8 $\pm$ 3.7	19.4 $\pm$ 2.0	11.3 $\pm$ 3.1	34.3 $\pm$ 6.5
0.10	20	-	-	19.3 $\pm$ 2.9	42.2 $\pm$ 2.9	11.0 $\pm$ 3.0	36.0 $\pm$ 6.6
0.15	20	-	-	16.3 $\pm$ 2.1	50.8 $\pm$ 2.2	10.5 $\pm$ 2.0	39.0 $\pm$ 5.0
0.20	20	-	-	12.8 $\pm$ 1.3	60.8 $\pm$ 1.7	9.8 $\pm$ 2.2	43.0 $\pm$ 6.4
LSD _{0.05}				8.5	9.8	9.3	13.0
<i>C. chinensis</i>:							
0.00	20	118.8 $\pm$ 8.2	0.0 $\pm$ 0.0	-	-	119.0 $\pm$ 6.0	0.0 $\pm$ 0.0
0.05	20	104.8 $\pm$ 4.4	11.1 $\pm$ 3.8	-	-	108.0 $\pm$ 6.1	9.3 $\pm$ 1.3
0.10	20	92.5 $\pm$ 5.9	21.8 $\pm$ 2.9	-	-	99.8 $\pm$ 4.6	16.1 $\pm$ 3.8
0.15	20	85.8 $\pm$ 3.5	27.3 $\pm$ 2.9	-	-	90.8 $\pm$ 3.3	23.6 $\pm$ 2.3
0.20	20	71.0 $\pm$ 7.2	40.5 $\pm$ 3.6	-	-	85.9 $\pm$ 3.2	27.8 $\pm$ 2.1
LSD _{0.05}		16.2	7.7	-	-	12.0	7.5

^a Final F_1 adult counts after 40 days for *C. chinensis* and 63 days for the other insects.

^b No F_1 adult insects emerged in Actellic 5EC (0.1% v/w), starved insects and soya oil (1.0% v/w) treated grains.

No data leaf (*R. dominica*), stem (*C. chinensis*) and fruit (*S. oryzae*) due to lack of test insects

For each insect, any two means in a column with overlapping SE values are not different at $\alpha = 0.05$ when separated by LSD and Tukey's HSD test.

Of the two plant parts assayed, the stem essential oil was the most efficacious against adult *R. dominica* and *C. chinensis* insects causing 8.37- 100% and 34.4- 63.7% mortalities, respectively (Table 5.5). The leaf oil, on the other hand, was more toxic against adult *S. oryzae* causing 6.3% kill than the stem oil (13.8% kill). Against *C. chinensis*, the leaf and stem oils were equally toxic causing 31.0- 55.0 and 34.0- 64.0% mortalities, respectively. *T. castaneum* was the most tolerant insect species with less than 10% mortality recorded for leaf and stem oils. Although the fruit essential oil was not assessed, significant ($P<0.0001$) intra-species variations in contact toxicity were observed against adults of all the four insect species tested. Significant ($P<0.0001$) concentration- and intra-species dependent reproduction inhibitory effects of essential oils extracted from leaves and succulent stems of *L. camara* were observed against all the test insects. The leaf oil (0.05-0.20% v/w) caused 16.0- 41.0, 18.0- 47.0, 32.0- 60.0 and 12.0- 45.0% reductions in adult F_1 progeny counts of *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis*, respectively (Table 5.6). Similarly, the stem essential oil achieved 20.0- 59.0, 24.0- 51.0, 14.0- 55.0 and 6.0- 35.0% reductions in adult F_1 progeny counts of the same four insects, respectively. These results exhibited marked intra-species variations in reproduction inhibitory effects of *L. camara* essential oils.

Significant ($P<0.0001$) concentration- and contact duration-dependent intra-species variations in the contact efficacy and reproduction inhibitory effects of *T. vogelii* essential oils against adult stages of the four test coleopteran pests of stored food grains were observed (Tables 5.7- 5.8). In all the contact toxicity bioassays, the LC_{50} values decreased with contact duration a manifestation of increased cumulative adult insect mortalities. Except for *C. chinensis* in which only leaf and stem oils were evaluated, the other test insects were exposed to grains treated with essential oils extracted from all the three aerial parts (leaf, stem and fruit) of *T. vogelii*. At the concentration range of 0.05-0.20% v/w and 10 days of contact, the leaf, stem and fruit essential oils achieved mortalities of 5.0- 15.0, 1.0- 8.0 and 27.0- 50.0% of adult *S. oryzae* compared to 8.0- 20.0, 8.0- 34.0 and 13.0- 27.0% kill of adult *R. dominica* insects by the same essential oils, respectively (Table 5.7). The corresponding LC_{50} values were 0.83, 2.15 and 0.23% v/w for leaf, stem and fruit oils, respectively, against adult *S. oryzae* compared to 1.33, 0.36 and 1.64% v/w for same oils against adult *R. dominica* insects (Table 5.7).

Table 5.5: Mean cumulative percent mortality of adult of four storage insects in grains treated with *Lantna camara* essential oils

Test insect/ Conc. (% v/w)	N	Cumulative percent adult mortality (mean±SE; n=4) ^a	
		PLANT PART ASSAYED	
		LEAF	STEM
<i>S. oryzae</i>:			
0.00	20	0.0±0.0	0.0±0.0
0.05	20	3.8±1.3	8.8±1.3
0.10	20	5.0±0.7	8.8±1.3
0.15	20	11.3±1.3	12.5±1.4
0.20	20	16.8±2.4	13.8±1.3
LSD _{0.05}		4.7	2.7
LC ₅₀ value ^b		1.02	22.0
95%FL		(0.45, 12.2)	(11.6, 121)
<i>R. dominica</i>:			
0.00	20	0.0±0.0	0.0±0.0
0.05	20	6.3±1.3	83.7±1.8
0.10	20	6.9±1.2	85.5±3.0
0.15	20	8.8±1.3	96.3±2.1
0.20	20	15.0±2.0	100.0±0.0
LSD _{0.05}		3.9	6.5
LC ₅₀ value ^b		1.28	0.02
95%FL		(0.49, 5.80)	(0.00, 0.06)
<i>T. castaneum</i>:			
0.00	20	0.0±0.0	0.0±0.0
0.05	20	1.3±0.6	0.0±0.0
0.10	20	3.8±1.3	0.0±0.0
0.15	20	5.0±0.9	3.4±1.1
0.20	20	8.8±2.4	5.0±1.7
LSD _{0.05}		4.7	3.3
LC ₅₀ value ^b		4.50	52.0
95%FL		(0.73, 10.0)	(30.43, 73.6)
<i>C. chinensis</i>:			
0.00	20	0.0±0.0	0.0±0.0
0.05	20	31.2±3.9	34.4±3.3
0.10	20	44.2±4.4	44.0±4.7
0.15	20	45.5±3.4	50.3±4.9
0.20	20	54.5±1.3	63.7±4.8
LSD _{0.05}		8.6	10.7
LC ₅₀ value ^c		0.16	0.12
95%FL		(0.12, 0.31)	(0.10, 0.17)

^a Cumulative mortality data recorded after 4 days for *C. chinensis* and 10 days for *S. oryzae*, *R. dominica* and *T. castaneum*.

^b LC₅₀ values were considered significantly different when 95% fiducial limits (FL) do not overlap. For each insect, any two means in a column with overlapping SE values are not different at $\alpha = 0.05$ when separated by LSD and Tukey's HSD test.

Table 5.6: Mean cumulative counts and percent reduction in F₁ adults of four storage insects in grains treated with graded levels of *L. camara* essential oils^a

Test insect/ Conc. (% v/w)	Cumulative counts and % reduction in F ₁ adult insect counts (mean±SE)				
	N	PLANT PART ASSAYED			
		LEAF		STEM	
		F ₁ count ^b	%Reduction	F ₁ count	Reduction
<u>S. oryzae:</u>					
0.00	20	67.8±6.0	0.0±0.0	55.8±6.5	0.0±0.0
0.05	20	56.5±3.8	16.0±3.6	44.8±6.3	20.0±4.4
0.10	20	53.5±4.5	20.8±3.0	35.3±5.3	36.0±7.8
0.15	20	45.8±3.0	31.8±3.9	29.3±4.2	47.3±5.1
0.20	20	39.8±2.4	40.8±2.6	22.0±1.6	59.0±5.6
LSD _{0.05}		11.8	7.7	12.9	15.4
<u>R. dominica:</u>					
0.00	20	21.5±2.2	0.0±0.0	18.5±4.3	0.0±0.0
0.05	20	17.8±2.6	18.4±3.7	13.5±2.4	24.2±4.4
0.10	20	14.8±1.7	31.5±2.9	11.8±2.1	33.8±5.2
0.15	20	12.3±1.4	43.2±2.4	10.3±1.6	40.8±7.0
0.20	20	11.5±1.3	46.6±2.4	8.5±1.2	50.8±5.3
LSD _{0.05}		5.1	7.5	9.5	13.8
<u>T. castaneum:</u>					
0.00	20	18.8±2.4	0.0±0.0	24.8±4.8	0.0±0.0
0.05	20	12.9±1.1	31.6±5.6	22.0±5.2	13.6±4.4
0.10	20	10.8±0.9	42.3±4.8	17.5±3.9	30.4±2.3
0.15	20	9.3±0.5	50.2±2.7	13.5±2.6	45.5±0.8
0.20	20	7.5±0.6	60.0±1.9	11.0±1.8	54.6±1.9
LSD _{0.05}		4.8	11.2	10.3	8.7
<u>C. chinensis:</u>					
0.00	20	129.8±3.7	0.0±0.0	198.3±12.4	0.0±0.0
0.05	20	114.5±4.0	11.7±2.8	186.0±9.6	5.8±2.9
0.10	20	95.8±5.0	26.1±3.7	169.5±9.8	14.3±2.5
0.15	20	79.3±6.4	38.5±6.2	153.5±14.8	22.8±4.1
0.20	20	70.5±5.9	44.9±9.6	129.3±13.6	35.1±3.8
LSD _{0.05}		12.6	18.9	29.2	8.2

^aFinal F₁ counts after 40 days for *C. chinensis* and 63 days for the other insects.

^bNo F₁ adults in Actellic 5EC (0.1% v/w), starved insects and soya oil (1.0% v/w) treated grains.

For each insect, any two means in a column with overlapping SE values are not different at $\alpha = 0.05$ when separated by LSD and Tukey's HSD test.

Adult *T. castaneum* and *C. chinensis* were the most tolerant and susceptible insect species, respectively. At the dose range of 0.05-0.20% v/w, the three essential oils (leaf, stem and fruit) were weakly toxic against adult *T. castaneum* insects with only 3.0- 10.0% mortality recorded. In the other bioassay with *C. chinensis*, the leaf and stem essential oils (0.05-0.20% v/w and 4 d) produced the best results with 63.0- 83.0 and 50.0- 64.0% mortality. On the basis of LC₅₀ and corresponding 95% FDL values, adult *C. chinensis* was the most susceptible insect species followed by *S. oryzae* and *R. dominica* which were equally and moderately tolerant and *T. castaneum* as the most tolerant insect species to the essential oil treatments.

A clear concentration-dependent ($P < 0.0001$) reproduction inhibitory effects of essential oils extracted from the leaves and fruits of *T. vogelii* were observed against adult F₁ progeny of *S. oryzae*, *R. dominica* and *T. castaneum* and similar response trends observed with leaf and stem oils against adult F₁ progeny of *C. chinensis* insects (Table 5.7). The leaf essential oil (0.05-0.20% v/w) caused 24.0- 67.0, 31.0- 58.0, 17.0- 42.0 and 36.0- 68.0% reductions in adult F₁ progeny counts of *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis*, respectively (Table 4.7). Similarly, the fruit essential oil achieved 20.0- 47.0, 19.0- 50.0 and 16.0- 47.0% reductions in adult F₁ progeny counts of *S. oryzae*, *R. dominica* and *T. castaneum*, respectively. The stem oils caused 18.0- 53.0% reduction in adult F₁ progeny counts of *C. chinensis* insects. The marked reductions in adult F₁ progeny counts of insects tested clearly manifested the existence of intra-species variations in bioactivity of *T. vogelii* oils.

The observed differential responses by the test insect species to *O. americanum*, *L. camara* and *T. vogelii* essential oils could be explained by individual and / or synergistic contact toxicity of their chemical constituents. Similarly, the toxicity differences of leaf, stem and fruit essential oils against different insect species were attributable to the existing intra-species variations in the quantitative chemical composition. The percent mortality observed could not be entirely accounted for by death due to starvation since the 100% mortality recorded after 8 d (*S. oryzae*, *R. dominica*) and 33.3% mortality of *T. castaneum* after 10 d were far beyond mortality levels observed in the present contact toxicity studies. In studies with essential oil constituents of *Ocimum* spp., Bekele and Hassanali (2001) reported significant synergistic toxic effects of 1, 8-cineole and *iso*-eugenol with 44 and 39% mortality of adult *S. zeamais* compared to 100% kill

the blend toxicity of the two compounds. In related contact toxicity studies using impregnated filter paper, eugenol and methyl eugenol showed strong contact toxic effects against female American cockroach, *Periplaneta americana* L. with LC_{50} values of 0.148 and 0.187 mg cm^{-2} , respectively (Ngoh *et al.*, 1998). In the same study, limonene, cineole and α -pinene showed weak contact toxicity and knockdown effects against the cockroach. In topical contact toxicity studies with essential oil constituents, Huang *et al.* (2002) reported that eugenol, isoeugenol and methyl eugenol had similar toxic effects against adult *S. zeamais* insects with LD_{50} value of approximately 30 $\mu g/mg$ insect but were differentially potent against *T. castaneum* with isoeugenol being most toxic followed by eugenol and methyl eugenol, respectively. Similarly, eugenol extracted from *O. suave* showed dose-dependent contact toxicity against four coleopteran pests with 100% kill at 1 ml/kg grain within 24 h (Obeng-Ofori and Reichmuth, 1997). Kéita *et al.* (2001), using aromatized kaolin powder against adult *Callosobruchus maculatus* insects, reported LC_{50} values of 65 and 116 $\mu l/g$ for *O. basilicum* and *O. gratissimum* essential oils, respectively, in a dose-dependent contact toxicity study. These aromatized were less toxic compared *O. americanum* leaf essential which had a LC_{50} value of 0.03% v/w ($\cong$ 0.3 $\mu l/g$) against adult *C. chinensis*.

In studies with two essential oil constituents, Prates *et al.* (1998) observed strong contact toxicity with LC_{50} values of 1.64 and 1.38 mg cm^{-2} for cineole against adult *R. dominica* and *T. castaneum* insects, respectively compared to 1.25 mg cm^{-2} for limonene against *T. castaneum*. However, *O. gratissimum* essential oil at a higher concentration of 1% v/w caused 98% kill of adult *S. zeamais* insects after 24 h (Kouninki *et al.*, 2005). Germacrene D, one of the major constituents of *O. americanum*, *L. camara* and *T. vogelii* essential oils, was found to be inactive against adult *T. castaneum* after 72 h in contact toxicity studies (Garcia *et al.*, 2005). In his recent review article, Isman (2006) observed that plant essential oils are produced commercially from several botanical sources many of which are members of the Lamiaceae family and that such oils are generally composed of complex mixtures of monoterpenes, biogenetically related phenols and sesquiterpenes. Hence, *Ocimum americanum*, a member of the Lamiaceae family, has potential industrial value for targetted exploitation as insect control agent.

Table 5.7: Mean cumulative percent mortality of adult *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis* in grains treated with *T. vogelii* essential oils^a

Insect species/ E. oil conc (% v/w)	N	Cumulative percent adult insect mortality (mean±SE)		
		PLANT PART ASSAYED		
		LEAF	STEM	FRUIT
<u>S. oryzae:</u>				
0.00	20	0.0±0.0	0.0±0.0	0.0±0.0
0.05	20	5.0±1.9	1.3±0.6	26.7±3.3
0.10	20	7.5±2.0	4.5±1.4	36.7±4.8
0.15	20	15.0±2.6	5.2±1.2	40.0±5.6
0.20	20	15.0±2.9	7.5±1.4	50.0±5.6
LSD _{0.05}		5.7	2.7	11.0
LC ₅₀ value		0.83	2.15	0.23
95%FL		(0.41, 10.9)	(0.59, 44.9)	(0.16, 0.73)
<u>R. dominica:</u>				
0.00	20	0.0±0.0	0.0±0.0	0.0±0.0
0.05	20	7.5±2.5	7.5±3.2	13.3±2.7
0.10	20	7.5±2.5	22.5±1.4	13.3±2.7
0.15	20	17.5±4.8	26.3±5.5	13.3±3.3
0.20	20	20.0±4.1	33.8±3.8	26.7±3.5
LSD _{0.05}		9.4	10.8	6.9
LC ₅₀ value		1.33	0.36	1.64
95%FL		(0.47, 53.9)	(0.25, 0.88)	(0.48, 57.1)
<u>T. castaneum:</u>				
0.00	20	0.0±0.0	0.0±0.0	0.0±0.0
0.05	20	5.0±1.9	0.0±0.0	3.3±1.3
0.10	20	5.0±1.9	1.3±0.6	3.3±1.3
0.15	20	7.5±2.0	5.0±0.0	3.3±1.3
0.20	20	10.0±3.1	6.3±1.3	3.3±1.3
LSD _{0.05}		6.1	2.5	2.5
LC ₅₀ value		22.7	30.7	212.7
95%FL		(10.9, 95.8)	(10.4, 121)	(99.5, 375)
<u>C. chinensis:</u>				
0.00	20	0.0±0.0	0.0±0.0	-
0.05	20	62.5±6.3	50.0±3.2	-
0.10	20	65.0±6.5	53.8±3.0	-
0.15	20	70.0±4.1	57.7±5.3	-
0.20	20	82.5±2.5	64.1±5.1	-
LSD _{0.05}		12.3	10.4	-
LC ₅₀ value		0.03	0.06	-
95%FL		(0.01, 0.05)	(0.03, 0.10)	-

^a Cumulative mortality data recorded after 4 days for *C. chinensis* and 10 days for *S. oryzae*, *R. dominica* and *T. castaneum*. ^b LC₅₀ values were considered significantly different when 95% fiducial limits (FL) do not overlap.

For each insect, any two means in a column with overlapping SE values are not different at $\alpha = 0.05$ when separated by LSD and Tukey's HSD test.

No mortality data for *T. vogelii* fruit essential oil (*C. chinensis*) due to lack of test insects

Table 5.8: Mean cumulative counts and percent reduction in F₁ adults of *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis* in grains treated with graded levels of essential oils obtained from aerial parts of *T. vogelii* Hook.^a

Insect species/ Ess oil conc (% v/w)	Cumulative counts and percent reduction in F1 adultsity (Mean±SE)						
	N	PLANT PART ASSAYED					
		LEAF		STEM		FRUIT	
		F ₁ count	%Red ^c	F ₁ count	%Red	F ₁ count	%Red
<i>S. oryzae</i>:							
0.00	20	21.5±1.3	0.0±0.0	-	-	33.3±3.8	0.0±0.0
0.05	20	16.3±1.7	24.2±3.1	-	-	26.7±2.8	19.8±2.5
0.10	20	12.0±1.4	44.2±3.8	-	-	22.5±2.3	32.4±3.7
0.15	20	9.5±0.6	55.8±6.4	-	-	19.6±1.7	41.1±3.9
0.20	20	7.0±2.4	67.4±3.7	-	-	17.9±1.5	46.2±4.3
LSD _{0.05}		4.7	12.6	-	-	7.5	8.5
<i>R. dominica</i>:							
0.00	20	17.8±2.1	0.0±0.0	-	-	24.7±3.1	0.0±0.0
0.05	20	12.3±0.6	30.9±3.3	-	-	20.0±2.6	19.0±1.8
0.10	20	9.8±1.0	44.9±4.1	-	-	16.3±1.7	34.0±2.6
0.15	20	7.8±1.3	56.2±4.7	-	-	14.5±1.8	41.2±3.5
0.20	20	7.5±0.3	57.9±4.5	-	-	12.3±1.5	50.2±4.6
LSD _{0.05}		4.1	9.3	-	-	6.7	9.2
<i>T. castaneum</i>:							
0.00	20	11.8±1.8	0.0±0.0	-	-	-	-
0.05	20	9.8±2.0	16.9±2.2	-	-	-	-
0.10	20	7.9±1.7	33.1±2.7	-	-	-	-
0.15	20	7.0±1.5	40.7±3.2	-	-	-	-
0.20	20	6.8±1.7	42.4±3.1	-	-	-	-
LSD _{0.05}		3.9	6.3	-	-	-	-
<i>C. chinensis</i>:							
0.00	20	104.5±9.3	0.0±0.0	160.5±7.7	0.0±0.0	-	-
0.05	20	67.3±8.7	35.6±3.6	132.0±10.9	17.8±2.9	-	-
0.10	20	59.8±3.4	42.8±3.9	122.5±9.4	23.7±3.3	-	-
0.15	20	36.3±3.8	65.2±5.7	94.0±8.9	41.4±3.6	-	-
0.20	20	33.5±3.4	67.9±5.2	75.3±6.6	53.1±4.7	-	-
LSD _{0.05}		18.3	11.2	21.5	9.5	-	-

^aFinal F₁ counts after 40 days for *C. chinensis* and 63 days for the other insects.

^bNo F₁ adults in Actellic 5EC (0.1% v/w), starved insects and soya oil (1.0% v/w) treated grains.

^c%Red = Percent inhibition of wheat seed germination

No data on counts and percent F₁ progeny reduction for *T. vogelii* stem (*S. oryzae*, *R. dominica*, *T. castaneum*) and fruit (*C. chinensis*) essential oils due to lack of test oil and insects, respectively

For each insect, any two means in a column with overlapping SE values are not different at $\alpha = 0.05$ when separated by LSD and Tukey's HSD test.

In the *L. camara* essential oil bioassays, except for *R. dominica* against which 100% mortality was observed for both starved insects and essential oil treatments, the percent mortality in *S. oryzae* and *T. castaneum* could not directly be accounted for by death due to starvation since the observed 100 and 33.3% mortalities recorded after 8 and 10 d, respectively, were far beyond mortality levels observed in the present contact toxicity studies. At the highest dosage (0.20% v/w) and 10 d of contact with treated grains, only 14.0- 17.0 and 5.0- 9.0% mortalities were recorded for *S. oryzae* and *T. castaneum*, respectively. It emerges from these results that essential oil treatments possibly had strong feeding deterrence against adult *R. dominica* but not against *S. oryzae* and *T. castaneum*. The nine major chemical constituents identified in the leaf and stem essential oils were α -pinene, sabinene, myrcene, 1,8-cineole, E-caryophyllene, α -humulene, bicyclogermacrene, germacrene D and (E)-nerolidol. The contact efficacy of 1, 8-cineole, α -pinene and germacrene D are as discussed above. Myrcene, one of the major constituents of *L. camara* essential oils, was found to be inactive against adult *T. castaneum* after 72 h in contact toxicity studies (Garcia *et al.*, 2005). However, the other main constituents of *L. camara* namely: sabinene, E-caryophyllene, α -humulene, bicyclogermacrene and (E)-nerolidol have not been evaluated for their possible contact efficacies according to current literature survey. Although existence of insecticidal effect has been demonstrated, results of current study were inferior to that of Bouda *et al.* (2001) who reported an LD₅₀ value of 0.16% v/w with essential oils obtained from leaves of *L. camara* in Cameroon against adult *S. zeamais*. According to literature survey, this differential bioactivity could be explained by existence of intra-plant variations in chemical composition of *L. camara* essential oils due to plant part assayed and geographical origin (Ngassoum *et al.*, 1999; Oyediji *et al.*, 2003; Randrianalijaona, 2006). However, scanty information is available on the potential of *L. camara* essential oils as contact insecticides against insect pests of stored agricultural products. Further in-depth studies are recommended to document the effect of spatial-temporal variations on the bioactivity of essential oils against insect pests and pathogenic organisms of stored food commodities. If bioassay guided research is logically followed and industrial value established, *L. camara* promises to be a cost-effective and an eco-friendly source of biopesticides for targetted exploitation in subsistence agriculture.

Against all the test insects, the percent mortalities caused by *T. vogelii* could not directly be accounted for by death due to starvation since the observed mortalities were far below mortality

levels in starvation studies (Fig. 5.1). At the highest dosage (0.20% v/w) and 10 d of contact with treated grains, only 8.0- 50.0, 20.0- 34.0 and 3.0- 10.0% mortalities of *S. oryzae*, *R. dominica* and *T. castaneum*, respectively, were recorded (Table 5.7). It emerges from these results that insects continued feeding even on essential oil treated grains. The eight major chemical constituents identified in the leaf, stem and fruit essential oils were α -pinene, limonene, E-caryophyllene, α -humulene, bicyclogermacrene, germacrene D, (E)-nerolidol and (E,Z)-Farnesol. The contact toxicity of α -pinene and germacrene D are as discussed under *O. americanum* above. In their contact and/or ingestion studies, Prates *et al.* (1998) reported that limonene had LC₅₀ values of 1.78 and 0.42 mg/g against adult *R. dominica* and *T. castaneum* insects, respectively, after 24 h. As observed for *L. camara*, the other constituents of *T. vogelii*: E-caryophyllene, α -humulene, bicyclogermacrene, (E)-nerolidol and (E,Z)-farnesol have not been evaluated for possible contact insecticidal and/or ingestion effects.

The differential bioactivity could be explained by existence of both intra-species and inter-plant variations in chemical composition of *T. vogelii* essential oils due to plant part assayed and geographical origin (Arriaga *et al.*, 2005). However, limited information is available on the potential of *T. vogelii* essential oils as contact insecticides against insect pests of stored agricultural products. Further in-depth studies are recommended to document the effect of spatial-temporal variations on the bioactivity of essential oils against insect pests and pathogenic organisms of stored food commodities. If bioassay guided research is logically followed and industrial value established, *T. vogelii* promises to be a cost-effective and an eco-friendly source of biopesticides for targetted exploitation in subsistence agriculture.

5.3.2 Residual contact toxicity on adult insects and F₁ progeny

The mean cumulative percent mortality of adult *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis* insects due to residual contact toxicity of selected *O. americanum* and *T. vogelii* essential oils are presented below (Tables 5.9- 5.11). Similarly, the percent reduction in adult F₁ progeny of *C. chinensis* in contact with grains treated with essential oils extracted from leaves of *O. americanum* and *T. vogelii* are presented in Figure 5.2. Residual contact toxicity and reproduction inhibition were significantly influenced ($P < 0.0001$) by storage duration, duration of contact with treated grains, essential oil concentration and corresponding factor interaction

effects except for *T. vogelii* leaf essential oil concentration effect on adult F₁ progeny of *C. chinensis*. The two positive controls, Actellic 5EC (0.1% v/w) and crude soya oil (1.0% v/w), had high residual contact toxicity with 100% kill of adult *S. oryzae*, *R. dominica* and *C. chinensis* insects and $\geq 95.0\%$ kill of adult *T. castaneum* insects within 10 days of contact with treated grains stored in sealed polythene bags over a 4-month period (Table 5.9). The positive control treatments caused 100% reduction in adult F₁ progeny of *C. chinensis*. Untreated and acetone-treated grains had no residual contact toxicity on all test insects and their F₁ progeny counts.

The results showed a significant ($P < 0.0001$) concentration-, insect species- and storage duration-dependent residual contact toxicity effects of *O. americanum* and *T. vogelii* fruit essential oils against adult *S. oryzae*, *R. dominica* and *T. castaneum* insects and the leaf essential oils of the two plants against adult *C. chinensis* insects (Tables 5.10- 5.11). At the highest concentration (0.20% v/w) and 90-120 days of grain storage, *O. americanum* fruit essential oil caused 18.0- 21.0, 15.0- 22.0 and 14.0- 18.0% mortality of adult *S. oryzae*, *R. dominica* and *T. castaneum*, respectively, with corresponding LC₅₀ values of 1.24- 1.78, 1.02- 4.16 and 1.01- 1.47% v/w (Table 5.10). At the same concentration and 120 days, *O. americanum* leaf essential oil caused 58.0- 75.0% kill of adult *C. chinensis* with an LC₅₀ value of 0.06-0.13% v/w (Table 5.10). Intra-species comparisons have shown that, when treated grains were stored in self-sealing polythene bags over a 4-month period (120 days), *O. americanum* fruit and leaf essential oils had strong residual contact toxicities against all the four insects tested. Temporal comparisons of data obtained from same essential oil treatments revealed 79.0- 81.0, 13.0- 40.0 and 14.0- 34.0 % reductions in contact efficacy of *O. americanum* fruit essential oil against adult *S. oryzae*, *R. dominica* and *T. castaneum* insects compared to a 0.0- 22.0% reduction in the efficacy of *O. americanum* leaf oil against adult *C. chinensis* after 90-120 days grain storage (Table 5.10). The magnitude of depressed contact toxicity was clearly manifested in increased LC₅₀ values with duration of grain storage. In the other residual contact toxicity bioassay, *T. vogelii* fruit essential oil (0.20% v/w) caused 31.0- 47.0 and 18.0- 23.0% mortality of adult *S. oryzae* and *R. dominica* after 120 days and 3.0- 5.0% kill of adult *T. castaneum* after 60 d grain storage. At the highest concentration (0.20% v/w) and 90 d grain storage, the *T. vogelii* leaf essential oil caused 37.0- 53.0% mortality of adult *C. chinensis* insects (Table 5.11). Temporal comparisons of instant and residual bioactivity data have produced 7.0- 38.0, 13.0- 34.0 and 0.0- 5.0 % reductions in contact

efficacy of *T. vogelii* fruit essential oil against adult *S. oryzae*, *R. dominica* and *T. castaneum* insects compared to a 35.8-55.6% reduction in the efficacy of *T. voeglii* leaf essential oil against adult *C. chinensis* insects after 60-120 d storage (see Table 5.7 above; Table 5.11).

Inter-plant comparisons have shown clear discernible variations in both instant and residual contact toxicities of *O. americanum* and *T. vogelii* essential oils. In the instant contact toxicity bioassay, the *O. americanum* fruit oil, at the highest concentration (0.20% v/w), achieved 21.0-96.0% mortality of adult *S. oryzae*, *R. dominica* and *T. castaneum* compared to 3.0- 50.0% mortality of the same insects in grains treated with *T. vogelii* fruit essential oil. However, at the same concentration, the *T. vogelii* leaf oil (83.0% mortality) was more toxic to adult *C. chinensis* than *O. americanum* leaf oil (74.0%). Although significant temporal reductions in efficacies were recorded, there were inter-insect species variations in residual susceptibility to *O. americanum* and *T. vogelii* essential oils. At the 0.05- 0.20% v/w dose range and a 4-month storage period, the *O. americanum* and *T. vogelii* fruit essential oils were equally efficacious against adult *R. dominica* and *T. castaneum*. However, the *T. vogelii* fruit essential oil had stronger residual contact toxicity (31.0- 47.0% kill) against adult *S. oryzae* than *O. americanum* fruit oil (18.0- 21.0% kill). The converse was true for *O. americanum* leaf essential oil that had a higher residual contact toxicity causing 58.0- 75.0% mortality of adult *C. chinensis* compared to 37.0- 53.0% mortality for *T. vogelii* leaf oil. These results concur with Demissie *et al.* (2008) who reported 33.0- 72.0% mortality of adult *S. zeamais*, after 3-month storage and 0.5% v/w, on grains treated with sunflower, corn and olive cooking oils.

Results of the cumulative counts and percent reduction in adult F₁ progeny of *C. chinensis* insects as a result of residual effects of *O. americanum* and *T. vogelii* leaf essential oils are presented in Fig. 5.2. Significant ($P<0.0001$) concentration- and storage duration-dependent residual inhibitory effects of *O. americanum* and *T. vogelii* leaf essential oils on the cumulative counts and percentage reduction in adult F₁ progeny of *C. chinensis* insects were observed. At the highest concentration (0.20% v/w) and 120 days of grain storage, *O. americanum* and *T. vogelii* leaf essential oils caused 28.0- 33.0 and 31.0- 40.0% reductions in adult F₁ progeny of *C. chinensis* insects, respectively (Fig. 5.2).

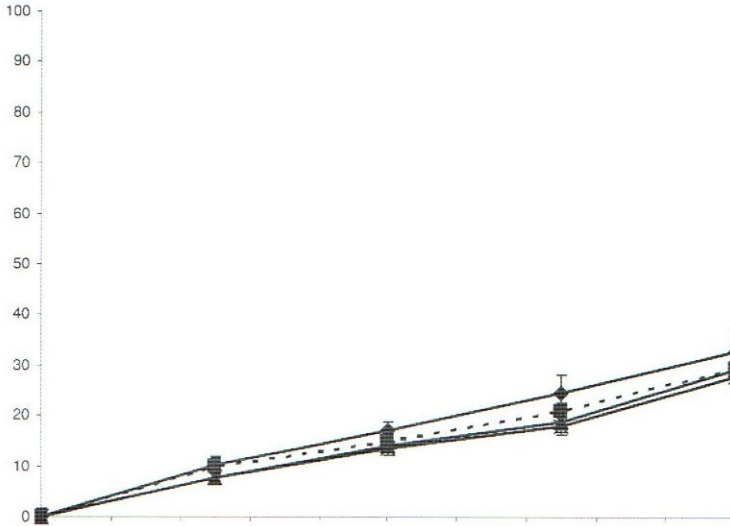
Table 59: End-point mean percent mortality of adult *S. oryzae*, *R. dominica*, *C. chinensis* and *T. castaneum* in control-treated grains in residual contact toxicity studies

Concentration (μ L/g grain)	N	PERCENT ADULT INSECT MORTALITY (MEAN $\pm$ SE) ^a			
		STORAGE DURATION (DAYS)			
		30	60	90	120
<i>S. oryzae</i>:					
1) Acetone 0.5	20	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
2) Actellic 1.0	20	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
3) Soya oil 10.0	20	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
<i>R. dominica</i>:					
1) Acetone 0.5	20	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
2) Actellic 1.0	20	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
3) Soya oil 10	20	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
<i>T. castaneum</i>:					
1) Acetone 0.5	20	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
2) Actellic 1.0	20	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
3) Soya oil 10	20	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
<i>C. chinensis</i>:					
1) Acetone 0.5	20	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
2) Actellic 1.0	20	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
3) Soya oil 10	20	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0

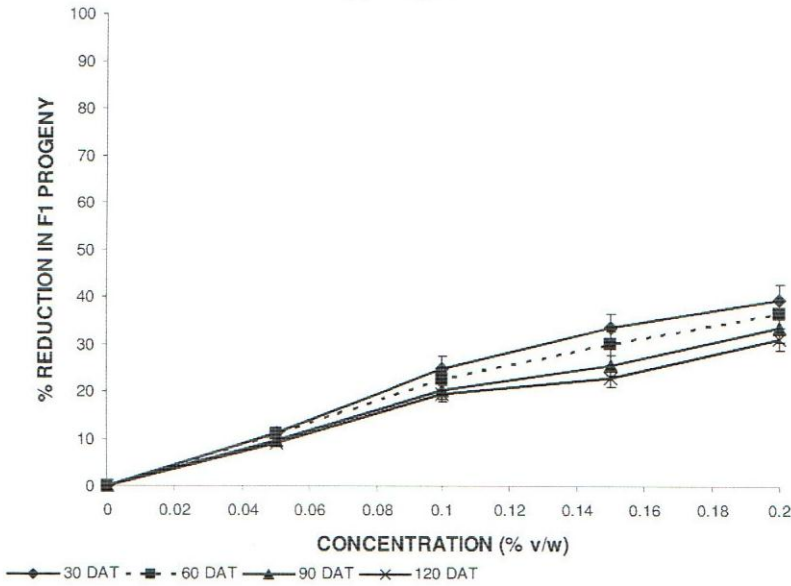
^aRecorded after 4 days for *C. chinensis* and 10 days for *S. oryzae*, *R. dominica* and *T. castaneum*.

Temporal comparisons of instant and residual bioactivity data showed 19.0- 32.0 and 42.0- 54.0% reductions in reproduction inhibition of *O. americanum* and *T. vogelii* leaf essential oils on the percent reduction in adult F₁ progeny of *C. chinensis* (Fig.5.2; Table 5.8). The observed differential residual contact toxic and reproduction inhibitory effects *O. americanum* and *T. vogelii* essential oils against four coleopteran pests of stored food grains could be explained by the individual and/or synergistic bioactivity of the major chemical constituents. Similarly, the intra-species and inter-plant variations in the qualitative and quantitative chemical compositions of essential oils could be attributed to the differential responses by the test insect species (Arriaga *et al.*, 2005). Although not directly investigated, the observed residual contact toxicity and reproduction inhibition could be extrapolated from the instant individual and/or synergistic bioactivity of 1,8-cineole, eugenol, methyl eugenol, limonene, α -pinene among other essential oil constituents (Obeng-Ofori and Reichmuth, 1997; Ngoh *et al.*, 1998; Prates *et al.*, 1998; Bekele and Hassanali, 2001; Huang *et al.*, 2002). It should be noted that no data on the residual bioactivity of essential oils and their constituents were available for comparison. However, in short-term residual bioactivity studies with crude powders and extracts, significant adult insect mortalities and reductions in oviposition and number of F₁ progeny of coleopteran pests of stored food commodities have been reported (Boeke *et al.*, 2003, 2004a; Koon, 2005; Koon *et al.*, 2007b). It emerges from these results that a full spectrum evaluation of intra-species and inter-plant bioactivity (instant and residual) of essential oils from aerial parts of *O. americanum* and *T. vogelii* against coleopteran pests of stored food grains be investigated. This will validate long-term essential oil use in grain protection and best plant-plant part(s) combination for optimal essential oil yield and bioactivity. The fact that *O. americanum* fruit essential oil achieved 96% kill of adult *S. oryzae* at 0.20% v/w within 10 days of contact with treated grains, is welcome scientific delight when compared to soya oil which caused 100% kill of the same insect within 2 days but at 5 times higher concentration (1.0% v/w). In addition to this positive development, with exception of rotenone with an oral rat LD₅₀ of 132 mg/kg and a LC₅₀ value of 458 $\mu\text{g l}^{-1}$ after 72 h against zebrafish embryos, the other essential oil constituents of *O. americanum*, *L. camara* and *T. vogelii* are fairly safe with LD₅₀ values of 500-4960 mg/kg (Golob *et al.*, 1999; Ekanem *et al.*, 2003). Hence, if appropriately harnessed and packaged, essential oils hold good promise for a cost-effective and eco-friendly control of stored-product insect pests.

(a) *O. americanum*



(b) *T. vogelii*



5.2: End-point mean percent reduction ($\pm$ SE, $n = 4$) in adult F_1 progeny of *C. chinensis* in contact with grains treated with (a) *O. americanum* and (b) *T. vogelii* leaf essential oils over a 4 month storage period

Table 5.10: Residual contact toxicity of *O. americanum* essential oils: cumulative percent mortality of adult *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis* in treated grains^a

Concentration (% v/w)/		PERCENT ADULT INSECT MORTALITY (Mean±SE; n=4) ^a			
Insect species	N	STORAGE DURATION (DAYS)			
		30	60	90	120
FRUIT OIL					
<i>S. oryzae</i>:					
0.00	20	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
0.05	20	7.7±2.2	6.4±1.3	6.1±1.4	5.7±2.2
0.10	20	12.8±2.1	10.1±1.2	11.0±1.3	10.4±2.4
0.15	20	17.9±2.3	12.5±2.1	12.2±1.5	11.7±1.3
0.20	20	20.5±3.5	19.2±3.2	18.9±2.7	18.2±1.3
LSD _{0.05}		6.9	6.4	5.5	4.9
LC ₅₀ value		1.24	1.39	1.56	1.78
95%FL		(0.46, 37.20)	(0.49, 48.4)	(0.52, 29.6)	(0.54, 11.6)
<i>R. dominica</i>:					
0.00	20	-	0.0±0.0	0.0±0.0	0.0±0.0
0.05	20	-	7.3±1.8	6.8±1.3	6.3±1.5
0.10	20	-	9.9±2.5	8.8±2.9	7.5±1.5
0.15	20	-	15.4±1.3	13.7±2.6	10.4±1.3
0.20	20	-	22.4±3.2	20.6±2.1	15.4±3.1
LSD _{0.05}		-	6.8	5.9	6.6
LC ₅₀ value		-	1.02	1.20	4.16
95%FL		-	(0.43, 44.3)	(0.46, 138.6)	(0.71, 10.0)
<i>T. castaneum</i>:					
0.00	20	0.0±0.0	0.0±0.0	0.0±0.0	-
0.05	20	3.9±1.2	2.8±1.8	2.7±1.2	-
0.10	20	7.8±2.6	7.5±2.1	7.1±1.7	-
0.15	20	11.5±2.9	9.7±2.2	8.8±2.3	-
0.20	20	17.8±3.5	15.0±2.8	13.8±2.7	-
LSD _{0.05}		7.2	5.8	5.6	-
LC ₅₀ value		1.01	1.24	1.47	-
95%FL		(0.44, 30.4)	(0.49, 12.5)	(0.52, 61.5)	-
LEAF OIL					
<i>C. chinensis</i>:					
0.00	20	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
0.05	20	48.6±5.9	47.4±6.2	43.6±4.7	40.3±4.2
0.10	20	56.7±2.3	51.1±2.3	49.5±2.2	44.2±3.5
0.15	20	65.0±4.6	57.3±4.7	54.3±5.2	50.3±3.5
0.20	20	74.7±2.6	69.2±3.5	64.7±3.4	58.2±2.3
LSD _{0.05}		11.7	13.4	10.6	8.5
LC ₅₀ value		0.06	0.07	0.09	0.13
95%FL		(0.03, 0.08)	(0.03, 0.10)	(0.04, 0.13)	(0.08, 0.33)

^a Grain sub-samples were obtained 30, 60, 90 and 120 days after treatment with essential oils and cumulative mortality data recorded after 4 days for *C. chinensis* and 10 days for *S. oryzae*, *R. dominica* and *T. castaneum*.

For each insect, any two means in a column with overlapping SE values are not different at $\alpha = 0.05$ when separated by LSD and Tukey's HSD test.

Table 5.11: Residual contact toxicity of *T. vogelii* essential oils: mean cumulative percent mortality of adult *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis* in treated grains^a

Concentration (% v/w)/		PERCENT ADULT INSECT MORTALITY (Mean±SE; n=4) ^a			
		STORAGE DURATION (DAYS)			
Insect species	N	30	60	90*	120*
FRUIT OIL					
<i>S. oryzae</i>:					
0.00	20	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
0.05	20	22.9±3.6	19.2±3.0	17.5±2.5	16.4±3.3
0.10	20	27.0±2.6	23.5±2.5	21.9±3.7	19.7±3.5
0.15	20	37.4±2.6	34.6±3.4	31.4±2.4	26.3±2.2
0.20	20	46.5±2.7	41.8±2.6	38.2±2.1	31.1±2.1
LSD _{0.05}		7.5	7.2	7.8	7.2
LC ₅₀ value		0.28	0.34	0.44	0.86
95%FL		(0.19, 0.83)	(0.22, 1.33)	(0.26, 3.27)	(0.35, 3.52)
<i>R. dominica</i>:					
0.00	20	-	0.0±0.0	0.0±0.0	0.0±0.0
0.05	20	-	10.5±1.2	10.2±1.1	10.2±1.2
0.10	20	-	11.2±1.5	10.8±1.4	10.5±1.3
0.15	20	-	12.7±2.0	12.1±1.9	10.9±1.7
0.20	20	-	23.3±2.1	21.0±2.1	17.7±1.9
LSD _{0.05}		-	4.7	4.6	4.1
LC ₅₀ value		-	3.08	4.11	18.3
95%FL		-	(0.61, 10.0)	(0.96, 11.4)	(11.75, 25.9)
<i>T. castaneum</i>:					
0.00	20	0.0±0.0	0.0±0.0	-	-
0.05	20	0.0±0.0	0.0±0.0	-	-
0.10	20	2.5±1.2	1.3±0.3	-	-
0.15	20	4.1±1.2	2.5±1.2	-	-
0.20	20	5.0±3.5	3.3±1.1	-	-
LSD _{0.05}		3.2	2.4	-	-
LC ₅₀ value		1.68	1.74	-	-
95%FL		(0.49, 19.5)	(0.52, 21.5)	-	-
LEAF OIL					
<i>C. chinensis</i>:					
0.00	20	0.0±0.0	0.0±0.0	0.0±0.0	-
0.05	20	31.8±2.9	26.8±2.7	21.3±2.6	-
0.10	20	39.4±3.5	28.1±3.5	24.0±1.3	-
0.15	20	43.9±2.9	32.4±2.3	29.3±2.9	-
0.20	20	53.0±2.9	42.7±2.6	36.6±2.7	-
LSD _{0.05}		7.2	7.2	6.0	-
LC ₅₀ value		0.19	0.56	0.69	-
95%FL		(0.14, 0.62)	(0.25, 9.91)	(0.30, 61.9)	-

^a Grain sub-samples were obtained 30, 60, 90 and 120 days after treatment with essential oils and cumulative mortality data recorded after 4 days for *C. chinensis* and 10 days for *S. oryzae*, *R. dominica* and *T. castaneum*. *Missing data due to lack of test insects

CHAPTER SIX

FUMIGANT TOXICITY OF PLANT ESSENTIAL OILS AND SELECTED VOLATILE CONSTITUENTS AGAINST FOUR INSECT PESTS OF STORED FOOD GRAINS

Abstract

The fumigant toxicity of essential oils extracted from aerial parts of three tropical plants, *O. americanum*, *L. camara* and *T. vogelii* and seven constituents were evaluated in space and grain fumigations against adult *S. oryzae* L., *R. dominica* F., *T. castaneum* (Herbst) and *C. chinensis* F. In the space fumigation tests, each test oil or constituent was evaluated at four rates (0, 1, 5 and 10 $\mu\text{L/L}$ air) with four replicates per concentration against all the four test insects. In the grain fumigation, the most active, *O. americanum* leaf essential oil, was assessed at five rates (0, 30, 50, 70 and 100 $\mu\text{L/L}$ air) with 4-6 replicates per concentration, against adult *S. oryzae*., *R. dominica* and *T. castaneum*. In space fumigation, fumigant efficacy of essential oil or constituent was dependent upon intra-species and inter-plant variations in chemical composition, concentration, time after application, insect species and corresponding factor interactions. *T. castaneum* and *C. chinensis* were the most tolerant and susceptible insect species, respectively, against all the essential oils and their terpenoid constituents. At 10 $\mu\text{L/L}$ air and 24 h after treatment, *O. americanum* essential oils achieved 22- 89, 38- 88, 81- 100 and 0- 11% kill against adult *S. oryzae*., *R. dominica*, *C. chinensis* and *T. castaneum*, respectively, compared to 10- 51, 12- 38, 96- 100 and 3- 8% mortality for *L. camara* essential oils against same insects. *T. vogelii* oils, on the other hand, caused 4- 30, 15- 29, 19- 96 and 1- 3% mortality against adult *S. oryzae*., *R. dominica*, *C. chinensis* and *T. castaneum*, respectively. Irrespective of plant part assayed, *O. americanum*, *L. camara* and *T. voeglii* essential oils achieved a 50% kill of test insects, except *T. castaneum*, at fairly low concentrations of 0.38- 34.68, 0.77- 64.10 and 0.44- 43.50 $\mu\text{L/L}$ air, respectively. These results are comparable to phosphine's recommended rate of 8- 13 $\mu\text{g/L}$. The six most active essential oil constituents, eugenol, (Z)- β -ocimene, caryophyllene oxide, 1,8-cineole, R-(+)- limonene and α -pinene had LC_{50} values of 0.03- 8.21, 0.28- 11.2, 29.6- 65.8, 7.8- 21.2, 18.2- 29.3 and 5.11- 36.8 $\mu\text{L/L}$ air, respectively against the four test insects. Except for *T. castaneum*, the end-point mortalities recorded 168 h after treatment were 80- 100, 70- 100 and 46- 90% for *O. americanum*, *L. camara* and *T. voeglii* oils, respectively against adult *S. oryzae* and *R. dominica* insects. In the grain fumigation studies with *O. americanum* leaf essential oil,

the percent adult insect mortality was significantly influenced ($P<0.001$) by the concentration, insect species, duration of exposure, post-fumigation time and corresponding factor interactions. Results showed that a concentration of 50 $\mu\text{L/L}$ air, 7 d exposure and 120 h post-fumigation time were enough to obtain 95.4-100% kill of *S. oryzae* and *R. dominica* and 65.5% mortality of *T. castaneum*. The fumigant potency is comparable to methyl bromide's recommended rate of 30-50 g/M^3 grain and that of the highly active Labiatae species oil, ZP51 (50 $\mu\text{L/L}$). Essential oils and their constituents hold good promise as effective alternatives to synthetic fumigants in the control of insect pests of stored grains. Further studies are needed to validate and upscale these results and ensure their biosafety and compatibility in the context of subsistence agriculture.

6.1 Introduction

Insect damage in stored food grains pose the greatest challenge to the provision of quality food with 10-60% losses in countries where modern storage technologies are yet to be fully adopted (Shaaya *et al.*, 1997; Ogendo *et al.*, 2003a). The magnitude of loss is dependent upon the insect species involved, storage duration and pest control methods among other factors. In the tropics, the gelechiid moths and coleopteran beetles are the major insect pests of stored cereal and legume grains with *Sitotroga cerealella* Olivier, *Sitophilus* spp., *Prostephanus truncatus* Horn, *Rhyzopertha dominica* F., *Tribolium castaneum* Herbst, *Acanthoscelides obtectus* Say and *Callosobruchus* spp. being the most destructive species (Ogendo *et al.*, 2003a; Gressel *et al.*, 2004). Currently, recommended pest control measures in durable stored food products rely heavily on use of synthetic insecticides which pose possible health hazards to warm-blooded animals, risk of environmental pollution, development of resistance by insects and pest resurgence are largely incompatible with subsistence agriculture (Shaaya *et al.*, 1997; Prates *et al.*, 1998; Ogendo *et al.*, 2003a; Shaaya and Kostyukovsky, 2006).

Fumigation is one of the most successful and cost-effective methods used in the protection of durable stored bulk grains with negligible residues left on grains (Faruki *et al.*, 2005). Currently, phosphine and methyl bromide are the two principal fumigants used worldwide for the disinfestations of food grains (Faruki *et al.*, 2005; Rajendran and Muralidharan, 2005). However, owing to the ozone-depleting properties of methyl bromide and reported development of resistance in many storage insect pests to phosphine (Zettler *et al.*, 1989; Zettler and Keever,

1994; Shaaya *et al.*, 1997; Faruki *et al.*, 2005), there is an urgent need to develop simple, convenient and safer alternatives with potential to replace these toxic synthetic fumigants. It is desirable that alternative fumigants identified are cheap, easy to apply, residue-free, non-carcinogenic, non-teratogenic and without adverse effects on quality of treated commodity and the environment (Rajendran and Muralidharan, 2005). In recent years, global research has focused on the possible use of plant secondary metabolites, especially essential oils, in protection of stored agricultural products. Essential oils and their major constituents, often monoterpenoids, are among the best known substances to have attracted research attention in recent years as potential alternatives to classical fumigants (Mondal and Khalequzzaman, 2006; Rozman *et al.*, 2007; Stamopoulos *et al.*, 2007). Their fumigant potencies have been tested against 20 coleopteran species, four lepidopterans and a liposcelid with special focus on adults of *T. castaneum*, *S. oryzae*, *S. zeamais*, *R. dominica* and bruchids (*Acanthoscelides obtectus*, *Callosbruchus* spp.) as the target insects (Rajendran and Sriranjini, 2008).

Past studies have demonstrated clear intra-species and inter-plant variations in the fumigant toxicity of essential oils based on chemical composition, pest susceptibility, degree of absorption of oil in the treated commodity and the route of entry of oil into target insects (Shaaya and Kostyukovsky, 2006; Talukder, 2006; Rajendran and Sriranjini, 2008). In various space fumigation studies, essential oils and their constituents extracted from plants in the Lamiaceae family were effective against several insect pests of stored durable agricultural products (Regnault-Roger, 1997; Shaaya *et al.*, 1997; Kostyukovsky *et al.*, 2002a; Shaaya and Kostyukovsky, 2006; Rajendran and Sriranjini, 2008). In studies with pure essential oils from *Ocimum basilicum* and *O. gratissimum* at a dose of 25 µl/vial, Kéita *et al.* (2001) observed 80 and 70% mortality of adult *C. maculatus* 12 h post-fumigation. In related studies four essential oil constituents (at 0.1 µl/720 ml volume), eugenol, 1,8-cineole, camphor and linalool caused 85-100, 80-100 and 0-13% mortality of adult *S. oryzae*, *R. dominica* and *T. castaneum* insects, respectively, 24 h after treatment (Rozman *et al.*, 2007). Similarly, Bekele and Hassanali (2001) reported that the toxic effects of essential oils were due to the combined effects of different components, either with or without significant individual toxic action of their own against stored-product insects. Although powder and liquid extracts have shown strong insecticidal potency against pre- and post-harvest insect pests (Baudoin and Smith, 2000; Belmain *et al.*, 2001;

Osorio, 2001; Karuru *et al.*, 2002; Ogendo *et al.*, 2003b; Kawuki *et al.*, 2005), there is little documented information on the fumigant toxicity of essential oils extracted from the aerial parts of *Tephrosia vogelii* and *Lantana camara*.

Compared with the recommended concentration of methyl bromide of 30-50 g/m³ grain, available data on past pilot studies have revealed that low concentrations of 50-100 g oil/M³ grain were effective in controlling gelechiid moths and coleopteran beetles (Kostyukovsky *et al.*, 2002a; Lee *et al.*, 2004). Other investigations aimed at seeking alternatives to methyl bromide showed that methyl iodide achieved 100% kill of *S. zeamais* at a dose of 3.02 mg/L which was comparable to a dose range of 50-150 mg/L with 24-120 h exposures for allyl acetate (Faruki *et al.*, 2005; Rajendran and Muralidharan, 2005). In their recent grain fumigation studies at 100 µl/L air, Lee *et al.* (2004) reported that LT₅₀ values of 16.2, 17.4 and 9.1 h were recorded for *Eucalyptus blakelyi*, *Melaleuca fulgens* and 1,8-cineole, respectively, against adult *S. oryzae* insects. It emerges from the above documented information that essential oils of botanical origin are promising alternatives to synthetic fumigants for managing insect pests of stored food commodities. Little or no local research efforts have been directed towards rationalized exploitation of essential oils of indigenous botanical plants that have served mankind for generations. The current study was therefore aimed at evaluating the fumigant efficacy of essential oils and selected constituents obtained from aerial parts of *O. americanum* L., *T. vogelii* Hook and *L. camara* L. against adult *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis*.

6.2 Materials and Methods

6.2.1 Mass rearing of test insects

The adult stages of *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis* used for contact toxicity and reproduction inhibition (F₁ progeny) studies were obtained from the insect cultures reared as described in Chapter 3 above.

6.2.2 Fumigant toxicity studies (Space fumigation)

Essential oils extracted from aerial parts of *O. americanum* L., *T. vogelii* Hook and *L. camara* L. (Section 4.3.2) and their selected major chemical constituents were evaluated for fumigant toxicity against adult stages of the four test insects (Section 4.2.1 above) in a space fumigation chamber according to Shaaya *et al.* (1991). A chamber comprising 3.4 litre flat-bottom glass

flask closed with a glass stopper and fitted with a hook was used (Fig. 6.1). Twenty (N_T) unsexed adult insects (5-10 d for *S. oryzae* and *R. dominica*; 0-3 d for *C. chinensis* and 30-60 d for *T. castaneum*) were placed in specially designed perforated cylindrical metallic cages (Length: 4 cm; diameter: 1.5 cm) with approximately 0.1 g of food grain and closed with a rubber stopper, lined with aluminium foil, fitted with hook wire. Each test essential oil or constituent was applied, at four rates (0, 1, 5 and 10 $\mu\text{l/L}$ air) with four replicates, to a piece of filter paper (Whatman No.1) and suspended together with test insects by hook in the glass stopper (Fig.6.1). A magnetic stirrer was used to ensure even distribution of fumigant in the chamber over a 24 h exposure period in a room maintained at $30\pm2^\circ\text{C}$, $68\pm2\%$ RH and 12D: 12D (total darkness). The numbers of dead (N_D) insects were recorded 24, 72, 120 and 168 h from onset of space fumigation. Actual and corrected percent adult insect mortalities were computed as described in Section 4.2.2 above.

6.2.3 Fumigant toxicity studies (Grain fumigation)

The *O. americanum* leaf essential oil, the most toxic, was further assayed in a 600-ml glass chambers filled to 70%, by volume, with whole wheat grains at moisture content of $\geq 12\%$ (Shaaya *et al.*, 1991). Inter-granular airspace was assumed to be 40% of the volume of wheat grains. Twenty (N_T) unsexed adult stages of each test insect, in a metallic cage sealed at one end by a metallic mesh and an aluminium lined rubber stopper at the other end, were introduced into the grain fumigation chamber (Fig.6.2) and exposed for 3, 5, 7 and 10 days. The test oil was assayed at five rates (0, 30, 50, 70 and 100 $\mu\text{l/L}$ air) with four replicates per concentration per exposure duration. At end of each exposure period, the glass stopper of each fumigation chamber was kept open for an hour for proper evacuation of fumigant (Faruki *et al.*, 2005). The numbers of dead (N_D) adult insects were recorded 24, 72, and 120 h post-grain fumigation. The actual and corrected percent adult mortalities were computed as described in Section 5.2.2 above.

6.3 Results and Discussion

6.3.1 Fumigant toxicity studies (Space fumigation)

Results showed that the fumigant efficacy of essential oils against adult insects were significantly ($P<0.001$) influenced by intra-species and inter-plant variations in chemical

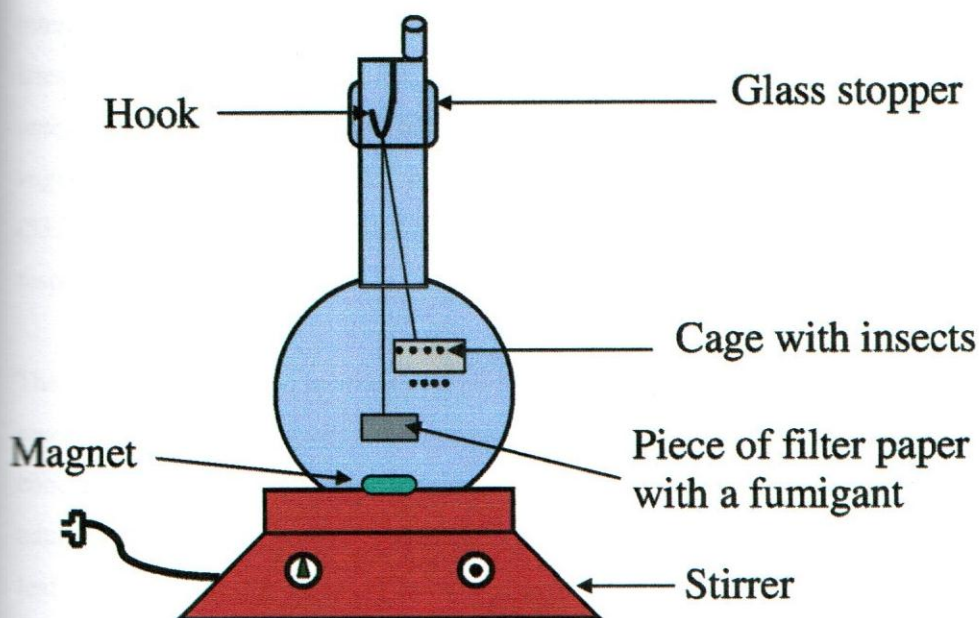


Fig.6.1: A 3.4 litre flat bottom glass (space) fumigation chamber

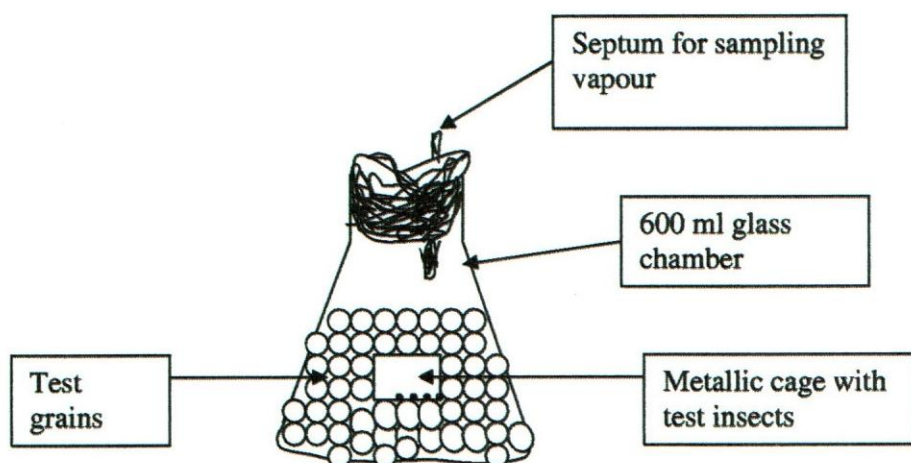


Fig.6.2: A 600 ml glass chamber filled with test grain

composition, time after application, concentration, insect species and corresponding factor interactions except plant part by time interaction effects on *S. oryzae* and *R. dominica*. Respective of test insect species, plant part assayed, time after application and concentration, *O. americanum* (59.4%) essential oil was the most toxic followed by *L. camara* (34.3%) and *T. angeli* (28.6%) essential oils, respectively, in decreasing order of toxicity. Similarly, leaf (53.7%) essential oils were the most toxic followed by stem (37.8%) and fruit (34.2%) oils, respectively (Fig. 6.3- 6.5).

The cumulative percent mortality of all insects tested were higher 168 h after treatment with *O. americanum* essential oils compared to 24 h (Figs. 6.3.1-6.3.2). Results showed clear concentration- dependent intra-species variations in the fumigant toxicity of essential oils against adult *S. oryzae*, *T. castaneum*, *R. dominica* and *C. chinensis*. Except for *C. chinensis* in fruit oil, leaf essential oil was the most toxic against the test insects, 24 h after treatment, followed by fruit and stem oils, respectively (Fig. 6.3.1; Table 6.1). *T. castaneum* and *C. chinensis* were the most tolerant and susceptible insect species, respectively. At the highest concentration (10 $\mu\text{L/L}$ air), 10.0, 0.0 and 3.0% adult *T. castaneum* mortalities were observed 24 h after treatment for essential oils extracted from leaves, stems and fruits, respectively, compared to 100, 100 and 90% mortality of adult *C. chinensis* (Fig. 6.3.1). The other two insects, *S. oryzae* and *R. dominica*, were equally susceptible against the leaf essential oil with 88% mortality at 10 $\mu\text{L/L}$ air. However, *R. dominica* was more susceptible than *S. oryzae* against the stem and fruit essential oils. Results of the probit regression analyses have revealed that the lethal concentration that killed 50% of test insects (LC_{50}) varied with plant part from which the essential oil was extracted and the insect species (Table 6.1). All test essential oils showed no response (no LC_{50} values) against *T. castaneum* whereas *C. chinensis* had LC_{50} values of 0.18, 1.15 and 3.70 $\mu\text{L/L}$ air 24h after treatment for leaf, fruit and stem oils, respectively. These results have revealed the existence of plant part by insect species interaction effects on adult mortality of test insects.

T. castaneum was the most tolerant insect species with 45, 38 and 8% kill for leaf, stem and fruit oils, respectively with corresponding LC_{50} values of 13.51, 25.02 and non-significant (NS), respectively (Table 6.1). Except for *T. castaneum* in which <50% mortality was recorded, the leaf and stem essential oils at 1 $\mu\text{L/L}$ air achieved 100% kill of adult *S. oryzae* and *R. dominica*

168 h after treatment. The fruit essential oil, on the other hand, achieved 100% kill of adult *S. oryzae* 168 h after treatment at ≥ 5 $\mu\text{L/L}$ air compared to 80 and $<10\%$ kill at 10 $\mu\text{L/L}$ air for *R. dominica* and *T. castaneum*, respectively (Table 6.1). Overall ranking of test insects, in order of increasing tolerance to *O. americanum* essential oils: *C. chinensis*, *R. dominica*, *S. oryzae* and *T. castaneum*. Similarly, the intra-species fumigant toxicities were ranked, in increasing order, as stem < fruit < leaf essential oils.

The fumigant toxicity of essential oils obtained from the aerial parts of *L. camara* against adult *S. oryzae*, *T. castaneum*, *R. dominica* and *C. chinensis* showed concentration-dependent intra-species variations in adult insect mortalities. Except for *C. chinensis* in which 100% kill was achieved at ≥ 5 $\mu\text{L/L}$ air 24 h after treatment, $<60\%$ kill of *S. oryzae*, *R. dominica* and *T. castaneum* adults were achieved for leaf, stem and fruit oils (Fig. 6.4.1). Strong plant part by insect species interactions ($P < 0.001$) were manifested for *S. oryzae* and *R. dominica* in leaf, stem and fruit oils. *T. castaneum* and *C. chinensis* were the most tolerant [LC_{50} value (fruit): 108.2 $\mu\text{L/L}$ air] and susceptible (LC_{50} values: 0.77-3.77 $\mu\text{L/L}$ air) insect species, respectively. The other two test insects, *S. oryzae* and *R. dominica*, showed significant variations in susceptibility to the leaf, stem and fruit essential oils. The LC_{50} values for *S. oryzae* were 64.10, 11.01 and 58.22 $\mu\text{L/L}$ air compared to 18.62, 32.60 and 42.50 $\mu\text{L/L}$ air for *R. dominica* 24 h after treatment with leaf, stem and fruit oils, respectively. The cumulative percent mortality of all insects was higher 168 h after treatment with *L. camara* oils compared to 24 h (Fig. 6.4.1- 6.4.2; Table 6.2). *T. castaneum* was the most tolerant insect with 28.0, 28.5 and 30.0% kill for leaf, stem and fruit oils, respectively, (Table 6.2). Stem oil was the most efficacious, 100% kill at ≥ 5 $\mu\text{L/L}$ air, with LC_{50} values of 0.61 and 0.08 $\mu\text{L/L}$ air against *S. oryzae* and *R. dominica*, respectively. However, the leaf and fruit oils, at 10 $\mu\text{L/L}$ air, achieved 78% and 70% kill against *S. oryzae* and *R. dominica*, respectively. Overall ranking of test insects, in order of increasing tolerance to *L. camara* essential oils: *C. chinensis*, *R. dominica*, *S. oryzae* and *T. castaneum*. Similarly, the intra-plant fumigant toxicities, in decreasing order: stem > leaf > fruit essential oils.

The fumigant toxicity of essential oils extracted from the aerial parts of *T. vogelii* (Fig. 6.5.1- 6.5.2; Table 6.3) against adult *S. oryzae*, *T. castaneum*, *R. dominica* and *C. chinensis* showed concentration-dependent intra-species variations in insect mortalities. Except for *C. chinensis* in

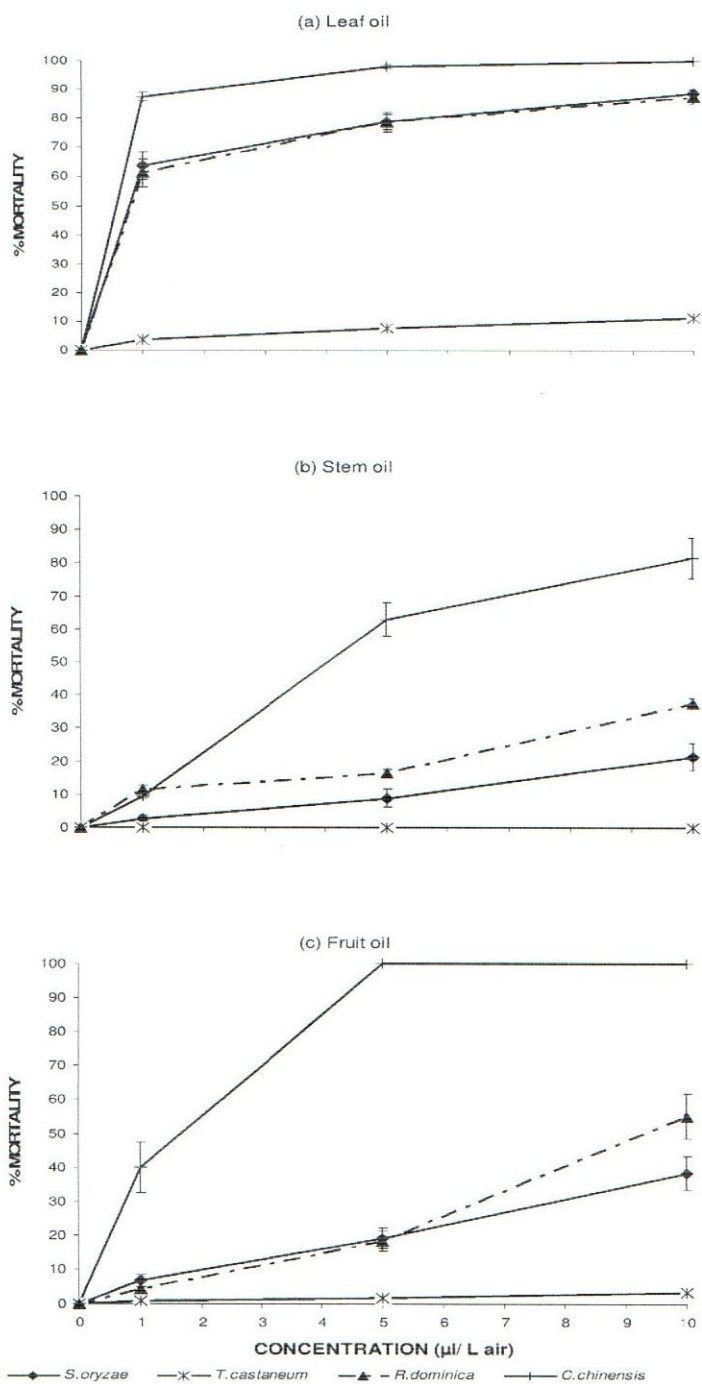


Fig.6.3.1: Mean percent adult mortality ($\pm SE$, $n = 4$) of four storage insect pests 24 h after treatment with essential oils extracted from aerial parts of *Ocimum americanum* L. in space fumigation chambers

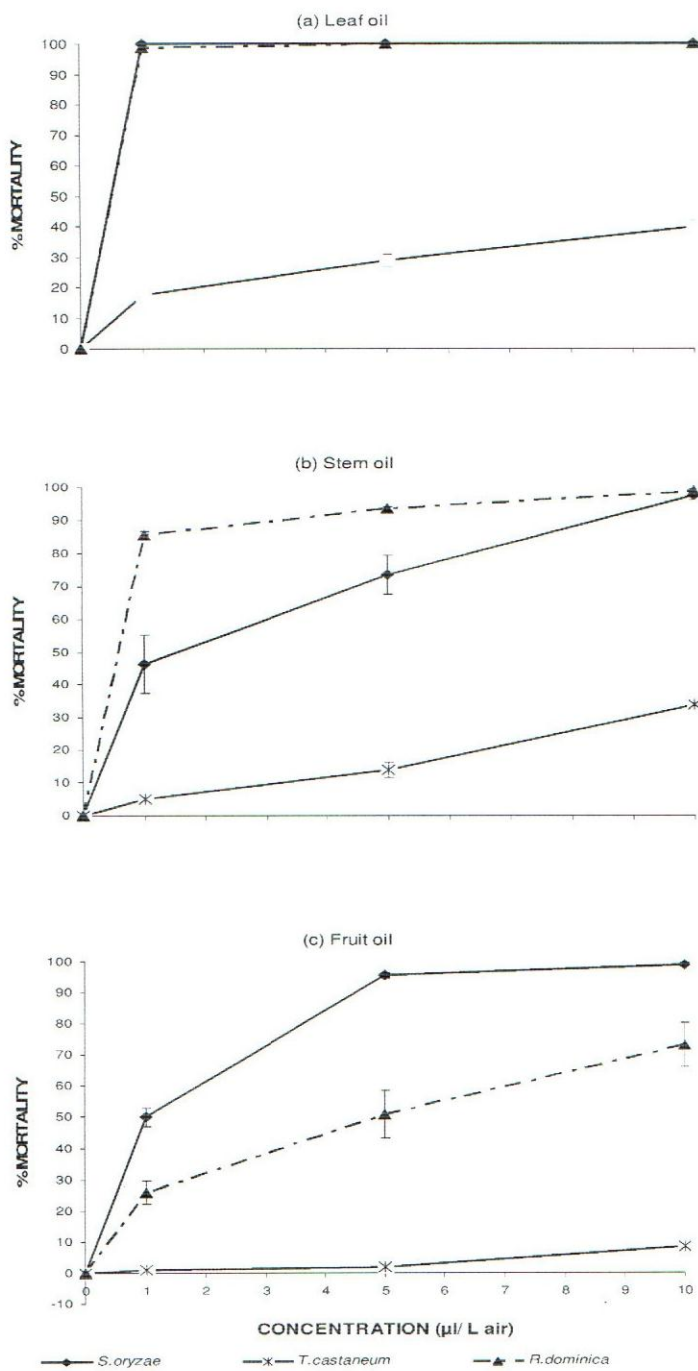


Fig. 6.3.2: Mean percent adult mortality ($\pm SE$, $n = 4$) of four storage insect pests 168 h after treatment with essential oils extracted from aerial parts of *Ocimum americanum* L. in space fumigation chambers

Table 6.2: Variation of *O. americanum* L. essential oil LC₅₀ values (µl/L air) with respect to plant part and time after treatment of four storage insects in space fumigation chambers.

Plant part/ Insect Species	N	LC ₅₀ values (µl/L air) based on plant part assayed and time (hours) after treatment					
		24		72		120	
		LC ₅₀	95%FL	LC ₅₀	95%FL	LC ₅₀	95%FL
Leaf:							
<i>S. oryzae</i>	20	0.38	(0.05, 0.83)	-	-	-	-
<i>R. dominica</i>	20	0.49	(0.11, 0.96)	0.27	(0.03, 0.68)	0.09	(0.01, 0.21)
<i>T. castaneum</i>	20	NS	-	109.10	(29.5, 315)	30.69	(18.9, 77.4)
<i>C. chinensis</i>	20	0.18	(0.01, 0.39)	0.01	(0.00, 0.03)	-	-
Stem:							
<i>S. oryzae</i>	20	10.74	(8.5, 15.4)	3.51	(2.22, 4.04)	2.34	(1.37, 3.79)
<i>R. dominica</i>	20	34.68	(22.7, 81.2)	7.09	(4.97, 12.1)	1.48	(0.60, 2.35)
<i>T. castaneum</i>	20	NS	-	63.50	(25.0, 122.4)	56.50	(24.0, 83.8)
<i>C. chinensis</i>	20	3.70	(3.09, 4.39)	0.64	(0.40, 0.84)	-	-
Fruit:							
<i>S. oryzae</i>	20	15.07	(9.88, 33.4)	1.02	(0.75, 1.28)	0.99	(0.73, 1.24)
<i>R. dominica</i>	20	10.61	(7.11, 13.9)	6.35	(5.1, 8.25)	4.11	(3.02, 5.67)
<i>T. castaneum</i>	20	NS	-	NS	-	NS	-
<i>C. chinensis</i>	20	1.15	(0.99, 1.34)	-	-	-	-

NS = Response to essential oil fumigation insignificant;

^a LC₅₀ values were considered significantly different when 95% fiducial limits did not overlap.

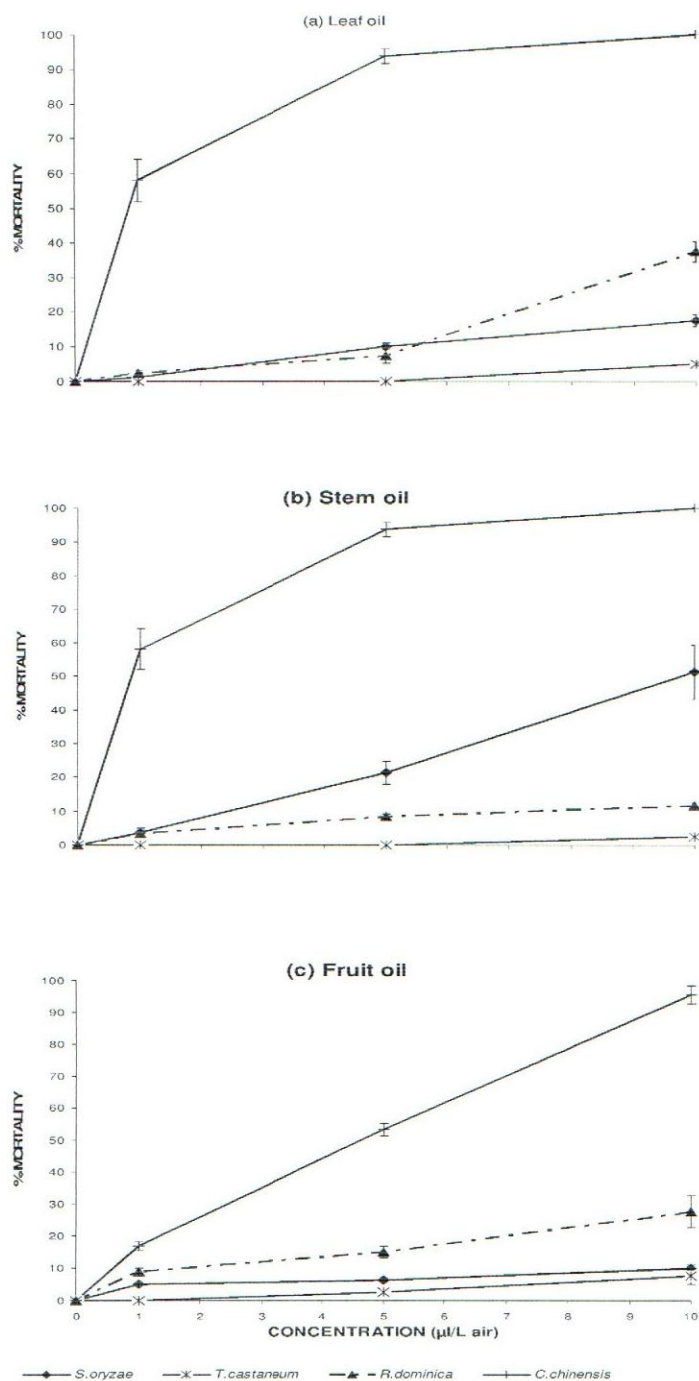


Fig.6.4.1: Mean percent adult mortality (± SE, n= 4) of four storage insect pests 24 h after treatment with essential oils extracted from aerial parts of *Lantana camara* L. in space fumigation chambers

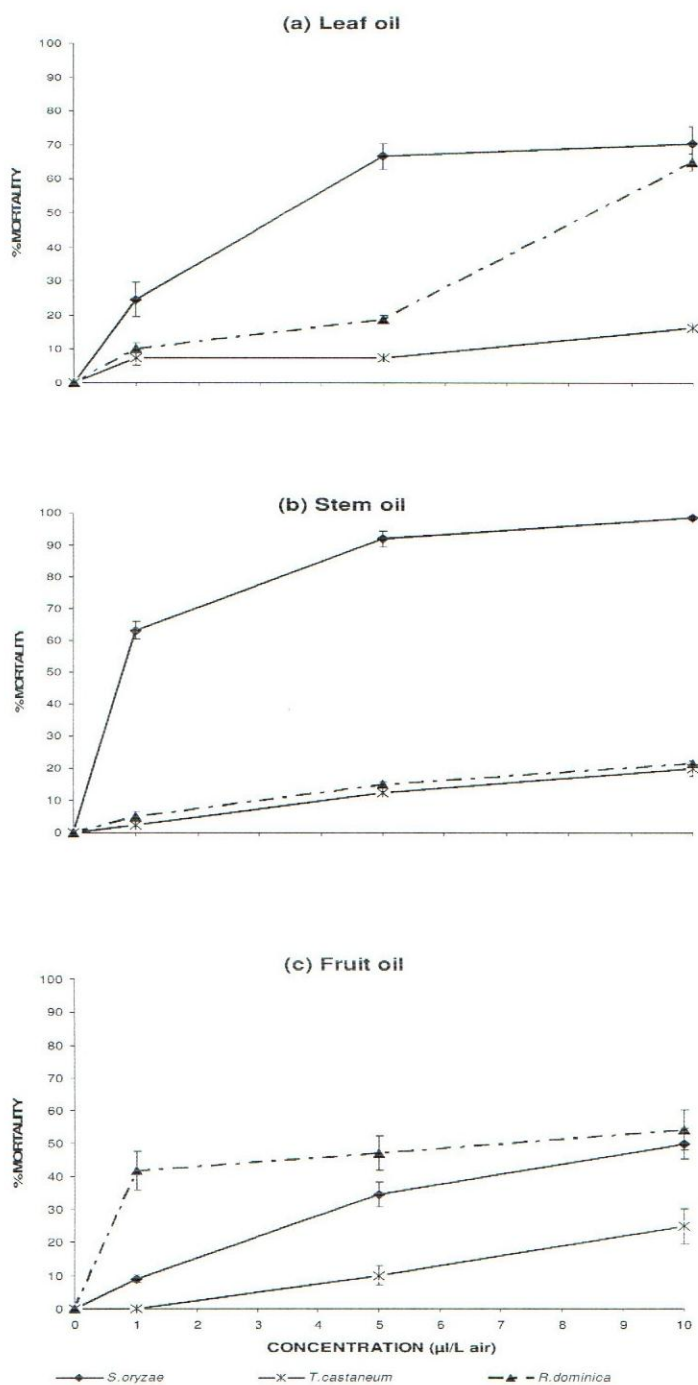


Fig.6.4.2: Mean percent adult mortality ($\pm$ SE, n= 4) of four stored-product insect pests 168 h after treatment with essential oils extracted from aerial parts of *Lantana camara* L. in space fumigation chambers.

Table 6.2: Variation of *Lantana camara* L. essential oil LC₅₀ values (µl/L air) with respect to plant part and time after treatment of four insect pests of stored food grains in space fumigation chambers¹.

Plant part/ Insect Species	N	LC ₅₀ values ^a (µl/L air) based on plant part assayed and time (hours) after treatment					
		24		72		120	
		LC ₅₀	95%FL	LC ₅₀	95%FL	LC ₅₀	95%FL
Leaf:							
<i>S. oryzae</i>	20	64.10	(25.5, 362)	4.91	(3.71, 6.76)	3.50	(2.57, 4.68)
<i>R. dominica</i>	20	18.62	(9.89, 42.7)	11.2	(5.56, 19.8)	10.1	(5.02, 16.7)
<i>T. castaneum</i>	20	NS	-	NS	-	NS	-
<i>C. chinensis</i>	20	0.77	(0.52, 1.01)	0.51	(0.26, 0.72)	-	-
Stem:							
<i>S. oryzae</i>	20	11.01	(8.62, 16.0)	2.62	(2.22, 3.04)	1.48	(1.18, 1.79)
<i>R. dominica</i>	20	32.60	(23.9, 77.4)	7.49	(4.45, 15.9)	1.29	(0.46, 2.12)
<i>T. castaneum</i>	20	NS	-	40.43	(20.4, 302)	36.50	(19.1, 378)
<i>C. chinensis</i>	20	3.77	(3.16, 4.46)	0.62	(0.37, 0.81)	-	-
Fruit:							
<i>S. oryzae</i>	20	58.22	(21.9, 79.5)	33.66	(22.6, 283)	17.30	(10.7, 42.9)
<i>R. dominica</i>	20	42.50	(25.6, 47.5)	31.95	(14.0, 242)	27.43	(8.21, 115)
<i>T. castaneum</i>	20	108.3	(77.9, 262)	58.13	(23.4, 225)	52.48	(22.1, 207)
<i>C. chinensis</i>	20	3.16	(1.97, 3.74)	0.58	(0.30, 0.86)	-	-

NS = Response to essential oil fumigation insignificant;

^a LC₅₀ values were considered significantly different when 95% fiducial limits did not overlap.

96.1% kill was achieved at 10 µl/L air 24 h after treatment, <60% kill of *S. oryzae*, *R. dominica* and *T. castaneum* adults were recorded for leaf, stem and fruit oils (Fig. 6.5.1). Strong plant part by insect species interaction effects were manifested for *S. oryzae* and *R. dominica* in the leaf, stem and fruit oils. The rust-red flour beetle, *T. castaneum*, and the cowpea beetle, *C. chinensis*, were the most tolerant [LC_{50} value: NS] and susceptible (LC_{50} values: 0.44- 8.20 µl/L air) insect species, respectively. Except for stem oil in which there was insignificant response, the other two test insects, *S. oryzae* and *R. dominica*, showed significant ($P<0.001$) variations in susceptibility to the leaf and fruit essential oils. The LC_{50} value for *S. oryzae* was 43.40 µl/L air for leaf essential oil compared to 16.90 and 18.24 µl/L air for *R. dominica* 24 h after treatment with leaf and fruit essential oils, respectively (Table 6.3).

The cumulative percent mortality of all insects was higher 168 h after treatment with *T. vogelii* essential oils compared to 24 h (Fig. 6.5.2; Table 5.3). *T. castaneum* was the most tolerant insect species with 12.5, 10.0 and 7.2 % kill for leaf, stem and fruit oils, respectively. Probit regression analyses showed that all LC_{50} values were insignificant (Table 6.3). Leaf oil was the most efficacious achieving 78- 89% kill at 10 µl/L air, with LC_{50} values of 1.23 and 1.86 µl/L air for *S. oryzae* and *R. dominica*, respectively. However, the stem essential oils, at 10 µl/L air, achieved 46.2% (LC_{50} value: 21.78 µl/L air) and 46.1% (LC_{50} value: 19.10 µl/L air) kill against *S. oryzae* and *R. dominica*, respectively compared to 55.4% (LC_{50} value: 10.20 µl/L air) and 63.1% (2.85 µl/L air) for fruit oil against the same insects, respectively. Overall ranking of test insects, in order of increasing tolerance: *C. chinensis*, *R. dominica*, *S. oryzae* and *T. castaneum*. Similarly, the intra-plant fumigant toxicities, in increasing order, were: stem < fruit < leaf oils.

The observed differential responses by different insect species to *O. americanum*, *L. camara* and *T. vogelii* essential oils could be attributed to individual and /or synergistic fumigant toxicity of their chemical constituents. Similarly, the marked differences in efficacies of leaf, stem and fruit oils against adults of individual insect species could be attributed to intra-species and inter-plant variations in the quantitative chemical composition. The GC-MS results identified eight major terpenoid constituents of *O. americanum*, four of which were screened for fumigant toxicity, namely 1,8-cineole, (Z)- β -ocimene, eugenol, E-caryophyllene, germacrene D, δ -bisabolene, elemicin and caryophyllene oxide. At a concentration of 10 µl/L air, eugenol was the most toxic

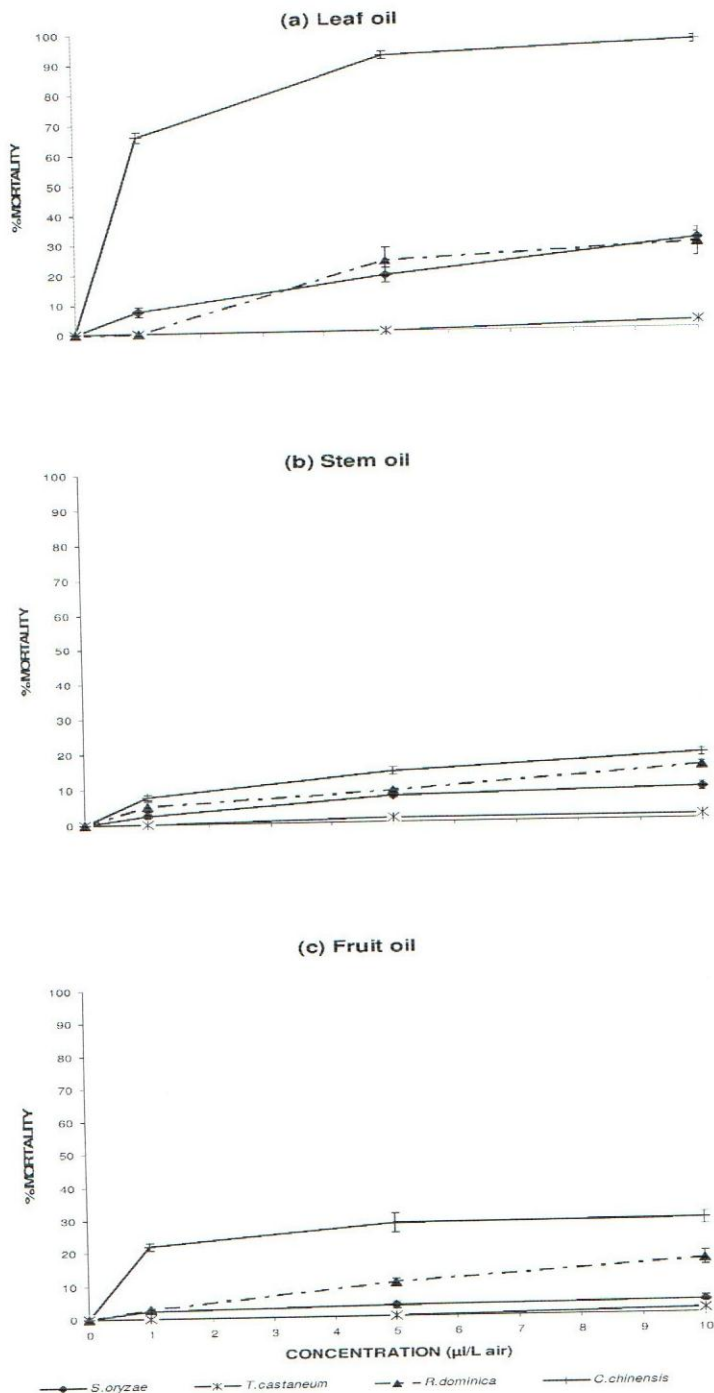


Fig.6.5.1: Mean percent adult mortality ($\pm$ SE, $n=4$) of four storage insect pests 24 h after treatment with essential oils extracted from aerial parts of *Tephrosia vogelii* Hook in space fumigation chambers

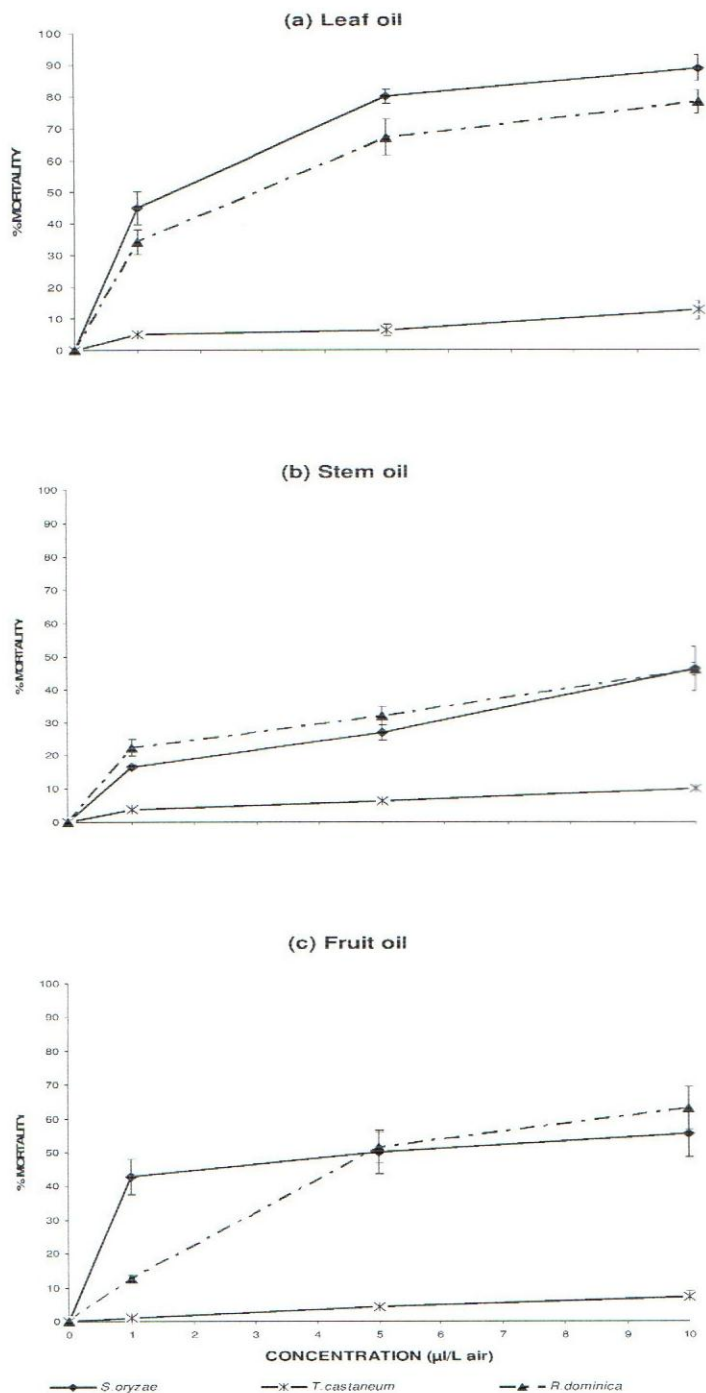


Fig.6.5.2: Mean percent adult mortality ($\pm$ SE, $n = 4$) of four storage insect pests 168 h after treatment with essential oils extracted from aerial parts of *Tephrosia vogelii* Hook in space fumigation chambers

Table 6.3: Variation of *T. vogelii* Hook essential oil LC₅₀ values (µl/L air) with respect to plant part and time after treatment of four storage insects in space fumigation chambers.

Plant part/ Insect Species	N	LC ₅₀ values (µl/L air) based on plant part assayed and time (hours) after treatment							
		24		72		120		168	
		LC ₅₀	95%FL	LC ₅₀	95%FL	LC ₅₀	95%FL	LC ₅₀	95%FL
<u>Leaf:</u>									
<i>S. oryzae</i>	20	43.40	(18.9, 47.4)	1.93	(1.37, 2.50)	1.70	(1.15, 2.26)	1.23	(0.77, 1.69)
<i>R. dominica</i>	20	16.90	(8.29, 25.5)	13.10	(6.76, 17.7)	5.49	(4.04, 8.01)	1.86	(1.17, 2.57)
<i>T. castaneum</i>	20	NS	-	NS	-	NS	-	23.2	(16.4, 98.7)
<i>C. chinensis</i>	20	0.44	(0.17, 0.73)	0.20	(0.01, 0.43)	-	-	-	-
<u>Stem:</u>									
<i>S. oryzae</i>	20	NS	-	17.68	(11.7, 39.3)	13.2	(9.29, 24.5)	21.78	(11.5, 205)
<i>R. dominica</i>	20	NS	-	NS	-	NS	-	19.10	(8.64, 42.0)
<i>T. castaneum</i>	20	NS	-	NS	-	NS	-	NS	-
<i>C. chinensis</i>	20	NS	-	1.52	(0.20, 2.94)	0.20	(0.01, 0.73)	-	-
<u>Fruit:</u>									
<i>S. oryzae</i>	20	NS	-	39.5	(20.0, 377)	23.13	(14.5, 266)	10.20	(7.19, 18.1)
<i>R. dominica</i>	20	18.24	(13.1, 45.4)	12.68	(9.32, 19.7)	4.90	(4.13, 5.82)	2.85	(2.44, 3.28)
<i>T. castaneum</i>	20	NS	-	NS	-	NS	-	NS	-
<i>C. chinensis</i>	20	8.20	(3.8, 12.5)	0.03	(0.01, 0.28)	-	-	-	-

NS = Response to essential oil fumigation insignificant;

^a LC₅₀ values were considered significantly different when 95% fiducial limits did not overlap.

enic compound causing 76.0, 94.0, 15.0 and 100% mortality of *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis*, respectively 24 h after treatment (Fig. 6.4.1) corresponding to LC₅₀ values of 8.21, 0.03, NS and 0.02 µl/L air, respectively (Table 6.4). Similarly, (Z)-β-ocimene, at 10 µl/L air, caused 88.0, 69.0, 42.0 and 100% kill of *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis*, respectively 24 h after treatment (Fig. 6.4.1) corresponding to LC₅₀ values of 11.20, 11.77, NS and 0.28 µl/L air, respectively (Table 6.4). In same study, 1,8-cineole (15 µl/L air) caused 66.0, 100.0 and 33.0% mortality of *S. oryzae*, *R. dominica* and *T. castaneum*, respectively (Fig. 6.5.1) 24 h after treatment with LC₅₀ values of 14.08, 7.80 and 21.20 µl/L air, respectively (Table 6.5). The fourth compound tested, caryophyllene oxide, was less toxic causing 10.0, 74.0, 10.0 and 40.0% kill of *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis*, respectively 24 h after treatment (Fig. 5.6.1) corresponding to LC₅₀ values of 65.8, 32.5, 58.2 and 29.6 µl/L air, respectively (Table 6.4).

The GC-MS analyses of *L. camara* essential oils identified seven terpenoid compounds likely to contribute towards the observed fumigant toxicities, namely α-pinene, sabinene, 1,8-cineole, E-caryophyllene, α-humulene, bicyclogermacrene and (E)-nerolidol (see Chapter 8). In particular, observed adult insect mortalities could be attributed to the strong fumigant activities of (+/-)-α-pinene, 1, 8-cineole and α-humulene, among other constituents, which individually showed varied degrees of efficacy against the four test insects (Fig. 6.6- 6.7). At a concentration of 10 µl/L air 24 h after treatment, (+)-α-pinene caused 8.0, 30.0 and 90.0% adult mortalities of *S. oryzae*, *R. dominica* and *C. chinensis*, respectively (Fig. 6.7.1) whose corresponding LC₅₀ values were NS, 36.81 and 5.11 µl/L air, respectively (Table 6.6). However, at the same concentration, (-)-α-pinene was slightly inferior causing 0.0, 24.0 and 42.0% adult mortalities of *S. oryzae*, *R. dominica* and *C. chinensis*, respectively (Fig. 6.7.1) whose corresponding LC₅₀ values were NS, 59.6 and 15.1 µl/L air, respectively. The other constituent tested, α-humulene, caused 2.0, 5.0 and 86.0% adult mortalities of *S. oryzae*, *R. dominica* and *C. chinensis*, respectively (Fig. 6.7.1) whose corresponding LC₅₀ values were 135.80, 106.30 and 0.48 µl/L air, respectively (Table 6.5). The fact that *L. camara* essential oils and their monoterpenoid constituents achieved 50% kill of all test insects, except *T. castaneum*, at concentrations of 0.8- 64.1, 5.1- 36.8, 7.8- 21.2 and 0.5- 135.8 µl/L air 24 h after treatment gives a glimmer of hope that these essential oils and their constituents are potential fumigants against insect pests of stored food commodities. In the

analysis of *T. vogelii* essential oils, six terpenoid compounds, namely, α -pinene, limonene, E-caryophyllene, germacrene D, E-nerolidol and (E, Z)-farnesol were identified as the major constituents and likely contributors to the observed varied adult insect mortalities (Fig. 6.4- 6.5). The bioactivity of (+/-)- α -pinene is as described for *L. camara* oils above. The other constituent, R-(+)-limonene, was tested against three adult insect species in this study. At a concentration of 20 μ L/L air, 17.5, 37.5 and 57.5% mortality of adult *S. oryzae*, *R. dominica* and *T. castaneum*, respectively, were recorded with corresponding LC₅₀ values of 29.30, 21.70 and 18.22 μ L/L air, respectively. Of the 21 major terpenoids identified in *O. americanum*, *L. camara* and *T. vogelii* essential oils, the following 8 were untested, namely; sabinene, E-caryophyllene, bicyclogermacrene, E-nerolidol, germacrene D, δ -bisabolene, elemicin and (E,Z)-farnesol. The bioactivity of these essential oil constituents, individually or in blends, needs to be ascertained.

The efficacy of essential oils and their constituent compounds were largely correlated and hence a possible explanation to the observed adult insect mortalities. Results of current study were inferior to Rozman *et al.* (2007) in which 1,8-cineole, at 0.14 μ L/L air, caused 100 and 97.5% kill of adult *S. oryzae* and *R. dominica*, respectively. In related studies, Ogendo *et al.* (2008) reported that *O. gratissimum* oil, eugenol and (Z)- β -ocimene had LC₅₀ values 0.20- 14.0, 0.01- 17.0 and 0.80- 23.0 μ L/L air, respectively, against all insects tested except *T. castaneum*. In earlier studies, Prates *et al.* (1998) reported strong insecticidal activity of two monoterpenes, 1, 8-cineole and R-(+)-limonene, against *R. dominica* and *T. castaneum*. The LC₅₀ values (21.2 μ L/L air) reported in this study for *T. castaneum* fall within the range (3.5 and 466 μ L/L air) observed against *T. confusum* in recent studies (Stamopoulos *et al.*, 2007). The fact that *O. americanum* essential oils and their terpenoid constituents achieved 50% kill of all insects tested, except *T. castaneum*, at concentrations of 0.4- 34.7, 0.02- 8.2, 0.3- 31.2 and 29.6- 65.8 μ L/L air 24 h after treatment for oil, eugenol, (Z)- β -ocimene and caryophyllene oxide, respectively, is welcome scientific promise that makes these constituents candidate fumigants against insect pests of stored food grains. In unrelated studies, α -pinene and limonene (at KD95: >6500 mg cm⁻²) had knockdown effect against the American cockroach (Ngoh *et al.*, 1998) and the former (α -pinene) caused 100% mortality of adult Colorado potato beetle 12 h after treatment (Kordali *et al.*, 2007). In their recent studies, Stamopoulos *et al.* (2007) reported that a 50% kill of *T. confusum* was achievable in space fumigation using R-(+)-limonene applied at 4.1 μ L/L air. Space fumigation studies

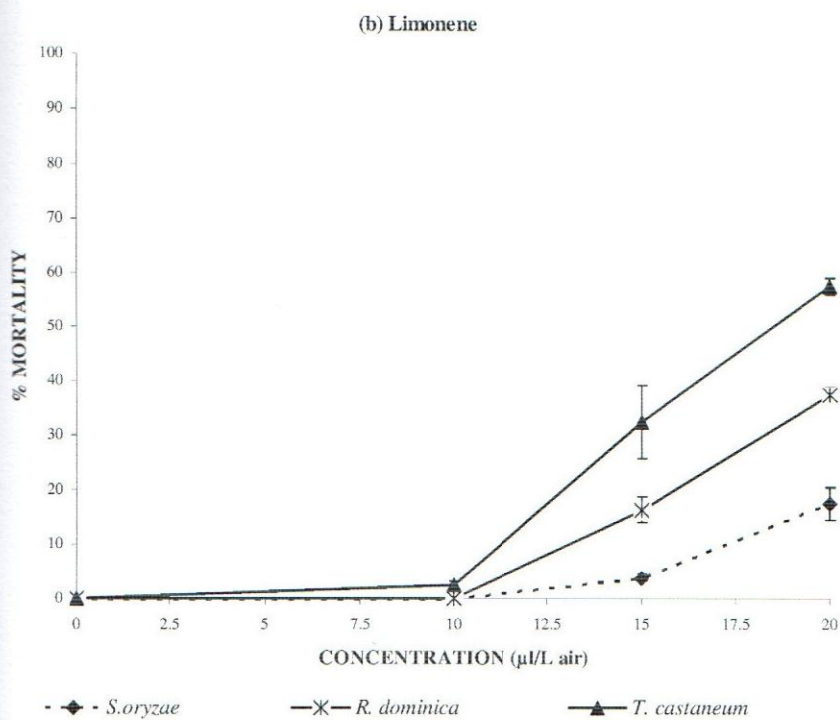
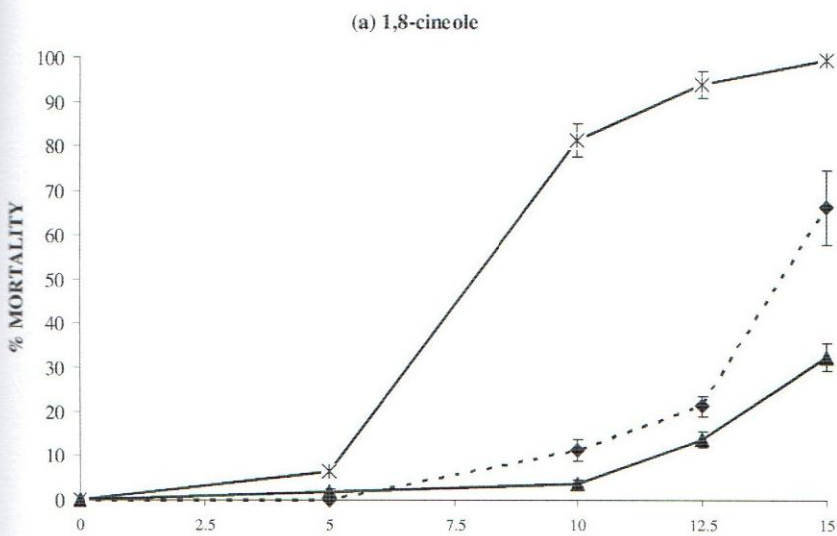


Fig.6.6.1: Mean percent adult mortality ($\pm$ SE, $n = 4$) of three storage insect pests 24 h after treatment with (a) 1, 8 cineole and (b) limonene in space fumigation chambers

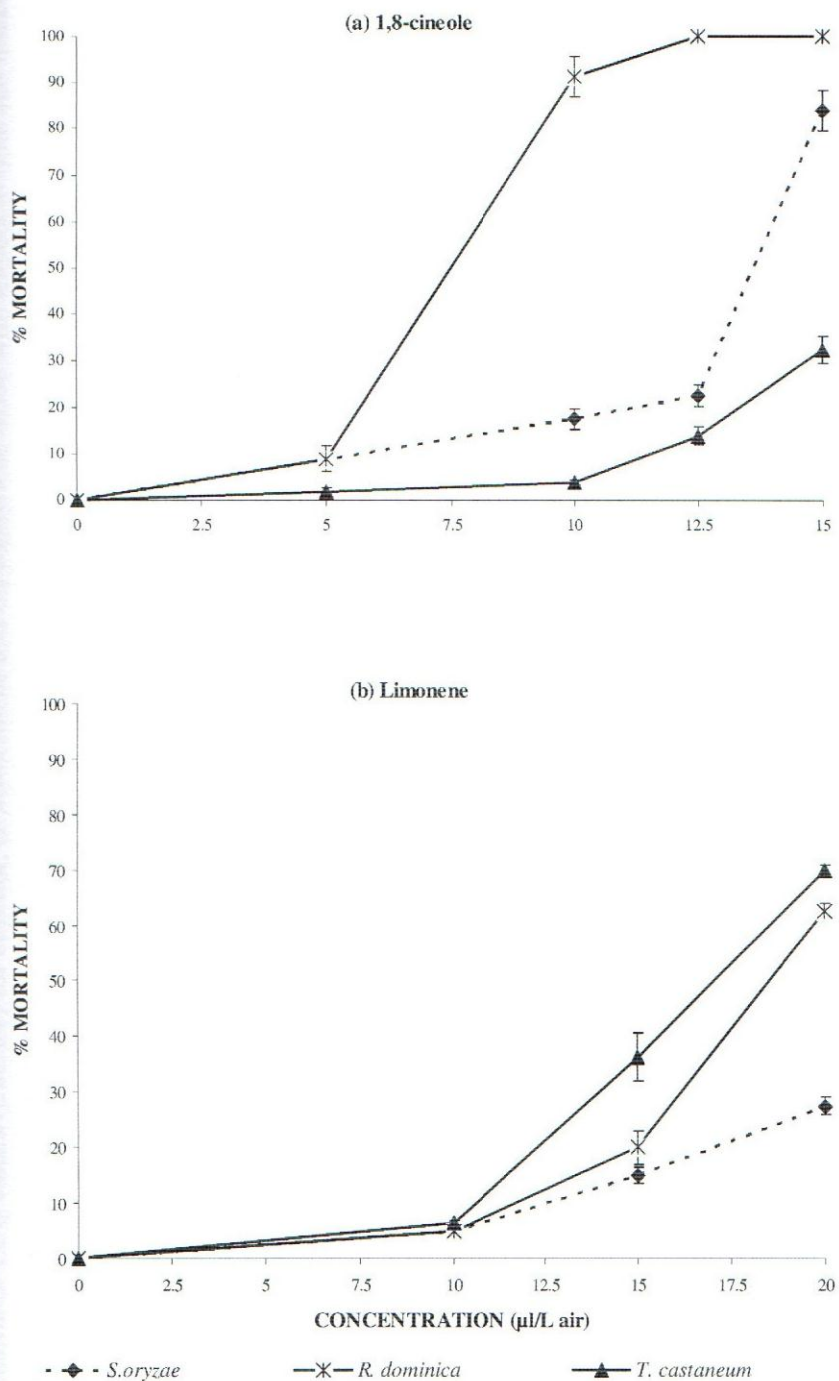


Fig.6.6.2: Mean percent adult mortality ($\pm$ SE, $n = 4$) of three storage insect pests 48 h after treatment with (a) 1,8 cineole and (b) limonene in space fumigation chambers

Table 6.4: Variation of LC₅₀ values (μl/L air) for eugenol, (Z)-β-ocimene and caryophyllene oxide with respect to time and insect species in space fumigation chambers.

Plant part/ Insect Species	N	LC ₅₀ values (μl/L air) based on essential oil constituent and time (hours) after treatment							
		24		72		120		168	
		LC ₅₀	95%FL	LC ₅₀	95%FL	LC ₅₀	95%FL	LC ₅₀	95%FL
<u>Eugenol:</u>									
<i>S. oryzae</i>	20	8.21	(3.09, 14.7)	0.28	(0.01, 0.97)	0.03	(0.00, 0.30)	0.02	(0.00, 0.22)
<i>R. dominica</i>	20	0.02	(0.01, 0.20)	0.02	(0.00, 0.23)	0.02	(0.00, 0.18)	0.02	(0.00, 0.18)
<i>T. castaneum</i>	20	NS	-	29.46	(20.8, 33.5)	23.10	(14.9, 39.4)	20.36	(14.0, 32.6)
<i>C. chinensis</i>	20	0.03	(0.01, 0.25)	-	-	-	-	-	-
<u>(Z)-β-Ocimene:</u>									
<i>S. oryzae</i>	20	11.20	(7.8, 15.9)	3.18	(1.79, 5.22)	1.98	(0.99, 2.98)	1.32	(0.79, 1.78)
<i>R. dominica</i>	20	11.07	(7.59, 20.1)	7.14	(5.11, 12.3)	1.50	(0.74, 2.21)	0.01	(0.00, 0.11)
<i>T. castaneum</i>	20	NS	-	14.87	(10.5, 27.6)	11.35	(8.25, 19.0)	7.58	(5.34, 10.9)
<i>C. chinensis</i>	20	0.28	(0.07, 0.51)	-	-	-	-	-	-
<u>Caryophyllene oxide:</u>									
<i>S. oryzae</i>	20	65.84	(34.2, 282)	47.10	(36.2, 56.7)	31.50	(22.4, 37.7)	28.40	(19.9, 33.4)
<i>R. dominica</i>	20	32.48	(14.9, 49.8)	0.52	(0.05, 1.42)	0.20	(0.03, 0.46)	0.07	(0.01, 0.28)
<i>T. castaneum</i>	20	58.22	(21.9, 62.0)	40.06	(28.8, 47.7)	28.30	(15.9, 32.7)	28.30	(15.9, 32.7)
<i>C. chinensis</i>	20	29.56	(14.0, 43.7)	5.25	(3.06, 7.04)	-	-	-	-

NS = Response to essential oil fumigation insignificant;

^a LC₅₀ values were considered significantly different when 95% fiducial limits did not overlap.

Table 6.5: Variation of LC₅₀ values (µl/L air) for 1,8-cineole, limonene and α-humulene with respect to time and insect species in space fumigation chambers.

Plant part/ Insect Species	N	LC ₅₀ values (µl/L air) based on essential oil constituent and time (hours) after treatment					
		24		72		120	
		LC ₅₀	95%FL	LC ₅₀	95%FL	LC ₅₀	95%FL
<u>1,8-cineole:</u>							
		<u>48 hr</u>		<u>48 hr</u>		<u>48 hr</u>	
<i>S. oryzae</i>	20	14.08	(9.94, 17.4)	13.39	(10.1, 16.9)	-	-
<i>R. dominica</i>	20	7.80	(7.31, 8.25)	6.99	(6.58, 7.42)	-	-
<i>T. castaneum</i>	20	21.20	(17.7, 25.7)	21.20	(17.7, 25.2)	-	-
<i>C. chinensis</i>	-	-	-	-	-	-	-
<u>Limonene:</u>							
<i>S. oryzae</i>	20	29.30	(23.6, 49.9)	26.75	(23.2, 40.3)	-	-
<i>R. dominica</i>	20	21.70	(20.1, 24.7)	18.52	(17.5, 20.0)	-	-
<i>T. castaneum</i>	20	18.22	(17.2, 19.7)	16.84	(15.9, 17.9)	-	-
<i>C. chinensis</i>	-	-	-	-	-	-	-
<u>α-humulene:</u>							
		<u>72 hr</u>		<u>72 hr</u>		<u>72 hr</u>	
<i>S. oryzae</i>	20	135.8	(47.6, 166)	6.91	(3.59, 9.72)	5.42	(3.77, 10.9)
<i>R. dominica</i>	20	106.30	(32.1, 149)	27.5	(14.1, 47.5)	22.2	(13.4, 63.6)
<i>T. castaneum</i>	-	-	-	-	-	-	-
<i>C. chinensis</i>	20	0.48	(0.08, 0.99)	0.01	(0.00, 0.10)	-	-

NS = Response to essential oil fumigation insignificant;

^a LC₅₀ values were considered significantly different when 95% fiducial limits did not overlap.

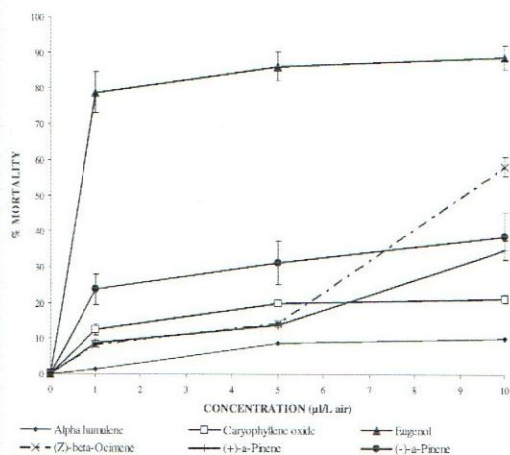
(a) *S. oryzae*



(c) *T. castaneum*



(c) *R. dominica*



(d) *C. chinensis*

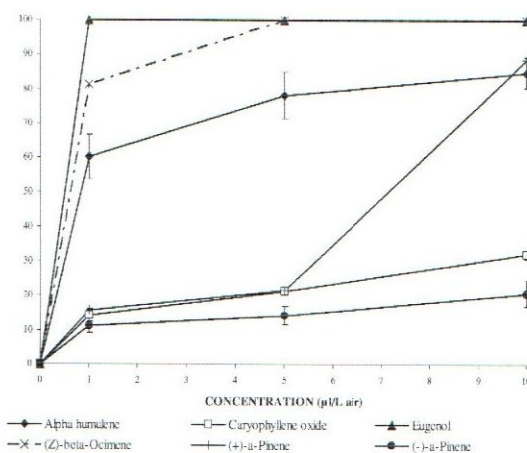


Fig.6.7.1: Mean percent adult mortality ($\pm SE$, $n = 4$) of four storage insect pests 24 h after treatment with six essential oil constituents in space fumigation chambers

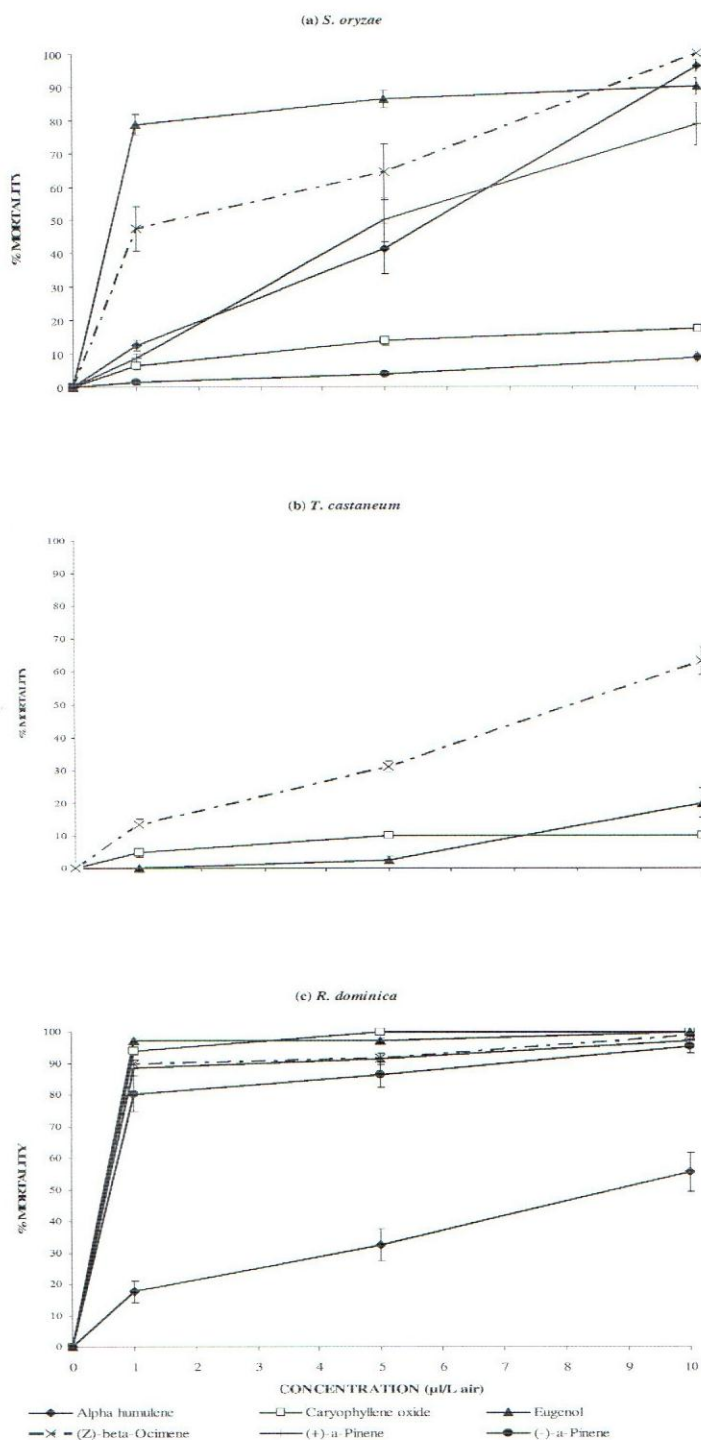


Fig.6.7.2: Mean percent adult mortality ($\pm$ SE, $n=4$) of three stored-product insect pests 168 h after treatment with six essential oil constituents in space fumigation chambers

Table 6.6: Variation of LC₅₀ values (μl/L air) for pinene compounds with respect to time and insect species in space fumigation chambers.

Plant part/ Insect Species	LC ₅₀ values (μl/L air) based on essential oil constituent and time (hours) after treatment								
	N	24		72		120		168	
		LC ₅₀	95%FL	LC ₅₀	95%FL	LC ₅₀	95%FL	LC ₅₀	95%FL
(+)-α-pinene:									
<i>S. oryzae</i>	20	NS	-	104.07	(30.3, 142)	6.87	(4.55, 8.95)	4.41	(3.68, 5.29)
<i>R. dominica</i>	20	36.81	(25.4, 55.8)	8.55	(3.90, 20.6)	0.47	(0.02, 1.18)	0.02	(0.00, 0.11)
<i>T. castaneum</i>	-	-	-	-	-	-	-	-	-
<i>C. chinensis</i>	20	5.11	(3.12, 7.25)	1.69	(0.87, 2.58)	-	-	-	-
(-)-α-pinene:									
<i>S. oryzae</i>	20	NS	-	74.57	(44.1, 141)	61.94	(36.9, 130)	31.97	(20.9, 53.0)
<i>R. dominica</i>	20	59.59	(14.4, 123)	2.14	(0.31, 4.38)	2.14	(0.31, 4.38)	0.06	(0.01, 0.30)
<i>T. castaneum</i>	-	-	-	-	-	-	-	-	-
<i>C. chinensis</i>	20	15.05	(9.77, 21.5)	0.87	(0.25, 2.41)	-	-	-	-
(+)-β-pinene:									
<i>S. oryzae</i>	20	51.95	(33.7, 65.9)	33.70	(28.3, 51.5)	10.58	(3.49, 15.6)	5.26	(2.80, 7.79)
<i>R. dominica</i>	20	12.30	(9.66, 21.4)	1.15	(0.11, 2.30)	0.12	(0.01, 0.41)	0.03	(0.00, 0.20)
<i>T. castaneum</i>	-	-	-	-	-	-	-	-	-
<i>C. chinensis</i>	20	2.94	(1.17, 3.06)	0.56	(0.14, 0.88)	-	-	-	-
(-)-β-pinene:									
<i>S. oryzae</i>	20	31.20	(16.8, 55.9)	12.62	(7.27, 20.3)	6.28	(4.33, 10.8)	5.16	(4.78, 9.77)
<i>R. dominica</i>	20	9.47	(5.26, 17.8)	0.24	(0.09, 4.22)	0.09	(0.01, 0.29)	0.09	(0.01, 0.29)
<i>T. castaneum</i>	-	-	-	-	-	-	-	-	-
<i>C. chinensis</i>	20	25.16	(17.8, 35.9)	2.47	(1.55, 3.98)	-	-	-	-

NS = Response to essential oil fumigation insignificant;

^a LC₅₀ values were considered significantly different when 95% fiducial limits did not overlap.

conducted earlier with R- (+)-limonene at 31.5 mg/L air caused 96.7 and 94.9% mortality of adult *R. dominica* and *T. castaneum*, respectively (Prates *et al.*, 1998).

The fact that, irrespective of plant part sampled, the essential oils of *O. americanum*, *L. camara* and *T. vogelii* achieved a 50% kill of the test insects 24 h after treatment, except *T. castaneum*, at fairly low concentrations of 0.4- 34.7, 0.8- 64.1 and 0.4- 43.4 $\mu\text{L/L}$ air, respectively, demonstrates the existence of strong fumigant toxicity and potential use as grain fumigants. These results were further strengthened by the screening of eight terpenoid constituents of these essential oils with LC_{50} values ranging between 0.03 and 135.80 $\mu\text{L/L}$ air against the test insects except *T. castaneum* [LC_{50} values: insignificant for eugenol, (Z)- β -ocimene and α -humulene]. With the most active, *O. americanum* leaf oil, a concentration of 10 $\mu\text{L/L}$ air and exposure time of 24 h was enough to obtain 88- 100% kill of all insects, except *T. castaneum*, in space fumigation studies. Although slightly inferior to the highly active Labiate sp. oil, ZP51 which caused 90% kill of *S. oryzae*, *R. dominica*, *O. surinamensis* and *T. castaneum* at a concentration of 1.4-4.5 $\mu\text{L/L}$ air (Shaaya *et al.*, 1997), these plant essential oils have great potential as fumigants against coleopteran pests of stored food grains. Hence, the need to conduct grain fumigation studies to establish the dose-response curves and appropriate exposure duration (days) against the major insect pests of stored food grains.

6.3.2 Fumigant toxicity studies (Grain fumigation)

The percent adult mortality and LC_{50} values for the fumigant toxicity of *O. americanum* leaf essential oil against three coleopteran pests in 600 ml glass chambers 70% filled with wheat grains are presented (Tables 6.7- 6.8). The fumigant efficacy of essential oil was significantly ($P<0.001$) influenced by concentration applied, exposure duration, post-fumigation time, insect species and corresponding factor interactions except for post-fumigation time by concentration interaction effects on adult *S. oryzae*. The results showed a significant ($P<0.001$) concentration-, insect species- and exposure duration-dependent toxicity against adult *S. oryzae*, *R. dominica* and *T. castaneum* insects. Percent adult mortalities for the three test insects increased with concentration, exposure duration and post-fumigation time. At a concentration range of 30- 100 $\mu\text{L/L}$ air, 3 days exposure and 120 h post-fumigation, 63.0- 100, 53.0- 93.0 and 48.0- 76.0 % kill were recorded for *S. oryzae*, *R. dominica* and *T. castaneum* insects, respectively (Table 6.7).

Highest mortality values of 78.0- 100, 100 and 67.0- 98.0% were recorded for *S. oryzae*, *R. dominica* and *T. castaneum*, respectively, at 30-100 $\mu\text{L/L}$ air, 10 d exposure and 120 h post-fumigation time. The same results revealed that *O. americanum* leaf essential oil, at a concentration of 50 $\mu\text{L/L}$ air, 7 d exposure and 120 h post-fumigation time were enough to obtain 95.0- 100% kill of *S. oryzae* and *R. dominica* and 66.0% for *T. castaneum*. Similar toxic effects from other Lamiaceae/Labiatae have been reported. For example, ZP51, a highly active essential oil from a Labiatae sp. applied at 50 $\mu\text{L/L}$ air in columns 70% filled with wheat grains and 7 d exposure caused 94.0- 100% mortality of adult *S. oryzae*, *R. dominica*, *O. surinamensis* and *T. castaneum* insects (Shaaya *et al.*, 1997). In grain fumigation studies using 300 ml conical flasks 22% filled with wheat grains, Lee *et al.* (2004) reported fumigant LT_{50} values of 16.20, 17.40 and 9.10 h for *Melaleuca fulgens* and *Eucalyptus blakelyi* essential oils and 1,8-cineole tested at a concentration of 100 $\mu\text{L/L}$ air against adult *S. oryzae* insects. In other studies seeking alternatives to methyl bromide showed that methyl iodide caused 100% kill of *S. zeamais* at a dose of 3.02 mg/L which was comparable to a dose range of 50-150 mg/L with 24-120 h exposures for allyl acetate (Faruki *et al.*, 2005; Rajendran and Muralidharan, 2005). The results of this study compares favourably with methyl bromide's recommended dose of 30-50 g/M^3 grain (Busvine, 1980; Kostyukovsky *et al.*, 2002a; Lee *et al.*, 2004). The strong fumigant activity exhibited by the *O. americanum* leaf essential oil against the three insects could possibly be attributed to individual and /or synergistic toxicity of the major monoterpenoid compounds in the leaves of test plant. In particular, 1,8-cineole, (Z)- β -ocimene and eugenol have been shown to have strong fumigant activity against various coleopteran pests of stored food grains (Prates *et al.*, 1998; Stamopoulos *et al.*, 2007; Ogendo *et al.*, 2008). By virtue of their structural characteristics, monoterpenoid compounds influence their insecticidal properties, degree to penetrate into the insect cuticle and their ability to move to and interact with an active site (Rice and Coats, 1994). Although not directly studied, the mode of action of the essential oils and their volatile constituents against insect pests of stored durable agricultural commodities in both space and grain fumigation could be due to knockdown effect, neurotoxicity and activation of octopamine (Kostyukovsky *et al.*, 2002b; Priestley *et al.*, 2003; Enan, 2005a, b; Garcia *et al.*, 2005; Jirovetz *et al.*, 2005; Shaaya and Kostyukovsky, 2006; Rajendran and Sriranjini, 2008). Additionally, a strong structure- insecticidal activity relationship against adult lice (Priestley *et al.*, 2006) and a correlation between total oxygenated monoterpenoid content and essential oil

insecticidal activity has been reported in which oxygenated monoterpenes exhibited higher activity than monoterpene hydrocarbons (Papachristos *et al.*, 2004). Stamopoulos *et al.* (2007) revealed insect growth regulator (IGR)-like properties of 1,8-cineole and R-(+)-limonene among five monoterpenoids, when applied to 3-day old pupae of *T. castaneum* resulting in adultoids and deformed adults. Hence, fumigant activity of the essential oils and their volatile monoterpene constituents are dependent upon their chemical structures, insect anatomy and the chemical contents of the oils.

Table 6.7: Mean percent mortality ($\pm SE$, $n = 4$) of three storage insect pests as influenced by fumigation duration (days), concentration ($\mu\text{L/L}$ air) of *O. americanum* leaf essential oil and post-fumigation time (hours) in wheat grains

Fumigation Duration (Days)	Insect Species/ Essential oil Conc ($\mu\text{L/L}$)	N	PERCENT MORTALITY (Mean $\pm$ SE) POST-FUMIGATION TIME (HOURS)		
			24	72	120
3	<u><i>S. oryzae</i>:</u>				
	0	20	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
	30	20	24.1 $\pm$ 6.4	62.8 $\pm$ 9.6	62.8 $\pm$ 9.6
	50	20	35.4 $\pm$ 5.2	66.7 $\pm$ 9.1	68.8 $\pm$ 8.8
	70	20	45.6 $\pm$ 6.2	73.1 $\pm$ 10.4	75.3 $\pm$ 9.9
	100	20	58.2 $\pm$ 8.8	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
	LSD _{0.05} 20		17.3	20.5	19.5
	<u><i>R. dominica</i>:</u>				
	0	20	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
	30	20	30.8 $\pm$ 4.4	48.8 $\pm$ 1.5	53.4 $\pm$ 0.8
	50	20	37.2 $\pm$ 5.7	57.3 $\pm$ 1.1	65.8 $\pm$ 2.0
	70	20	55.6 $\pm$ 1.6	65.6 $\pm$ 0.7	78.4 $\pm$ 1.9
	100	20	61.5 $\pm$ 5.8	80.0 $\pm$ 3.2	93.2 $\pm$ 1.7
	LSD _{0.05} 20		11.4	6.6	4.5
	<u><i>T. castaneum</i>:</u>				
	0	20	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
	30	20	10.0 $\pm$ 2.0	47.8 $\pm$ 2.5	47.8 $\pm$ 2.5
	50	20	17.5 $\pm$ 3.1	55.1 $\pm$ 1.0	55.1 $\pm$ 1.0
	70	20	50.0 $\pm$ 6.1	65.0 $\pm$ 1.0	65.0 $\pm$ 1.0
	100	20	63.8 $\pm$ 9.6	76.4 $\pm$ 0.6	76.4 $\pm$ 0.6
	LSD _{0.05}		18.9	5.9	5.9
5	<u><i>S. oryzae</i>:</u>				
	0	20	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
	30	20	51.9 $\pm$ 6.2	60.3 $\pm$ 7.7	69.7 $\pm$ 6.0
	50	20	61.0 $\pm$ 6.8	65.7 $\pm$ 7.7	76.7 $\pm$ 4.4
	70	20	70.6 $\pm$ 4.3	74.0 $\pm$ 4.6	78.6 $\pm$ 4.8
	100	20	93.5 $\pm$ 2.5	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
	LSD _{0.05}	20	13.4	15.2	11.9
	<u><i>R. dominica</i>:</u>				
	0	20	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
	30	20	41.0 $\pm$ 4.8	53.2 $\pm$ 4.6	63.8 $\pm$ 4.5
	50	20	44.9 $\pm$ 5.8	69.3 $\pm$ 2.3	72.6 $\pm$ 3.0
	70	20	65.6 $\pm$ 1.6	76.0 $\pm$ 3.4	80.8 $\pm$ 3.3
	100	20	85.9 $\pm$ 4.8	85.9 $\pm$ 4.8	93.2 $\pm$ 2.6
	LSD _{0.05}	20	11.4	9.5	8.9
	<u><i>T. castaneum</i>:</u>				
	0	20	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
	30	20	40.0 $\pm$ 16.0	56.8 $\pm$ 1.4	58.4 $\pm$ 0.6
	50	20	47.5 $\pm$ 7.5	60.0 $\pm$ 1.0	62.5 $\pm$ 0.7
	70	20	57.5 $\pm$ 8.5	68.8 $\pm$ 0.7	72.5 $\pm$ 1.2
	100	20	75.7 $\pm$ 4.2	85.0 $\pm$ 1.0	88.4 $\pm$ 0.6
	LSD _{0.05}	20	16.8	6.7	6.5

For each insect, any two means in a column whose SE values overlap are not different at $\alpha = 0.05$ by LSD and Tukey's HSD test

Table 6.8: Variation of LC₅₀ values (µl/L air) for *O.americanum* leaf essential oil exposure duration (days) and post-fumigation time (hours) in wheat grain fumigation studies¹.

Exposure Duration/ Insect Species	N	LC ₅₀ values (µl/L air) based on fumigation duration and post-fumigation time (hours)					
		24		72		120	
		LC ₅₀	95%FL	LC ₅₀	95%FL	LC ₅₀	95%FL
<u>3 Days:</u>							
<i>S. oryzae</i>	20	78.94	(66.4, 104)	38.24	(23.5, 65.6)	35.06	(26.8, 40.7)
<i>R. dominica</i>	20	56.12	(38.8, 79.6)	34.64	(22.7, 60.9)	33.19	(24.5, 47.7)
<i>T. castaneum</i>	20	70.95	(48.6, 163)	65.39	(59.4, 72.7)	61.71	(56.0, 68.5)
<u>5 Days:</u>							
<i>S. oryzae</i>	20	53.51	(45.6, 61.9)	28.30	(19.9, 34.7)	25.83	(20.2, 30.9)
<i>R. dominica</i>	20	53.59	(36.6, 69.7)	25.28	(17.2, 33.9)	21.29	(14.7, 28.4)
<i>T. castaneum</i>	20	67.67	(29.8, 181)	62.04	(56.7, 68.2)	56.34	(51.3, 61.8)
<u>7 Days:</u>							
<i>S. oryzae</i>	20	32.80	(21.9, 49.8)	26.42	(22.8, 28.6)	19.94	(13.8, 24.3)
<i>R. dominica</i>	20	9.70	(0.94, 17.1)	7.20	(1.12, 12.8)	7.20	(1.12, 12.8)
<i>T. castaneum</i>	20	50.38	(35.8, 64.5)	45.09	(23.0, 62.9)	43.58	(25.2, 70.2)
<u>10 Days:</u>							
<i>S. oryzae</i>	20	26.75	(23.4, 29.0)	26.21	(20.6, 29.5)	19.79	(14.0, 23.9)
<i>R. dominica</i>	20	7.16	(0.30, 15.8)	5.21	(0.05, 13.6)	5.06	(0.03, 12.5)
<i>T. castaneum</i>	20	47.17	(12.6, 75.8)	31.53	(25.5, 39.4)	30.14	(26.7, 37.2)

¹Twenty unsexed adult test insects, in 4-6 replicates, were exposed to fumigant in wheat grains for a 3, 5, 7 and 10 days.

^aLC₅₀ values were considered significantly different when 95% fiducial limits did not overlap.

CHAPTER SEVEN

REPELLENT EFFECTS OF *ocimum americanum* L., *Lantana camara* L. AND *Tephrosia vogelii* HOOK ESSENTIAL OILS AGAINST FOUR COLEOPTERAN PESTS OF STORED FOOD GRAINS

Abstract

Laboratory studies were conducted to evaluate the instant and residual repellent activity of *O. americanum*, *L. camara* and *T. vogelii* essential oils and a monoterpene, eugenol, against adult *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis* insects. Each essential oil or constituent at four rates (0.5, 1.0, 1.5 and 2.0 $\mu\text{L/g}$ grain), Actellic 5EC (1.0 $\mu\text{L/g}$ grain), crude soya oil (10 $\mu\text{L/g}$ grain) and untreated control were evaluated against the four test insects in a CRD with 4 replicates per treatment. Essential oils obtained from leaves, succulent stems and fruits of *O. americanum*, *L. camara* and *T. vogelii* and eugenol were separately evaluated for instant repellence against adult stages of the four test insects. In the residual repellency bioassays, the fruit essential oils of *O. americanum* and *T. vogelii* were selectively evaluated against adult *S. oryzae*, *R. dominica* and *T. castaneum* whereas the leaf essential oils of the same plants were tested against adult *C. chinensis* over a 4-month storage duration. Results showed that instant repellency significantly ($P < 0.0001-0.025$) depended upon intra-species and inter-plant variations, exposure time, concentration, insect species and corresponding factor interactions. Irrespective of plant part assayed, exposure time and concentration, *L. camara* (PR value: 79.0-84.0%) was the most repellent against *S. oryzae* and *T. castaneum* followed by *T. vogelii* (PR value: 75.0- 76.0%) and *O. americanum* (PR value: 60.0- 72.0%), respectively. Against adult *R. dominica*, *T. vogelii* (PR value: 84.0%) was the most repellent oil followed by *L. camara* (PR value: 80.0%) and *O. americanum* (PR value: 66.0%). However, *O. americanum* oils were the most repellent (PR value: 83.0%) against adult *C. chinensis* followed by *L. camara* (PR value: 79.0%) and *T. vogelii* (PR value: 78.0%), respectively. Stem essential oils were the most repellent with PR values of 79.0- 88.0% (*S. oryzae*, *R. dominica*, *T. castaneum*) followed by leaf oil with PR value of 80.9% (*C. chinensis*) and fruit oils with PR values of 75.0- 80.0% for same three insects. The soya oil-treated grains had PR values of 93.0, 74.0 and 41.0% against adult *S. oryzae*, *T. castaneum* and *R. dominica*, respectively. In the residual choice bioassays, repellency

was significantly influenced ($P < 0.0001-0.048$) by inter-plant variations, storage duration, exposure time, concentration, insect species and corresponding factor interactions. Irrespective of storage duration, exposure time and concentration, *O. americanum* (PR value: 75.0%) and *T. vogelii* (PR value: 76.0%) were equally repellent against adult *S. oryzae* insects. However, *O. americanum* was more repellent against adult *R. dominica* and *T. castaneum* with PR values of 76.0- 80.0% compared to 67.0- 75.0% for *T. vogelii* against the two insects. Against adult *C. chinensis*, *T. vogelii* (PR value: 79.0%) was marginally more repellent than *O. americanum* (PR value: 77.0%). Except for *T. castaneum* against which the PR values remained unchanged over the 24 h exposure time (75.0- 78.0%), the repellency of the two botanical plants decreased with the duration of grain storage and exposure time against all insects. The PR values for soya oil-treated grains decreased from 79.0- 54.0 and 76.0- 61.0% against adult *S. oryzae* and *T. castaneum* but increased from 41.0- 79.0 and 56.0- 66.0% against adult *R. dominica* and *C. chinensis*, respectively, compared to 58.0- 46.0, 53.0- 44.0 and 69.0- 61.0% for Actellic 5EC against adult *S. oryzae*, *R. dominica* and *T. castaneum*, respectively. The strong instant and residual repellency of essential oils against coleopteran pests, under sealed storage, gives a glimmer of hope for their potential use as grain protectants.

7.1 Introduction

Aromatic plants, known to contain volatile compounds which give them an odour and characteristic flavour, have enormous socio-economic value in flavouring (salad or cooked food), cosmetics and perfumery, confectionery, aromatherapy and medicinal preparations (Magness *et al.*, 2006; Regnault-Roger, 1997; PFAF, 2000; Isman, 2006; NIIR, 2006). Essential oils are one of the fractions of aromatic plants most frequently used for industrial applications and has shown good promise for use in IPM (Isman, 2006). Essential oils are particularly abundant in some families of plants: Conifers, Rutaceae, Umbelliferae, Myrtaceae and Lamiaceae among others (Regnault-Roger *et al.*, 1993; Regnault-Roger, 1997).

Essential oils, naturally occurring substances, have a broad spectrum bioactivity due to the presence of several active ingredients with varied modes of action. In recent years, essential oils have attracted enormous attention as an alternative pest control option particularly due to their specificity to pests, biodegradable nature, potential for commercial application and ecosocio-

compatibility (Liu *et al.*, 2006). As part of an integrated stored-product protection, phytochemicals may be used for pest prevention and repelling pests of agricultural products (López *et al.*, 2008). An insect repellent is a chemical that acts in the vapour phase and prevents an insect from reaching a target to which it would otherwise be attracted (Murugan *et al.*, 2007). Repellents from plant origins are considered safe pest control agents due to their minimal pesticide residues, biosafety to man, environment and wildlife (Talukder and Howse, 1995; Talukder, 2006). Various plant extracts, powders and essential oils have been reported as repellent to different economically important insect pests of field and stored food commodities (Ogendo *et al.*, 2003b; Isman, 2006; Liu *et al.*, 2006; Ogendo *et al.*, 2008). Essential oils are generally composed of complex mixtures of monoterpenes, biogenetically related phenols and sesquiterpenes such as 1, 8-cineole, eugenol, thymol and menthol (Isman, 2006). Plant-based products have traditionally been used for protection of stored durable agricultural products in the tropics owing to their insecticidal and repellent properties (Bekele and Hassanali, 2001; Belmain *et al.*, 2001; Bouda *et al.*, 2001; Kéita *et al.*, 2001; Koona, 2005; Koona *et al.*, 2007a,b). Additionally, these products have shown useful repellence principles against biting insects such as mosquitoes (Dua *et al.*, 1996, 2003; Pålsson and Jaenson, 1999; Moore and Lenglet, 2004). Plants in the Lamiaceae, Fabaceae and Verbenaceae are among botanicals that have been used traditionally for generations in protection against biting insects and as grain protectants in storage (Pålsson and Jaenson, 1999; Papachristos and Stamopoulos, 2002a; Dua *et al.*, 2003; Ogendo *et al.* 2003a; Waka *et al.*, 2004; Erler *et al.*, 2006). Although the protection principle of these products is not well known, it is generally believed that they repel insects through their aromatic volatile essential oils and terpenoid constituents. Little or no local scientific interventions have pursued the use of essential oils as repellents of insect pests of stored food commodities. The objective of this study was to selectively evaluate the instant and residual repellent properties of essential oils extracted from aerial parts of *O. americanum*, *L. camara* and *T. vogelii* and a monoterpene constituent, eugenol, against adult stages of *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis*.

7.2 Materials and Methods

7.2.1 Mass rearing of test insects

The adult stages of *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis* insects used for contact toxicity and reproduction inhibition (F_1 progeny) studies were obtained from the insect cultures reared as described in Section 3.1 above.

7.2.2 Repellence studies (Choice bioassay)

(a) Instant Repellence

Essential oils extracted from aerial parts of *O. americanum*, *L. camara* and *T. vogelii* and an essential oil constituent, eugenol, were evaluated for instant repellent effects against the adult stages of *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis* in a choice bioassay system according to Ogendo *et al.* (2008). The base of a 14-cm diameter plastic Petri dish, lined with aluminium foil, was divided into four equal parts (Fig. 7.1) and 2.0 g whole/broken wheat (or 4.0 g chickpea for *C. chinensis*) grain samples placed in each quarter equidistant from the centre. Each essential oil or constituent, dissolved in 1 mL acetone, was evaluated at five rates (0, 0.5, 1.0, 1.5 and 2.0 $\mu\text{L/g}$) as an alternate untreated (control)-treated arrangement with four replicates per concentration.

Control treatments consisted of a no-choice all untreated and choice bioassays with acetone (0.5 $\mu\text{L/g}$), Actellic 5EC (1.0 $\mu\text{L/g}$) and crude soya oil (10.0 $\mu\text{L/g}$). The treated grains were kept for 1 h to allow acetone to evaporate completely. Twenty unsexed adult stages of each test insect were then released at the centre and the top secured by its plastic cover. The number of insects present in the control (N_C) and treated (N_T) grains were recorded after 1, 3, 5 and 24 h exposure. The choice bioassay tests were conducted in a room maintained at $30\pm 2^\circ\text{C}$, $68\pm 2\%$ RH and 12D: 12D (total darkness). Percent repellence (PR) values were computed according to Asawalam *et al.* (2006):

$$\text{PR} = \frac{(N_C - N_T)}{(N_C + N_T)} \times 100 \quad \text{Equation 3}$$

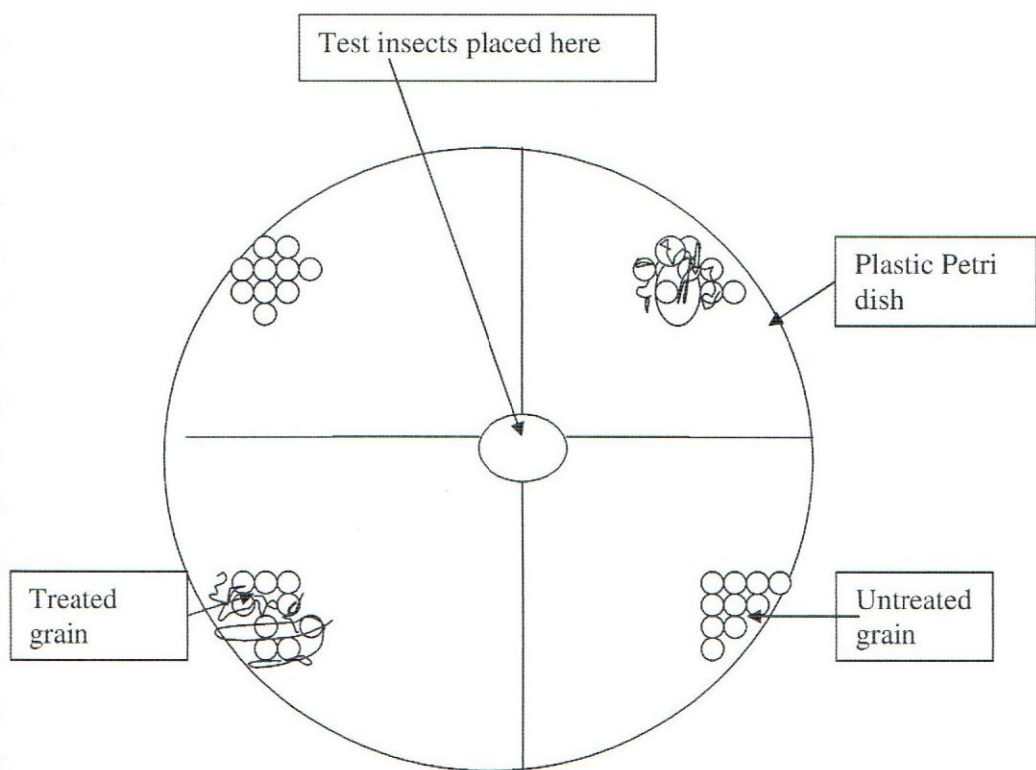


Fig. 7.1: An alternate untreated-treated choice bioassay system.

(b) Residual repellence (Choice bioassay)

The residual repellent effects of test essential oils against the adult stages of *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis* were evaluated over a 120-day storage period in a choice bioassay system as described in Section 6.2.2 (a) above. Each test essential oil, dissolved in acetone AR (99.8% GC), was applied to 20 g wheat (or 40 g chickpea) grain samples in special self-sealing polythene bags (20 cm x 25 cm; 2 Litre) at four rates (0.5, 1.0, 1.5 and 2.0 $\mu\text{L/g}$). The negative controls consisted of untreated and acetone (0.5 $\mu\text{L/g}$) treated grains whereas Actellic 5EC (1.0 $\mu\text{L/g}$) and crude soya oil (10.0 $\mu\text{L/g}$) were the positive controls. All treated grains were kept in fume chamber for 24 h to allow acetone to evaporate completely before being transferred into a room maintained at $30\pm 2^\circ\text{C}$, $68\pm 2\%$ RH and 12D: 12D (total darkness) for long term hermetic storage. Unbiased grain sub-samples (2 g wheat and 4 g chickpea) were then drawn from each experimental unit containing treated grains 30, 60, 90 and 120 days after treatment. Each essential oil concentration and controls were then assayed for repellence in an alternate untreated (control)-treated arrangement with four replicates per concentration as described in section 6.2.2 (a) above. Twenty unsexed adult stages of each test insect were released at the centre and the top secured by its plastic cover. The number of insects present in the control (N_C) and treated (N_T) grains were recorded after 1, 3, 5 and 24 h exposure. The choice bioassay tests were conducted in a room maintained at $30\pm 2^\circ\text{C}$, $68\pm 2\%$ RH and 12D: 12D (total darkness). Percent repellence (PR) values were computed as described in Equation 3 in Section 6.2.2 (a) above.

7.3 Results and Discussion

7.3.1 Instant Repellence

Instant repellent activity of essential oils against adult insects were significantly influenced ($P < 0.0001 - 0.025$) by intra-species and inter-plant variations in composition, exposure time, concentration of essential oil, insect species and corresponding factor interactions except plant species by plant part (*S. oryzae*), plant part by exposure time (*S. oryzae*, *T. castaneum*, *C. chinensis*), exposure time by concentration (all four test insects) and plant part by concentration (*T. castaneum*, *C. chinensis*). Irrespective of plant part assayed, exposure time and concentration of essential oil, *L. camara* (PR value: 79.0%) was the most repellent against adult *S. oryzae*

followed by *T. vogelii* (PR value: 75.0%) and *O. americanum* (PR value: 72.0%), respectively (Fig. 7.2-7.4; Tables 7.1-7.4). The same response trend was observed in adult *T. castaneum* insects with PR values of 84.0, 76.0 and 60.0% for *L. camara*, *T. vogelii* and *O. americanum* oils, respectively. Against adult *R. dominica* insects, *T. vogelii* (PR value: 84.0%) was the most repellent oil followed by *L. camara* (PR value: 80.0%) and *O. americanum* (PR value: 66.0%). However, *O. americanum* oils were the most repellent (PR value: 83.0%) against adult *C. chinensis* followed by *L. camara* (PR value: 79.0%) and *T. vogelii* (PR value: 78.0%), respectively. Essential oils extracted from succulent stems, irrespective of plant species, were the most repellent with PR values of 79.0- 88.0% (*S. oryzae*, *R. dominica*, *T. castaneum*) followed by fruit oils (PR values: 75.0- 80.0% against *S. oryzae*, *R. dominica*, *T. castaneum*) and leaf oils (PR value: 80.9% against adult *C. chinensis*). The soya oil-treated grains produced PR values of 41.0- 93.0% after 24 h exposure, with strongest repellence observed against *S. oryzae* (93.0%) and *T. castaneum* (74.0%) and least repellence against *R. dominica* (41.0%) (Fig.7.2; Table 7.1). PR values for acetone- and untreated-controls were zero and hence excluded from the results.

The results showed the existence of marked dose-dependent intra-specific variations in the instant repellent activity of essential oils obtained from the aerial parts of *O. americanum* against adult *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis*. Although complete factorial combination of plant part and test insects were not evaluated, essential oil extracted from the fruits showed the highest repellence against adult *S. oryzae* and *R. dominica* with PR values of 81.0 and 94.0%, respectively, at the highest concentration (2.0 μ L/g grain) and 24 h exposure compared to 70.0 and 74.0%, respectively, for the leaf oil (Fig. 7.2; Table 7.1). Under the same conditions, the leaf oil had PR values of 51.0 and 83.0% against adult *T. castaneum* and *C. chinensis*, respectively. However, the leaf oil produced PR values that decreased with dosage and a corresponding dose-dependent mortality of adult *T. castaneum* on treated wheat grains. An essential oil constituent, eugenol, was strongly repellent with PR values of 100, 65 and 100% against adult *S. oryzae*, *R. dominica* and *T. castaneum* insects, respectively, at 2.0 μ L/g grain and 24 h exposure. Against adult *C. chinensis* insects, eugenol produced dose-dependent decreasing PR values (83.0-45.0%) and a corresponding dose-dependent increasing mortality on treated chickpea grains.

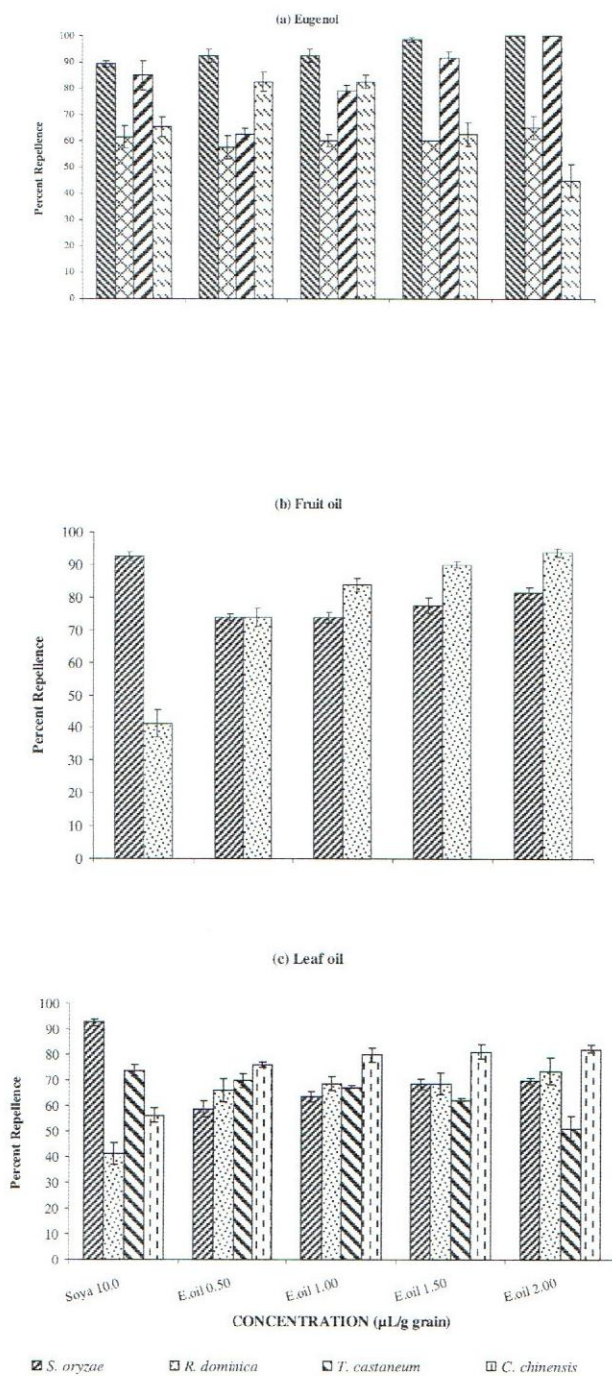


Fig.7.2: End-point mean percent repellence ($\pm$ SE, n=4) after 24 h exposure of four storage insects to (a) eugenol, an essential oil constituent and essential oils extracted from (b) fruits and (c) leaves of *O. americanum* L.

Table 7.1: Mean percent repellence ($\pm$ SE, n=4) of *Ocimum americanum* essential oils against four coleopteran pests of stored food grains^a

Essential oil treatment (μ l/g grain)		N	Percent Repellence (PR%) (Mean $\pm$ SE)			
			Exposure Time (Hours) after treatment with essential oils			
			1	3	5	24
LEAF ESSENTIAL OIL:						
<u><i>S. oryzae</i>:</u>						
Soya oil	10.00	20	81.3 $\pm$ 5.1	91.9 $\pm$ 4.6	93.2 $\pm$ 3.5	92.6 $\pm$ 2.8
E. oil	0.50	20	66.3 $\pm$ 2.4	65.0 $\pm$ 7.9	63.8 $\pm$ 5.5	58.8 $\pm$ 6.3
	1.00	20	67.5 $\pm$ 7.8	68.8 $\pm$ 7.5	67.5 $\pm$ 2.5	63.8 $\pm$ 4.3
	1.50	20	70.0 $\pm$ 8.4	68.8 $\pm$ 10.1	67.5 $\pm$ 6.0	68.8 $\pm$ 4.3
	2.00	20	72.5 $\pm$ 1.4	70.0 $\pm$ 8.4	71.3 $\pm$ 3.8	70.0 $\pm$ 2.0
	LSD _{0.05}		14.2	11.3	7.1	8.6
<u><i>R. dominica</i>:</u>						
Soya oil	10.00	20	55.0 $\pm$ 8.5	53.2 $\pm$ 9.0	52.6 $\pm$ 7.9	41.3 $\pm$ 8.4
E. oil	0.50	20	51.3 $\pm$ 2.4	51.3 $\pm$ 5.5	56.3 $\pm$ 3.1	66.3 $\pm$ 9.2
	1.00	20	51.3 $\pm$ 2.9	52.5 $\pm$ 3.2	66.3 $\pm$ 6.6	68.8 $\pm$ 5.2
	1.50	20	55.0 $\pm$ 10.8	57.5 $\pm$ 12.7	68.8 $\pm$ 4.3	68.8 $\pm$ 8.5
	2.00	20	55.0 $\pm$ 6.5	75.0 $\pm$ 6.8	71.3 $\pm$ 12.3	73.8 $\pm$ 10.1
	LSD _{0.05}		16.7	18.8	18.5	9.8
<u><i>T. castaneum</i>:</u>						
Soya oil	10.00	20	85.0 $\pm$ 5.4	73.8 $\pm$ 5.2	80.0 $\pm$ 7.4	73.8 $\pm$ 4.7
E. oil	0.50	20	48.8 $\pm$ 6.6	61.3 $\pm$ 6.3	68.8 $\pm$ 4.3	70.0 $\pm$ 5.4
	1.00	20	55.0 $\pm$ 12.6	53.8 $\pm$ 7.9	66.3 $\pm$ 10.7	67.5 $\pm$ 1.4
	1.50	20	56.3 $\pm$ 4.3	46.3 $\pm$ 10.7	46.3 $\pm$ 10.7	62.5 $\pm$ 1.4
	2.00	20	57.5 $\pm$ 14.5	37.5 $\pm$ 9.9	37.5 $\pm$ 9.9	51.3 $\pm$ 9.4
	LSD _{0.05}					
<u><i>C. chinensis</i>:</u>						
Soya oil	10.00	20	65.0 $\pm$ 3.5	68.8 $\pm$ 6.3	63.8 $\pm$ 4.3	56.3 $\pm$ 5.5
E. oil	0.50	20	88.8 $\pm$ 6.6	91.3 $\pm$ 3.8	86.3 $\pm$ 4.7	76.3 $\pm$ 2.4
	1.00	20	90.0 $\pm$ 4.6	92.5 $\pm$ 3.2	86.3 $\pm$ 3.8	80.0 $\pm$ 5.4
	1.50	20	91.3 $\pm$ 3.8	95.0 $\pm$ 3.5	87.5 $\pm$ 5.2	81.3 $\pm$ 5.5
	2.00	20	97.5 $\pm$ 1.4	95.0 $\pm$ 2.9	90.0 $\pm$ 4.6	82.5 $\pm$ 2.5
	LSD _{0.05}		10.4	6.9	3.1	6.4
FRUIT ESSENTIAL OIL:						
<u><i>S. oryzae</i>:</u>						
Soya oil	10.00	20	81.3 $\pm$ 5.1	91.9 $\pm$ 4.6	93.2 $\pm$ 3.5	92.6 $\pm$ 2.8
E. oil	0.50	20	82.5 $\pm$ 5.2	78.8 $\pm$ 4.3	77.5 $\pm$ 2.5	73.8 $\pm$ 2.4
	1.00	20	83.8 $\pm$ 4.3	80.0 $\pm$ 3.5	82.5 $\pm$ 7.8	73.8 $\pm$ 3.1
	1.50	20	83.8 $\pm$ 4.7	80.0 $\pm$ 3.7	82.5 $\pm$ 5.2	77.5 $\pm$ 4.8
	2.00	20	92.5 $\pm$ 1.4	85.0 $\pm$ 1.6	88.8 $\pm$ 5.2	81.3 $\pm$ 3.1
	LSD _{0.05}		7.7	5.4	10.3	4.7
<u><i>R. dominica</i>:</u>						
Soya oil	10.00	20	55.0 $\pm$ 8.5	53.2 $\pm$ 9.0	52.6 $\pm$ 7.9	41.3 $\pm$ 8.4
E. oil	0.50	20	72.5 $\pm$ 4.3	77.5 $\pm$ 1.4	76.3 $\pm$ 2.4	73.8 $\pm$ 5.5
	1.00	20	81.3 $\pm$ 3.8	81.3 $\pm$ 3.1	83.8 $\pm$ 2.4	83.8 $\pm$ 4.3
	1.50	20	87.5 $\pm$ 4.3	82.5 $\pm$ 3.2	87.5 $\pm$ 1.4	90.0 $\pm$ 2.0
	2.00	20	92.5 $\pm$ 1.4	90.0 $\pm$ 2.0	90.0 $\pm$ 2.0	93.8 $\pm$ 2.4
	LSD _{0.05}		5.9	3.6	4.8	4.6

^aPercent repellence in an alternate untreated (control) – treated choice bioassay.

For each insect, any two means in a column whose SE values overlap are not different at $\alpha = 0.05$ by LSD and Tukey's HSD test

Table 7.2: Mean percent repellence ($\pm$ SE, n=4) of eugenol, an essential oil constituent against four coleopteran pests of stored food grains^a

Eugenol concentration (μ l/g grain)	N	Percent Repellence (PR%) (Mean $\pm$ SE)			
		Exposure Time (Hours) after treatment with essential oils			
		1	3	5	24
<u>S. oryzae:</u>					
Soya oil 10.00	20	81.3 $\pm$ 5.1	91.9 $\pm$ 4.6	93.2 $\pm$ 3.5	92.6 $\pm$ 2.8
Eugenol 0.50	20	82.5 $\pm$ 4.8	95.0 $\pm$ 5.0	95.0 $\pm$ 3.0	92.5 $\pm$ 3.3
1.00	20	87.5 $\pm$ 4.8	95.0 $\pm$ 2.9	97.5 $\pm$ 1.5	92.5 $\pm$ 2.9
1.50	20	90.0 $\pm$ 4.1	95.0 $\pm$ 2.9	97.5 $\pm$ 1.5	98.5 $\pm$ 0.9
2.00	20	90.0 $\pm$ 7.1	97.5 $\pm$ 2.5	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
LSD _{0.05}		5.9	5.1	7.1	6.7
<u>R. dominica:</u>					
Soya oil 10.00	20	55.0 $\pm$ 8.5	53.2 $\pm$ 9.0	52.6 $\pm$ 7.9	41.3 $\pm$ 8.4
Eugenol 0.50	20	62.5 $\pm$ 4.8	55.0 $\pm$ 6.5	57.5 $\pm$ 4.8	57.5 $\pm$ 8.6
1.00	20	65.0 $\pm$ 2.9	57.5 $\pm$ 8.6	60.0 $\pm$ 4.1	60.0 $\pm$ 4.1
1.50	20	67.5 $\pm$ 6.3	65.0 $\pm$ 6.5	62.5 $\pm$ 4.8	60.0 $\pm$ 4.3
2.00	20	72.5 $\pm$ 6.3	70.0 $\pm$ 4.1	70.0 $\pm$ 4.1	65.0 $\pm$ 8.7
LSD _{0.05}		6.8	9.7	7.6	9.1
<u>T. castaneum:</u>					
Soya oil 10.00	20	85.0 $\pm$ 5.4	73.8 $\pm$ 5.2	80.0 $\pm$ 7.4	73.8 $\pm$ 4.7
Eugenol 0.50	20	75.0 $\pm$ 4.4	91.7 $\pm$ 3.4	79.2 $\pm$ 5.5	62.5 $\pm$ 4.2
1.00	20	79.2 $\pm$ 4.0	95.9 $\pm$ 2.4	83.4 $\pm$ 1.9	79.2 $\pm$ 4.2
1.50	20	95.9 $\pm$ 2.2	100.0 $\pm$ 0.0	91.7 $\pm$ 5.4	91.7 $\pm$ 4.8
2.00	20	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	95.9 $\pm$ 2.2	100.0 $\pm$ 0.0
LSD _{0.05}		10.7	9.7	10.9	9.6
<u>C. chinensis:</u>					
Soya oil 10.00	20	65.0 $\pm$ 3.5	68.8 $\pm$ 6.3	63.8 $\pm$ 4.3	56.3 $\pm$ 5.5
Eugenol 0.50	20	92.5 $\pm$ 4.8	95.0 $\pm$ 2.9	95.0 $\pm$ 2.9	82.5 $\pm$ 6.3
1.00	20	87.5 $\pm$ 6.3	95.0 $\pm$ 2.9	92.5 $\pm$ 2.5	82.5 $\pm$ 4.8
1.50	20	85.0 $\pm$ 2.9	85.0 $\pm$ 5.0	87.5 $\pm$ 2.5	62.5 $\pm$ 6.5
2.00	20	82.5 $\pm$ 4.8	80.0 $\pm$ 4.1	85.0 $\pm$ 1.9	45.0 $\pm$ 9.6
LSD _{0.05}		6.7	6.7	4.9	9.5

^aPercent repellence in an alternate untreated (control) – treated choice bioassay.

For each insect, any two means in a column whose SE values overlap are not different at $\alpha = 0.05$ by LSD and Tukey's HSD test

Results showed the existence of strong dose-dependent intra-specific variations in the instant repellent activity of essential oils obtained from leaves and succulent stems of *L. camara* against adult *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis* insects. At 2.0 $\mu\text{L/g}$ grain and 24 h exposure, stem essential oil had superior PR values of 90.0, 80.0 and 84.0% against adult *S. oryzae*, *T. castaneum* and *C. chinensis* insects, respectively, compared to 78.0, 73.0 and 69.0% for leaf essential oil against the same three insects, respectively. At the same concentration and exposure period, the leaf essential oil was more repellent with PR value of 85.0% against adult *C. chinensis* compared to 69.0% for the stem oil (Fig. 7.3; Table 7.2). However, the leaf oil produced dose-dependent decreasing PR values (84.0- 69.0%) and a corresponding dose-dependent increasing mortality of adult *C. chinensis* in treated chickpea grains.

Results of instant repellence studies with *T. vogelii* essential oils showed strong dose-dependent intra-species and inter-insect species variability against adult *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis* insects. *T. vogelii* fruit oil, at 2.0 $\mu\text{L/g}$ grain and 24 h exposure, were more repellent against *S. oryzae*, *R. dominica* and *T. castaneum* with PR values of 86.0, 93.0 and 95.0%, respectively, compared to 71.0, 88.0 and 71.0% for the leaf oil against the same insects (Fig. 7.4; Table 7.3). Plant part by insect species interaction was evident when, the leaf essential oil at same concentration and exposure was more repellent against adult *C. chinensis* (PR value: 94.0%) than the fruit oil (PR value: 75.0%). Irrespective of plant parts assayed and at highest concentration (2.0 $\mu\text{L/g}$ grain) and 24 h exposure, *T. vogelii* essential oils were the most repellent (PR values: 71.0- 95.0%) followed by *O. americanum* (PR values: 51.0- 94.0%) and *L. camara* (PR values: 69.0- 85.0%), respectively. These results provide scientific basis for preferential targeted exploitation of these plant volatiles as repellents of storage insect pests.

7.3.2 Residual Repellence

Results showed that residual repellence of essential oils against adult insects were significantly influenced ($P < 0.0001$ -0.0484) by inter-plant variations, storage duration, exposure time, concentration, insect species and corresponding factor interactions except plant species by exposure time (all insects tested), plant species by concentration (*S. oryzae*, *T. castaneum*, *C. chinensis*), storage duration by exposure time (*R. dominica*, *T. castaneum*, *C. chinensis*), exposure time (*T. castaneum*) and storage duration by concentration (*T. castaneum*). Due to

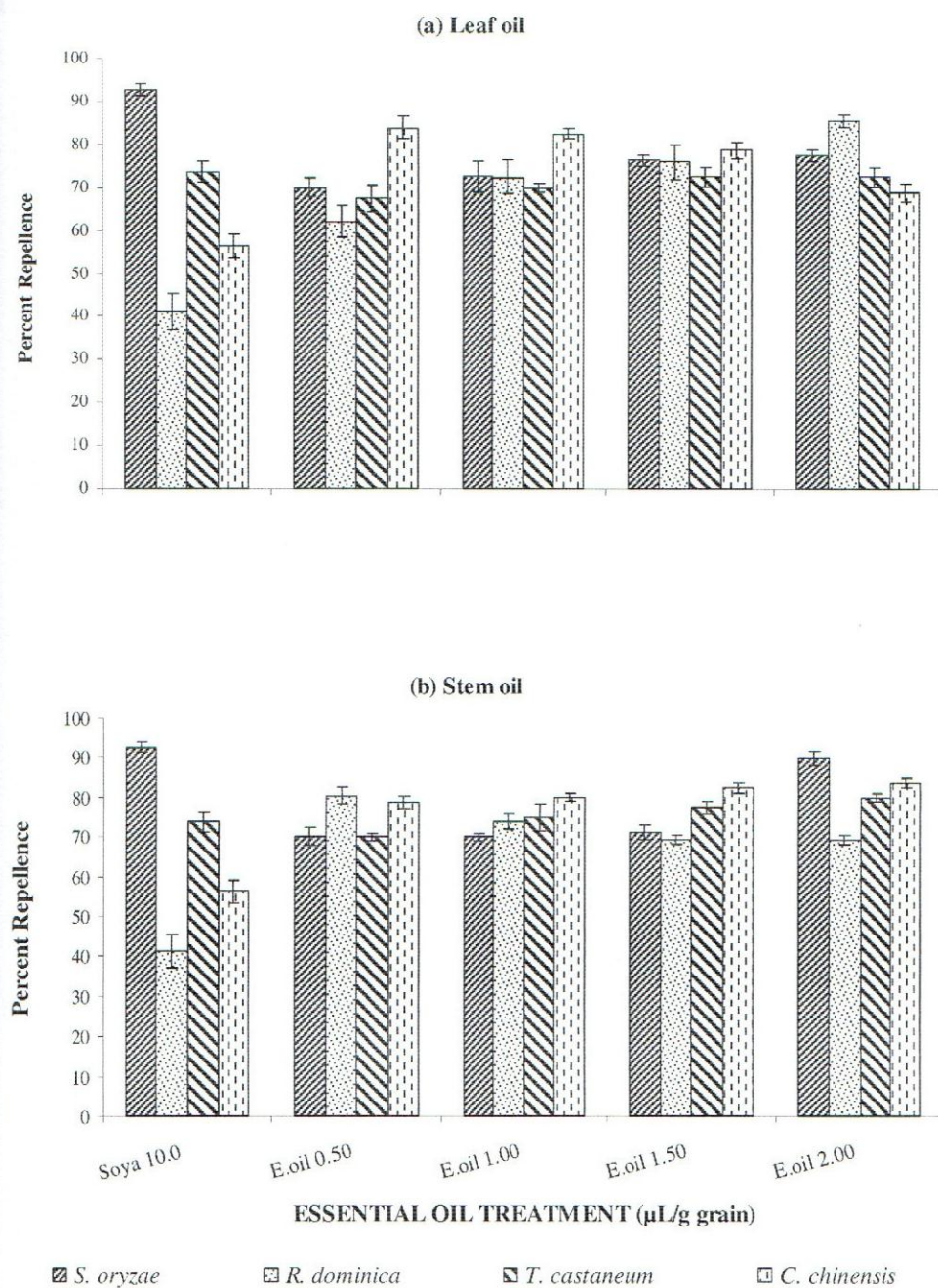


Fig.7.3: End-point mean percent repellence ($\pm\text{SE}$, $n=4$) after 24 h exposure of four storage insects to essential oils extracted from (a) leaves and (b) stems of *L. camara* L.

Table 7.3 (a): Mean percent repellence ($\pm$ SE, n=4) of *Lantana camara* leaf essential oil against four coleopteran pests of stored food grains^a

Essential oil treatment (μ l/g grain)/ Insect species			Percent Repellence (PR%) (Mean $\pm$ SE) Exposure Time (Hours) after treatment with essential oils			
N			1	3	5	24
<u><i>S. oryzae</i>:</u>						
Soya oil	10.00	20	81.3 $\pm$ 5.1	91.9 $\pm$ 4.6	93.2 $\pm$ 3.5	92.6 $\pm$ 2.8
E. oil	0.50	20	81.3 $\pm$ 5.2	64.3 $\pm$ 7.9	68.8 $\pm$ 5.5	70.0 $\pm$ 4.6
	1.00	20	82.5 $\pm$ 3.2	71.3 $\pm$ 3.1	75.0 $\pm$ 5.0	72.5 $\pm$ 7.2
	1.50	20	82.5 $\pm$ 6.0	78.8 $\pm$ 3.1	76.3 $\pm$ 5.2	76.3 $\pm$ 2.4
	2.00	20	86.3 $\pm$ 4.3	83.8 $\pm$ 1.3	78.8 $\pm$ 6.9	77.5 $\pm$ 2.5
	LSD _{0.05}		5.7	13.1	6.9	9.6
<u><i>R. dominica</i>:</u>						
Soya oil	10.00	20	55.0 $\pm$ 8.5	53.2 $\pm$ 9.0	52.6 $\pm$ 7.9	41.3 $\pm$ 8.4
E. oil	0.50	20	73.4 $\pm$ 5.2	67.7 $\pm$ 9.7	63.0 $\pm$ 6.6	62.0 $\pm$ 7.2
	1.00	20	78.1 $\pm$ 4.6	73.4 $\pm$ 3.7	71.9 $\pm$ 3.1	72.4 $\pm$ 8.0
	1.50	20	84.9 $\pm$ 6.2	86.5 $\pm$ 5.2	80.7 $\pm$ 7.7	76.0 $\pm$ 8.1
	2.00	20	96.9 $\pm$ 3.1	89.6 $\pm$ 4.4	91.2 $\pm$ 3.8	85.4 $\pm$ 2.8
	LSD _{0.05}		10.8	12.1	9.6	11.2
<u><i>T. castaneum</i>:</u>						
Soya oil	10.00	20	85.0 $\pm$ 5.4	73.8 $\pm$ 5.2	80.0 $\pm$ 7.4	73.8 $\pm$ 4.7
E. oil	0.50	20	80.0 $\pm$ 2.9	83.8 $\pm$ 2.4	72.5 $\pm$ 7.8	67.5 $\pm$ 5.9
	1.00	20	81.3 $\pm$ 5.5	85.0 $\pm$ 3.5	81.3 $\pm$ 5.5	70.0 $\pm$ 2.0
	1.50	20	86.3 $\pm$ 2.4	85.0 $\pm$ 3.5	86.3 $\pm$ 4.7	72.5 $\pm$ 4.5
	2.00	20	90.0 $\pm$ 4.1	87.5 $\pm$ 5.2	93.8 $\pm$ 3.8	72.5 $\pm$ 4.7
	LSD _{0.05}		6.3	5.7	7.9	7.7
<u><i>C. chinensis</i>:</u>						
Soya oil	10.00	20	65.0 $\pm$ 3.5	68.8 $\pm$ 6.3	63.8 $\pm$ 4.3	56.3 $\pm$ 5.5
E. oil	0.50	20	85.0 $\pm$ 4.1	90.0 $\pm$ 2.0	88.8 $\pm$ 5.2	83.8 $\pm$ 5.2
	1.00	20	81.3 $\pm$ 5.8	86.3 $\pm$ 2.4	85.0 $\pm$ 2.9	82.5 $\pm$ 2.5
	1.50	20	78.8 $\pm$ 3.8	80.0 $\pm$ 4.6	83.8 $\pm$ 5.5	78.8 $\pm$ 3.8
	2.00	20	76.3 $\pm$ 4.3	78.8 $\pm$ 2.4	77.5 $\pm$ 5.2	68.8 $\pm$ 4.3
	LSD _{0.05}		4.7	8.6	5.3	6.1

^aPercent repellence in an alternate untreated (control) – treated choice bioassay
For each insect, any two means in a column whose SE values overlap are not different at $\alpha = 0.05$ by LSD and Tukey's HSD test

Table 7.3. (b): Mean percent repellence ($\pm$ SE, n=4) of *Lantana camara* stem essential oil against four coleopteran pests of stored food grains^a

Essential oil treatment (μ l/g grain)/ Insect species			Percent Repellence (PR%) (Mean $\pm$ SE) Exposure Time (Hours) after treatment with essential oils			
	N		1	3	5	24
<u>S. oryzae:</u>						
Soya oil 10.00	20		81.3 $\pm$ 5.1	91.9 $\pm$ 4.6	93.2 $\pm$ 3.5	92.6 $\pm$ 2.8
E. oil 0.50	20		81.3 $\pm$ 6.3	66.5 $\pm$ 7.9	75.0 $\pm$ 5.4	70.0 $\pm$ 4.6
1.00	20		83.8 $\pm$ 1.3	76.3 $\pm$ 3.8	77.5 $\pm$ 3.2	70.0 $\pm$ 2.0
1.50	20		85.0 $\pm$ 5.4	81.3 $\pm$ 3.1	77.5 $\pm$ 4.3	71.3 $\pm$ 3.8
2.00	20		90.0 $\pm$ 2.9	82.5 $\pm$ 6.0	78.8 $\pm$ 3.1	90.0 $\pm$ 3.5
LSD _{0.05}			10.1	9.6	4.7	5.3
<u>R. dominica:</u>						
Soya oil 10.00	20		55.0 $\pm$ 8.5	53.2 $\pm$ 9.0	52.6 $\pm$ 7.9	41.3 $\pm$ 8.4
E. oil 0.50	20		96.3 $\pm$ 2.4	87.5 $\pm$ 7.2	87.9 $\pm$ 7.4	80.4 $\pm$ 3.9
1.00	20		96.7 $\pm$ 2.0	93.3 $\pm$ 3.5	92.5 $\pm$ 3.5	73.8 $\pm$ 3.6
1.50	20		96.7 $\pm$ 2.0	95.9 $\pm$ 4.1	92.9 $\pm$ 2.4	69.2 $\pm$ 2.2
2.00	20		97.9 $\pm$ 2.0	98.8 $\pm$ 1.1	98.7 $\pm$ 1.1	69.2 $\pm$ 2.2
LSD _{0.05}			12.8	15.6	13.4	12.3
<u>T. castaneum:</u>						
Soya oil 10.00	20		85.0 $\pm$ 5.4	73.8 $\pm$ 5.2	80.0 $\pm$ 7.4	73.8 $\pm$ 4.7
E. oil 0.50	20		81.3 $\pm$ 3.1	83.8 $\pm$ 4.3	68.8 $\pm$ 6.3	70.0 $\pm$ 2.0
1.00	20		87.5 $\pm$ 3.2	85.0 $\pm$ 2.0	77.5 $\pm$ 5.2	75.0 $\pm$ 6.8
1.50	20		91.3 $\pm$ 4.3	87.5 $\pm$ 4.3	81.3 $\pm$ 3.1	77.5 $\pm$ 3.2
2.00	20		95.0 $\pm$ 2.0	87.5 $\pm$ 1.4	82.5 $\pm$ 6.0	80.0 $\pm$ 2.0
LSD _{0.05}			6.9	7.6	8.5	9.6
<u>C. chinensis:</u>						
Soya oil 10.00	20		65.0 $\pm$ 3.5	68.8 $\pm$ 6.3	63.8 $\pm$ 4.3	56.3 $\pm$ 5.5
E. oil 0.50	20		77.5 $\pm$ 4.2	73.8 $\pm$ 4.3	81.3 $\pm$ 5.5	78.8 $\pm$ 3.1
1.00	20		80.0 $\pm$ 2.0	77.5 $\pm$ 1.4	82.5 $\pm$ 4.3	80.0 $\pm$ 2.0
1.50	20		82.5 $\pm$ 3.2	78.8 $\pm$ 3.8	82.5 $\pm$ 4.3	82.5 $\pm$ 2.5
2.00	20		85.0 $\pm$ 4.1	86.3 $\pm$ 2.4	83.8 $\pm$ 2.1	83.8 $\pm$ 2.1
LSD _{0.05}			4.5	9.7	6.9	7.1

^aPercent repellence in an alternate untreated (control) – treated choice bioassay

For each insect, any two means in a column whose SE values overlap are not different at $\alpha = 0.05$ by LSD and Tukey's HSD test

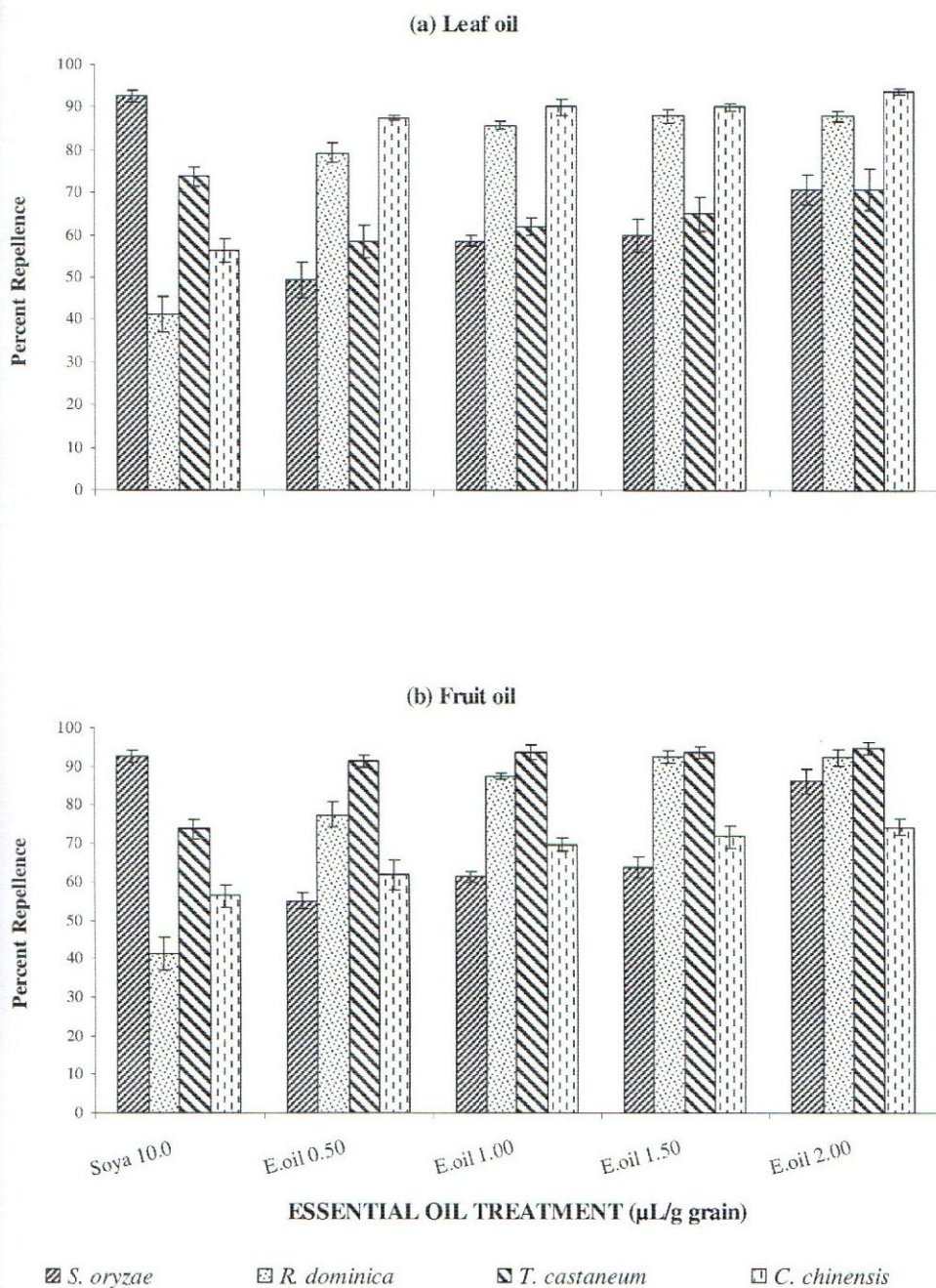


Fig.7.4: End-point mean percent repellence ($\pm\text{SE}$, $n=4$) after 24 h exposure of four storage insects to essential oils extracted from (a) leaves and (b) fruits of *T. vogelii* Hook.

Table 7.4. (a): Mean percent repellence ($\pm$ SE, n=4) of *Tephrosia vogelii* leaf essential oil against four coleopteran pests of stored food grains^a

Essential oil treatment (μ l/g grain)/ Insect species		N	Percent Repellence (PR%) (Mean $\pm$ SE) Exposure Time (Hours) after treatment with essential oils			
			1	3	5	24
<i>S. oryzae</i>:						
Soya oil	10.00	20	81.3 $\pm$ 5.1	91.9 $\pm$ 4.6	93.2 $\pm$ 3.5	92.6 $\pm$ 2.8
E. oil	0.50	20	72.5 $\pm$ 12.7	80.0 $\pm$ 9.7	63.8 $\pm$ 10.0	49.3 $\pm$ 8.3
	1.00	20	77.5 $\pm$ 12.7	80.0 $\pm$ 4.6	73.8 $\pm$ 4.7	58.6 $\pm$ 2.4
	1.50	20	82.5 $\pm$ 8.8	82.5 $\pm$ 4.3	76.3 $\pm$ 9.4	60.0 $\pm$ 7.7
	2.00	20	85.0 $\pm$ 11.7	83.8 $\pm$ 4.3	83.8 $\pm$ 8.4	70.7 $\pm$ 7.1
	LSD _{0.05}		15.1	10.9	12.8	11.7
<i>R. dominica</i>:						
Soya oil	10.00	20	55.0 $\pm$ 8.5	53.2 $\pm$ 9.0	52.6 $\pm$ 7.9	41.3 $\pm$ 8.4
E. oil	0.50	20	83.8 $\pm$ 3.1	75.0 $\pm$ 6.1	81.3 $\pm$ 6.7	79.3 $\pm$ 4.6
	1.00	20	86.3 $\pm$ 3.8	85.0 $\pm$ 3.5	83.8 $\pm$ 2.4	85.7 $\pm$ 2.0
	1.50	20	90.0 $\pm$ 2.2	85.0 $\pm$ 4.1	85.0 $\pm$ 2.9	87.9 $\pm$ 3.1
	2.00	20	90.0 $\pm$ 3.5	86.3 $\pm$ 3.8	91.3 $\pm$ 2.4	87.9 $\pm$ 2.5
	LSD _{0.05}		12.4	10.9	10.9	12.6
<i>T. castaneum</i>:						
Soya oil	10.00	20	85.0 $\pm$ 5.4	73.8 $\pm$ 5.2	80.0 $\pm$ 7.4	73.8 $\pm$ 4.7
E. oil	0.50	20	72.5 $\pm$ 12.7	80.3 $\pm$ 7.4	68.3 $\pm$ 7.9	58.6 $\pm$ 7.8
	1.00	20	77.5 $\pm$ 12.7	80.7 $\pm$ 4.3	74.7 $\pm$ 4.7	62.1 $\pm$ 4.2
	1.50	20	82.5 $\pm$ 8.8	82.5 $\pm$ 4.3	77.5 $\pm$ 7.5	65.0 $\pm$ 8.2
	2.00	20	85.0 $\pm$ 11.7	83.8 $\pm$ 2.3	85.8 $\pm$ 9.4	70.7 $\pm$ 10.1
	LSD _{0.05}		14.5	10.1	9.4	11.7
<i>C. chinensis</i>:						
Soya oil	10.00	20	65.0 $\pm$ 3.5	68.8 $\pm$ 6.3	63.8 $\pm$ 4.3	56.3 $\pm$ 5.5
E. oil	0.50	20	88.8 $\pm$ 2.4	90.0 $\pm$ 2.0	90.0 $\pm$ 2.0	87.5 $\pm$ 1.4
	1.00	20	90.0 $\pm$ 3.5	91.3 $\pm$ 2.4	91.3 $\pm$ 2.4	90.0 $\pm$ 3.6
	1.50	20	92.5 $\pm$ 1.4	93.8 $\pm$ 1.3	92.5 $\pm$ 1.4	90.0 $\pm$ 2.0
	2.00	20	93.8 $\pm$ 1.3	95.0 $\pm$ 2.0	95.0 $\pm$ 2.0	93.8 $\pm$ 1.3
	LSD _{0.05}		4.4	9.9	5.8	8.3

^aPercent repellence in an alternate untreated (control) – treated choice bioassay

For each insect, any two means in a column whose SE values overlap are not different at $\alpha = 0.05$ by LSD and Tukey's HSD test

Table 7.4.(b): Mean percent repellence ($\pm$ SE, n=4) of *Tephrosia vogelii* fruit essential oil against four coleopteran pests of stored food grains^a

Essential oil treatment (μ l/g grain)/ Insect species			Percent Repellence (PR%) (Mean $\pm$ SE) Exposure Time (Hours) after treatment with essential oils			
		N	1	3	5	24
<u><i>S. oryzae</i>:</u>						
Soya oil	10.00	20	81.3 $\pm$ 5.1	91.9 $\pm$ 4.6	93.2 $\pm$ 3.5	92.6 $\pm$ 2.8
E. oil	0.50	20	75.0 $\pm$ 5.4	73.8 $\pm$ 4.3	70.0 $\pm$ 5.4	55.0 $\pm$ 4.1
	1.00	20	78.8 $\pm$ 8.5	75.0 $\pm$ 7.1	72.5 $\pm$ 3.2	61.3 $\pm$ 2.4
	1.50	20	90.0 $\pm$ 0.0	80.0 $\pm$ 3.5	77.5 $\pm$ 9.5	63.8 $\pm$ 5.2
	2.00	20	92.5 $\pm$ 1.4	82.5 $\pm$ 3.2	80.0 $\pm$ 4.1	86.3 $\pm$ 5.9
	LSD _{0.05}		16.7	7.7	12.4	7.1
<u><i>R. dominica</i>:</u>						
Soya oil	10.00	20	55.0 $\pm$ 8.5	53.2 $\pm$ 9.0	52.6 $\pm$ 7.9	41.3 $\pm$ 8.4
E. oil	0.50	20	91.3 $\pm$ 3.8	87.5 $\pm$ 5.2	83.8 $\pm$ 3.1	77.5 $\pm$ 6.6
	1.00	20	92.5 $\pm$ 4.8	88.8 $\pm$ 6.6	91.3 $\pm$ 3.1	87.5 $\pm$ 1.4
	1.50	20	93.8 $\pm$ 3.8	91.3 $\pm$ 3.1	93.8 $\pm$ 6.3	92.5 $\pm$ 3.2
	2.00	20	93.8 $\pm$ 3.8	93.8 $\pm$ 3.8	93.8 $\pm$ 3.8	92.5 $\pm$ 4.3
	LSD _{0.05}		9.3	11.7	9.6	13.9
<u><i>T. castaneum</i>:</u>						
Soya oil	10.00	20	85.0 $\pm$ 5.4	73.8 $\pm$ 5.2	80.0 $\pm$ 7.4	73.8 $\pm$ 4.7
E. oil	0.50	20	75.0 $\pm$ 5.4	76.3 $\pm$ 9.0	80.0 $\pm$ 7.1	91.3 $\pm$ 3.1
	1.00	20	77.5 $\pm$ 8.3	81.3 $\pm$ 8.3	86.3 $\pm$ 2.4	93.8 $\pm$ 3.8
	1.50	20	91.3 $\pm$ 1.3	87.5 $\pm$ 7.8	92.5 $\pm$ 3.2	93.8 $\pm$ 3.1
	2.00	20	93.8 $\pm$ 1.3	87.5 $\pm$ 6.6	92.5 $\pm$ 1.4	95.0 $\pm$ 2.9
	LSD _{0.05}		13.9	9.5	11.3	3.7
<u><i>C. chinensis</i>:</u>						
Soya oil	10.00	20	65.0 $\pm$ 3.5	68.8 $\pm$ 6.3	63.8 $\pm$ 4.3	56.3 $\pm$ 5.5
E. oil	0.50	20	72.5 $\pm$ 1.4	78.8 $\pm$ 2.4	73.3 $\pm$ 1.9	61.8 $\pm$ 7.5
	1.00	20	81.3 $\pm$ 4.3	80.0 $\pm$ 5.4	79.0 $\pm$ 2.3	69.8 $\pm$ 3.4
	1.50	20	82.5 $\pm$ 4.3	81.3 $\pm$ 2.4	81.0 $\pm$ 4.0	71.8 $\pm$ 6.1
	2.00	20	83.8 $\pm$ 3.1	83.8 $\pm$ 6.6	83.8 $\pm$ 5.5	74.5 $\pm$ 4.1
	LSD _{0.05}		5.9	8.3	7.2	8.1

^aPercent repellence in an alternate untreated (control) – treated choice bioassay

For each insect, any two means in a column whose SE values overlap are not different at $\alpha = 0.05$ by LSD and Tukey's HSD test

unavoidable lack of some test insects and essential oils, full factorial combinations of plant species, plant parts and duration of storage on test insect species were not studied. Irrespective of storage duration, exposure time and concentration, *O. americanum* (PR value: 75.0%) and *T. vogelii* (PR value: 76.0%) were equally repellent against adult *S. oryzae*. However, *O. americanum* was more repellent against adult *R. dominica* and *T. castaneum* with PR values of 76.0 and 80.0%, respectively compared to 67.0 and 75.0% for *T. vogelii* against the same insects, respectively. *T. vogelii* (PR value: 79.0%) marginally repellent more *C. chinensis* than *O. americanum* (PR value: 77.0%). Except for *T. castaneum* against which the PR values remained unchanged over the 24 h exposure time (75.0- 78.0%), the repellency of the two botanical plants decreased with the duration of grain storage and exposure time against all insects.

Results showed existence of concentration-, storage duration- and exposure time-dependent residual repellent activity of *O. americanum* and *T. vogelii* fruit oils against adult *S. oryzae*, *R. dominica* and *T. castaneum* and their leaf oils against adult *C. chinensis*. The PR values for the *O. americanum* fruit oil (at 2.0 μ L/g grain and 24 h exposure), against adult *S. oryzae*, *R. dominica* and *T. castaneum* decreased from 73.0- 63.0, 94.0- 72.0 and 99.0- 96.0%, respectively, over a 120-day storage period (Fig.7.5; Table 7.5). The same result trends were observed with *T. vogelii* fruit oil in which PR values decreased from 86.0- 71.0, 84.0- 73.0 and 89.0- 79.0% against the same insects, respectively (Fig. 7.6; Table 7.6). Similarly, at 2.0 μ L/g grain and 24 h exposure, the PR values for essential oil extracted from leaves of *O. americanum* (Fig. 7.6; Table 6.5) and *T. vogelii* (Fig. 7.7; Table 7.6) against adult *C. chinensis* insects decreased from 85.0- 77.5 and 93.8-86.3%, respectively, after 60-90 days grain storage. The soya oil- and Actellic 5EC-treated grains produced varying PR values against the four test insects that decreased with storage duration and exposure time (Table 7.7). In the soya oil-treated grains, the PR values after 24 h exposure decreased from 79.0- 54.0 and 76.0- 61.0% against adult *S. oryzae* and *T. castaneum* but increased from 41.0- 79.0 and 56.0- 66.0% against adult *R. dominica* and *C. chinensis*, respectively, over the 120 days grain storage duration. In the Actellic 5EC-treated grains, the PR values after 24 h exposure decreased from 57.5-45.8, 52.5-43.8 and 68.8-61.3% against adult *S. oryzae*, *R. dominica* and *T. castaneum*, respectively.

Although the intra-species and inter-plant comparisons of instant and residual bioassays have revealed differential temporal decreases in repellency, all essential oils tested maintained fairly high ($\geq 63.0\%$) PR values against the four test insects over the 120-day hermetic grain storage period. This is a welcome scientific development based on the promise it holds for adoption of volatile plant constituents for long term protection of durable agricultural products. In a Y-tube olfactometer repellency studies, Erler *et al.* (2006) reported that *O. basilicum* essential oil, at 10 μ l and 3 h exposure, had a PR value of 100% against *Culex pipiens*. Similarly, essential oils of two *Ocimum* spp. and selected constituents were found to be more repellent than a commercial arthropod repellent, N, N-diethyl-*m*-toluamide (DEET), against *Anopheles gambiae* (Odalo *et al.*, 2005). In a push-pull strategy, van Tol *et al.* (2006) reported a PR value of 71% for *O. gratissimum* essential oil against onion thrips, *Thrips tabaci*. A related indoor study in Guinea Bissau showed that fresh *O. canum* Sims achieved a PR value of 64.0% against mosquitoes (Pålsson and Jaenson, 1999). Results of this study concur with several others in which *Ocimum* spp. have shown strong instant repellent action against insect pests of stored agricultural products (Kéita *et al.*, 2000, 2001; Papachristos and Stamopoulos, 2002b; Moretti *et al.*, 2003).

Recent local studies showed that crude powders of *L. camara* and *T. vogelii* (10% w/w) had moderate to strong repellency against adult *S. zeamais* with PR values of 62.5 and 87.5%, respectively, after 24 h exposure (Ogendo *et al.*, 2003b). In repellency tests with live potted plants, Seyoum *et al.* (2003) observed that *L. camara* and a combination of *O. americanum* and *L. camara* repelled 32.4 and 31.6% of *Anopheles gambiae*, respectively. Strong intra-part variations in repellency index have been observed. Studies with different fractions isolated from *L. camara* flowers produced PR range of 76.0- 100% against adult *Aedes* mosquitoes (Dua *et al.*, 1996; Dua *et al.*, 2003). In grain storage adaptability studies, Koona *et al.* (2007) reported an 80% reduction in damage, by bruchid beetles over a 6-month period, of seeds stored in jute bags impregnated with aqueous extracts of *L. camara*. Studies conducted elsewhere showed that extracts of *T. vogelii* and its mixture with petroleum oil had strong repellent action against the banana aphid, *Pentalonia nigronervosa* (Liang *et al.*, 2002).

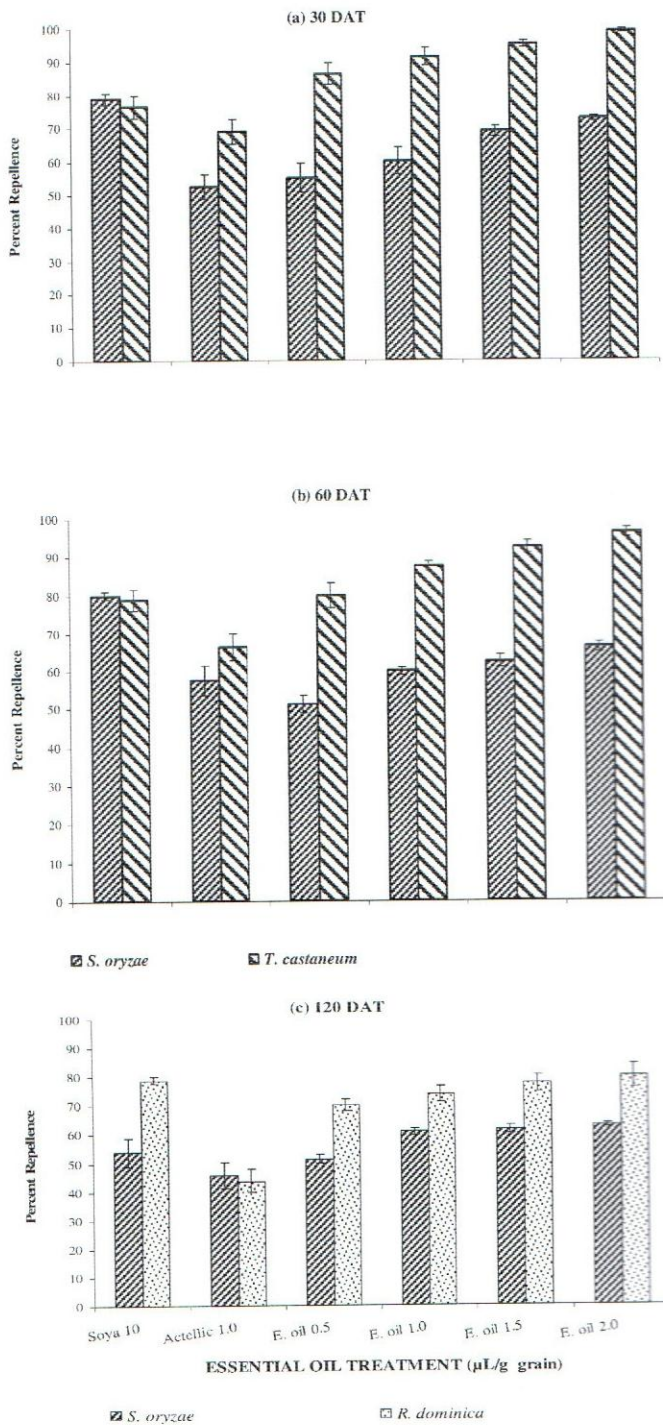


Fig. 7.5: End-point residual mean percent repellence ($\pm$ SE, n=4) after 24 h exposure to *O. americanum* fruit essential oil against four coleopteran pests over a 120-day storage period.

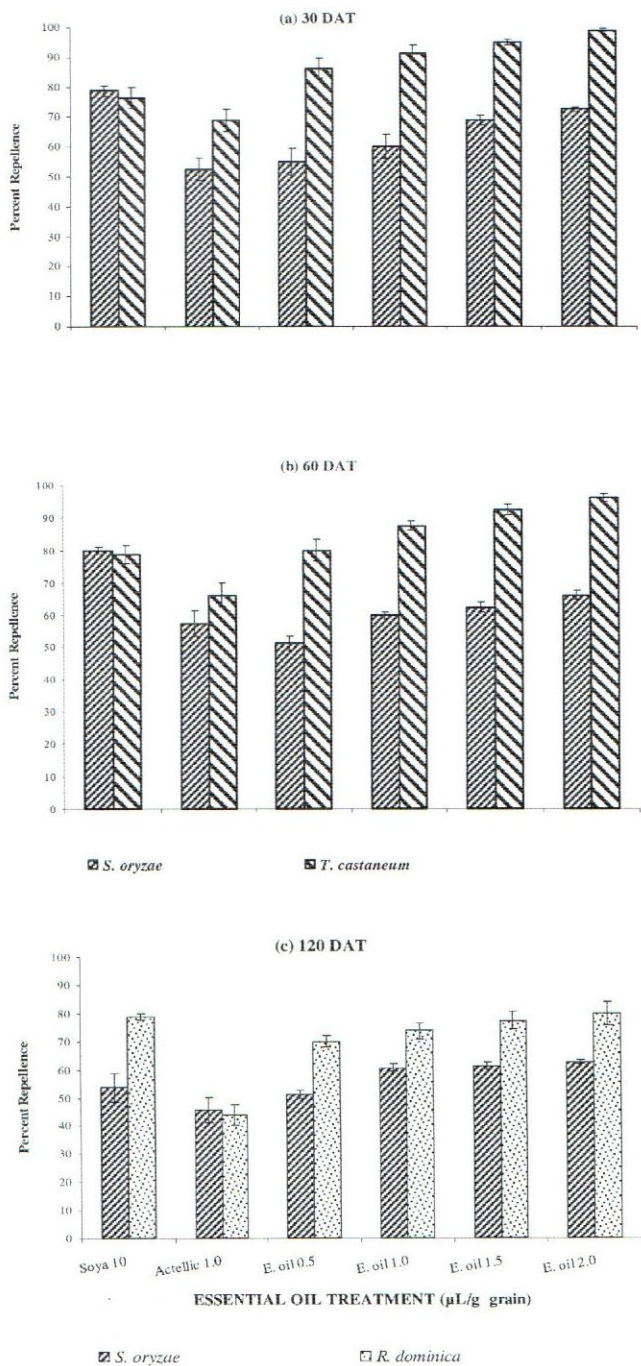


Fig. 7.6: End-point residual mean percent repellence ($\pm$ SE, n=4) after 24 h exposure to *T. vogelii* fruit essential oil against four coleopterian insects over a 120-day storage period

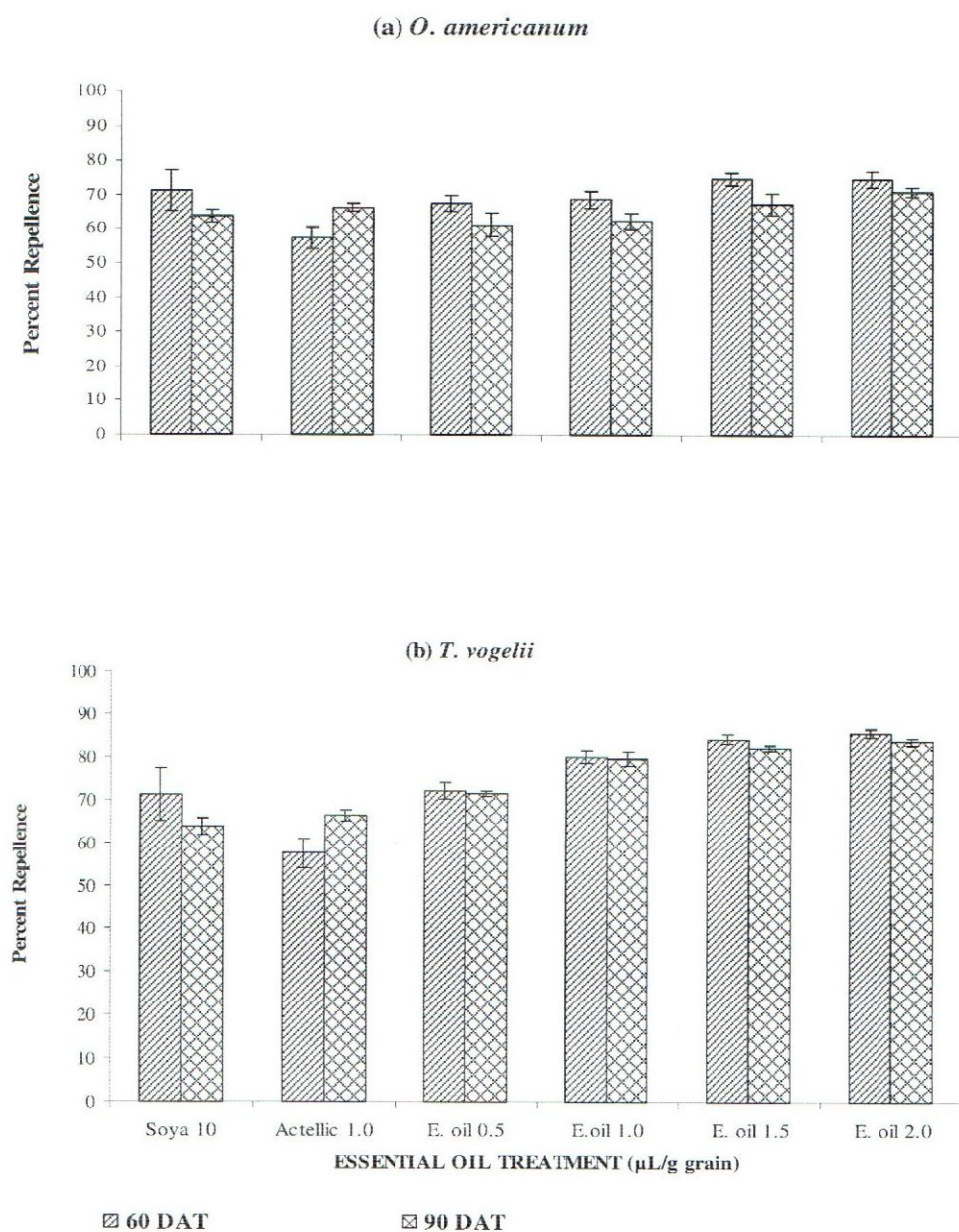


Fig.7.7: End-point residual mean percent repellence ($\pm$ SE, n=4) after 24 h exposure to essential oils extracted from leaves of (a) *O. americanum* and (b) *T. vogelii* against adult *C. chinensis* insects 60-90 days after grain treatment (DAT)

Table 7.5: Residual mean percent repellence ($\pm$ SE, n=4) of *Ocimum americanum* essential oils against four insect pests of stored food grains over a 4-month period^a

Storage duration/ Conc. (μ l/g grain)/		Percent Repellence (PR%) (Mean $\pm$ SE)			
Insect species		Exposure Time (Hours) after treatment with essential oils			
	N	1	3	5	24
FRUIT ESSENTIAL OIL:					
30 DAYS: <i>S. oryzae</i>:					
0.50	20	68.8 $\pm$ 3.8	62.5 $\pm$ 4.8	53.8 $\pm$ 4.3	55.0 $\pm$ 8.7
1.00	20	73.8 $\pm$ 2.4	65.0 $\pm$ 4.6	66.3 $\pm$ 1.3	60.0 $\pm$ 7.9
1.50	20	77.5 $\pm$ 4.3	65.0 $\pm$ 5.0	67.5 $\pm$ 3.2	68.8 $\pm$ 3.1
2.00	20	78.8 $\pm$ 2.4	65.5 $\pm$ 2.0	68.8 $\pm$ 5.5	72.5 $\pm$ 1.4
LSD _{0.05}		5.5	6.2	8.3	14.4
<i>T. castaneum</i>:					
0.50	20	83.8 $\pm$ 1.3	82.5 $\pm$ 6.3	80.0 $\pm$ 2.9	86.3 $\pm$ 6.3
1.00	20	85.0 $\pm$ 2.0	85.0 $\pm$ 5.0	85.0 $\pm$ 5.4	91.3 $\pm$ 5.2
1.50	20	87.5 $\pm$ 3.2	90.0 $\pm$ 2.9	90.0 $\pm$ 6.1	95.0 $\pm$ 2.0
2.00	20	92.5 $\pm$ 1.4	91.3 $\pm$ 5.9	92.5 $\pm$ 3.2	98.8 $\pm$ 1.0
LSD _{0.05}		3.9	6.8	6.5	9.9
60 DAYS: <i>S. oryzae</i>:					
0.50	20	61.3 $\pm$ 4.3	56.3 $\pm$ 5.9	56.3 $\pm$ 5.9	51.3 $\pm$ 4.4
1.00	20	72.5 $\pm$ 3.2	61.3 $\pm$ 3.1	62.5 $\pm$ 2.5	60.0 $\pm$ 2.0
1.50	20	72.5 $\pm$ 6.0	63.8 $\pm$ 2.4	63.8 $\pm$ 3.1	62.5 $\pm$ 3.2
2.00	20	76.3 $\pm$ 5.2	66.3 $\pm$ 3.1	67.5 $\pm$ 2.5	66.3 $\pm$ 2.4
LSD _{0.05}		5.5	7.1	6.9	4.7
<i>T. castaneum</i>:					
0.50	20	77.5 $\pm$ 2.5	82.5 $\pm$ 2.5	82.5 $\pm$ 6.3	80.0 $\pm$ 6.5
1.00	20	80.0 $\pm$ 5.0	87.5 $\pm$ 6.0	86.3 $\pm$ 5.2	87.5 $\pm$ 2.5
1.50	20	80.0 $\pm$ 5.0	92.5 $\pm$ 3.2	88.8 $\pm$ 3.8	92.5 $\pm$ 3.2
2.00	20	87.5 $\pm$ 3.2	96.3 $\pm$ 2.4	95.0 $\pm$ 2.9	96.3 $\pm$ 2.4
LSD _{0.05}		5.2	7.3	6.9	8.1
120 DAYS: <i>S. oryzae</i>:					
0.50	20	53.8 $\pm$ 3.5	50.8 $\pm$ 2.4	50.8 $\pm$ 2.4	51.3 $\pm$ 3.1
1.00	20	56.3 $\pm$ 2.4	52.0 $\pm$ 4.6	53.1 $\pm$ 4.6	60.7 $\pm$ 2.4
1.50	20	62.5 $\pm$ 1.4	55.5 $\pm$ 2.5	57.5 $\pm$ 2.5	61.5 $\pm$ 2.5
2.00	20	63.8 $\pm$ 1.3	59.5 $\pm$ 2.5	60.5 $\pm$ 2.5	62.8 $\pm$ 1.4
LSD _{0.05}		4.5	4.4	4.4	3.5
<i>R. dominica</i>:					
0.50	20	66.3 $\pm$ 7.2	68.8 $\pm$ 6.3	72.3 $\pm$ 3.8	60.0 $\pm$ 4.1
1.00	20	75.0 $\pm$ 6.1	71.3 $\pm$ 7.1	76.0 $\pm$ 4.1	63.8 $\pm$ 5.5
1.50	20	76.3 $\pm$ 6.6	72.5 $\pm$ 3.2	80.0 $\pm$ 7.5	68.3 $\pm$ 6.0
2.00	20	86.3 $\pm$ 2.4	82.5 $\pm$ 1.4	84.8 $\pm$ 4.3	71.5 $\pm$ 8.4
LSD _{0.05}		12.2	11.7	10.1	8.3
LEAF ESSENTIAL OIL:					
60 DAYS: <i>C. chinensis</i>:					
0.50	20	75.0 $\pm$ 2.0	71.3 $\pm$ 4.3	76.3 $\pm$ 2.4	67.5 $\pm$ 4.3
1.00	20	85.0 $\pm$ 2.0	81.3 $\pm$ 3.1	81.3 $\pm$ 3.1	68.8 $\pm$ 5.5
1.50	20	86.3 $\pm$ 5.9	82.5 $\pm$ 4.8	82.5 $\pm$ 1.4	75.0 $\pm$ 3.5
2.00	20	87.5 $\pm$ 4.8	86.5 $\pm$ 1.7	83.8 $\pm$ 2.4	75.0 $\pm$ 4.6
LSD _{0.05}		7.7	6.3	3.5	4.1
90 DAYS: <i>C. chinensis</i>:					
0.50	20	78.8 $\pm$ 5.2	80.0 $\pm$ 2.0	68.8 $\pm$ 5.2	61.3 $\pm$ 6.6
1.00	20	82.5 $\pm$ 2.5	82.5 $\pm$ 1.4	72.5 $\pm$ 2.5	62.5 $\pm$ 4.8
1.50	20	82.5 $\pm$ 4.3	82.5 $\pm$ 3.2	78.8 $\pm$ 4.7	67.5 $\pm$ 6.5
2.00	20	87.5 $\pm$ 1.4	87.5 $\pm$ 1.4	80.0 $\pm$ 2.9	71.3 $\pm$ 2.4
LSD _{0.05}		5.9	3.6	5.4	8.3

^aGrain sub-samples collected after 30, 60, 90 and 120 days were subjected to alternate untreated – treated choice bioassay tests

Table 7.6 (a): Residual percent repellence (mean±SE, n=4) of *Tephrosia vogelii* fruit essential oil against three insect pests of stored food grains over a 4-month period^a

Storage duration/ Conc. (µl/g grain)/ Insect species		N	Percent Repellence (PR%) (Mean±SE) Exposure Time (Hours) after treatment with essential oils			
			1	3	5	24
30 DAYS:						
<i>S. oryzae</i> :	0.50	20	75.0±5.4	73.8±4.3	70.0±5.4	55.0±4.1
	1.00	20	78.8±7.5	75.0±7.1	72.5±3.2	61.3±2.4
	1.50	20	85.0±2.0	80.0±3.5	77.5±7.5	63.8±5.2
	2.00	20	91.3±1.3	82.5±3.2	80.0±4.1	86.3±5.9
	LSD _{0.05}		12.3	7.7	8.6	7.1
<i>T. castaneum</i> :	0.50	20	66.3±5.9	75.0±4.6	70.0±5.4	57.5±6.0
	1.00	20	81.3±3.1	77.5±7.5	73.8±3.8	58.8±2.4
	1.50	20	87.5±4.8	81.3±3.8	73.8±8.7	71.3±5.5
	2.00	20	92.5±3.2	83.8±3.1	81.3±4.3	88.8±3.8
	LSD _{0.05}		6.9	8.7	9.7	7.2
60 DAYS:						
<i>S. oryzae</i> :	0.50	20	76.3±4.7	76.3±2.4	68.8±2.4	61.3±7.7
	1.00	20	81.3±3.1	80.0±2.0	73.8±4.3	62.5±4.3
	1.50	20	86.3±1.3	87.5±4.3	77.5±2.5	76.3±2.4
	2.00	20	87.5±2.5	92.5±1.4	85.0±3.5	76.3±4.3
	LSD _{0.05}		6.9	5.9	3.7	10.5
<i>R. dominica</i> :	0.50	20	70.0±5.4	68.8±5.9	68.8±5.9	65.0±5.0
	1.00	20	73.8±1.3	71.3±6.6	72.5±7.8	68.8±4.3
	1.50	20	75.0±1.7	81.3±2.4	76.3±4.7	76.3±2.4
	2.00	20	82.5±1.4	83.8±3.8	81.3±3.1	83.8±1.3
	LSD _{0.05}		8.1	8.3	9.3	7.5
<i>T. castaneum</i> :	0.50	20	67.5±4.3	71.3±7.5	69.8±5.8	54.5±9.4
	1.00	20	75.0±4.1	78.8±5.5	71.8±4.7	56.9±4.8
	1.50	20	78.8±2.4	80.0±2.9	73.0±6.1	69.3±3.5
	2.00	20	81.3±2.4	82.0±5.4	80.0±1.5	81.5±5.2
	LSD _{0.05}		3.8	9.2	9.0	11.7
120 DAYS:						
<i>S. oryzae</i> :	0.50	20	68.8±3.1	67.5±3.2	65.0±5.4	60.0±4.1
	1.00	20	70.0±2.0	71.3±3.8	65.0±2.6	65.0±2.9
	1.50	20	71.3±3.1	71.3±2.4	67.5±3.2	65.0±2.0
	2.00	20	71.3±1.3	75.0±3.5	67.5±2.5	71.3±1.3
	LSD _{0.05}		3.6	2.9	5.7	5.5
<i>R. dominica</i> :	0.50	20	57.5±1.4	60.0±3.5	58.8±1.3	65.0±2.0
	1.00	20	62.5±4.3	63.8±2.4	61.3±4.3	68.8±2.4
	1.50	20	66.3±2.4	66.3±2.4	63.8±2.4	68.8±1.3
	2.00	20	70.0±2.8	68.8±4.3	70.0±3.5	72.5±2.5
	LSD _{0.05}		5.9	3.8	4.5	2.5
<i>T. castaneum</i> :	0.50	20	60.0±7.4	66.3±3.1	72.5±2.5	62.5±2.5
	1.00	20	73.8±5.9	72.5±4.8	80.0±2.9	65.0±0.0
	1.50	20	75.0±4.1	75.0±6.1	82.5±2.5	77.5±1.4
	2.00	20	75.0±4.1	85.0±6.1	87.5±1.4	78.8±2.4
	LSD _{0.05}		6.7	6.2	3.1	4.9

^aGrain sub-samples collected after 30, 60, 90 and 120 days used in an alternate untreated (control) – treated choice bioassay

For each insect, any two means in a column whose SE values overlap are not different at $\alpha = 0.05$ by LSD and Tukey's HSD test

No data for *C. chinensis* due to lack of test essential oil at time of setting up of residual grain storage study.

Table 7.6 (b): Residual mean percent repellence ($\pm$ SE, n=4) of *Tephrosia vogelii* leaf essential oil against adult *Callosobruchus chinensis* insects over a 4-month period^a

Storage duration/ Conc. (μ l/g grain)/ Insect species	N	Percent Repellence (PR%) (Mean $\pm$ SE)			
		Exposure Time (Hours) after treatment			
		1	3	5	24
60 DAYS:					
0.50	20	75.0 $\pm$ 2.0	81.3 $\pm$ 1.3	82.5 $\pm$ 4.8	72.1 $\pm$ 3.9
1.00	20	85.0 $\pm$ 4.9	83.8 $\pm$ 3.8	85.0 $\pm$ 2.9	80.0 $\pm$ 2.7
1.50	20	88.8 $\pm$ 3.8	85.0 $\pm$ 4.6	86.3 $\pm$ 3.1	84.3 $\pm$ 2.0
2.00	20	88.8 $\pm$ 3.8	85.0 $\pm$ 7.4	91.3 $\pm$ 5.8	85.7 $\pm$ 1.7
LSD _{0.05}		5.9	12.1	5.4	7.6
90 DAYS:					
0.50	20	72.3 $\pm$ 2.4	78.8 $\pm$ 1.3	80.0 $\pm$ 1.3	71.5 $\pm$ 1.4
1.00	20	75.8 $\pm$ 4.3	80.0 $\pm$ 3.5	81.3 $\pm$ 1.3	79.6 $\pm$ 3.2
1.50	20	82.5 $\pm$ 1.4	81.3 $\pm$ 2.4	85.0 $\pm$ 3.5	82.3 $\pm$ 1.3
2.00	20	83.8 $\pm$ 1.3	84.5 $\pm$ 3.2	86.3 $\pm$ 1.3	83.7 $\pm$ 1.3
LSD _{0.05}		5.9	4.3	4.3	3.7

^aGrain sub-samples collected after 30, 60, 90 and 120 days used in an alternate untreated (control) – treated choice bioassay
For each insect, any two means in a column whose SE values overlap are not different at $\alpha = 0.05$ by LSD and Tukey's HSD test
No data on other test insects due to lack of *T. vogelii* leaf essential oil;
No data *C. chinensis* 30 and 120 days due to insufficient insect supplies.

Table 7.7: Residual mean percent repellence ($\pm$ SE, n=4) of positive controls, soya oil and Actellic 5EC, against four storage insect pests over a 4-month period^a

Storage Duration(Days)	Control treatment/ Insect	N	Percent Repellence (PR%) (Mean $\pm$ SE)			
			Exposure Time (Hours) after treatment with essential oils			
			1	3	5	24
30	<u>Soya oil (10.0 μL/g):</u>					
	<i>S. oryzae</i>	20	92.5 $\pm$ 4.8	81.3 $\pm$ 1.3	85.0 $\pm$ 5.4	78.8 $\pm$ 3.1
	<i>T. castaneum</i>	20	85.0 $\pm$ 7.4	77.5 $\pm$ 2.5	80.0 $\pm$ 9.1	76.3 $\pm$ 6.9
	<u>Actellic 5EC (1.0 μL/g):</u>					
	<i>S. oryzae</i>	20	85.0 $\pm$ 4.1	75.0 $\pm$ 6.5	60.0 $\pm$ 10.8	52.5 $\pm$ 7.8
	<i>R. dominica</i>	20	81.3 $\pm$ 3.8	76.3 $\pm$ 3.1	70.0 $\pm$ 4.6	43.8 $\pm$ 7.5
60	<i>T. castaneum</i>	20	90.0 $\pm$ 6.1	73.8 $\pm$ 7.2	67.5 $\pm$ 8.5	68.8 $\pm$ 7.5
	<u>Soya oil (10.0 μL/g):</u>					
	<i>S. oryzae</i>	20	91.3 $\pm$ 3.1	77.5 $\pm$ 3.2	76.3 $\pm$ 3.1	80.0 $\pm$ 2.0
	<i>T. castaneum</i>	20	85.0 $\pm$ 0.0	76.3 $\pm$ 10.7	86.3 $\pm$ 1.3	78.8 $\pm$ 5.5
	<i>C. chinensis</i>	20	78.8 $\pm$ 2.4	81.3 $\pm$ 2.4	87.5 $\pm$ 3.2	71.3 $\pm$ 12.5
	<u>Actellic 5EC (1.0 μL/g):</u>					
90	<i>S. oryzae</i>	20	80.0 $\pm$ 3.5	76.3 $\pm$ 5.9	66.3 $\pm$ 6.3	57.5 $\pm$ 7.8
	<i>R. dominica</i>	20	80.0 $\pm$ 2.0	66.3 $\pm$ 4.3	71.3 $\pm$ 4.7	52.5 $\pm$ 6.0
	<i>T. castaneum</i>	20	75.0 $\pm$ 5.8	70.0 $\pm$ 3.5	67.1 $\pm$ 8.9	66.3 $\pm$ 7.2
	<i>C. chinensis</i>	20	75.0 $\pm$ 5.4	61.3 $\pm$ 6.6	60.0 $\pm$ 6.8	57.5 $\pm$ 6.6
	<u>Soya oil (10.0 μL/g):</u>					
	<i>C. chinensis</i>	20	78.8 $\pm$ 3.8	86.3 $\pm$ 3.8	87.5 $\pm$ 3.2	63.8 $\pm$ 3.8
120	<u>Actellic 5EC (1.0 μL/g):</u>					
	<i>C. chinensis</i>	20	81.3 $\pm$ 3.1	78.8 $\pm$ 4.3	70.0 $\pm$ 3.5	66.3 $\pm$ 2.4
	<u>Soya oil (10.0 μL/g):</u>					
	<i>S. oryzae</i>	20	87.5 $\pm$ 2.5	72.5 $\pm$ 3.2	70.0 $\pm$ 2.0	53.8 $\pm$ 10.3
	<i>R. dominica</i>	20	68.8 $\pm$ 5.2	77.5 $\pm$ 3.2	77.5 $\pm$ 3.2	78.8 $\pm$ 2.4
	<i>T. castaneum</i>	20	68.8 $\pm$ 3.8	83.8 $\pm$ 3.1	68.8 $\pm$ 3.8	61.3 $\pm$ 9.7
	<u>Actellic 5EC (1.0 μL/g):</u>					
	<i>S. oryzae</i>	20	87.5 $\pm$ 3.2	75.0 $\pm$ 7.1	48.8 $\pm$ 6.0	45.8 $\pm$ 8.8
	<i>R. dominica</i>	20	81.3 $\pm$ 3.8	76.3 $\pm$ 3.1	70.0 $\pm$ 4.6	43.8 $\pm$ 7.5
	<i>T. castaneum</i>	20	75.0 $\pm$ 4.1	63.8 $\pm$ 4.3	68.8 $\pm$ 2.4	61.3 $\pm$ 5.5

^a Grain sub-samples collected after 30, 60, 90 and 120 days were subjected to alternate untreated – treated choice bioassay tests
For each control treatment, any two means with overlapping SE values are not different at $\alpha=0.05$ by LSD test.
Missing data points were due to lack of test insects at time of setting up bioassays.

The differential repellency responses by different insect species to *O. americanum*, *L. camara* and *T. vogelii* essential oils could be attributed to individual and / or synergistic repellent activity of their terpenoid constituents. Similarly, the marked differences in the repellency of leaf and fruit essential oils against adults of individual insect species could be explained by the intra-species variations in chemical composition. Plants containing terpenes may be used as repellents without modification by rubbing fresh leaves on skin to release the oils. Most monoterpenes are volatile constituents that deter phytophagous insects by acting in the vapour form on the olfactory receptors (Moore and Lenglet, 2004). Monoterpenes and sesquiterpenes, such as α - and β -pinenes, limonene, linalool and terpinene-4-ol, are well-known repellents of biting insects (Stuart and Stuart, 1998; Debboun *et al.*, 2007; Wang *et al.*, 2008). Similarly, Hori (2004) reported that α -terpineol, linalool and (-)-perillaldehyde were strongly repellent against adult *Lasioderma serricorne* whereas (-)-caryophyllene oxide and α -humulene were weakly repellent against the same insect. Except for *C. chinensis* (PR values: 83.0- 45.0%) in which repellence decreased with dosage, a major *O. americanum* essential oil constituent, eugenol (12.59-49.22%), produced increasing PR values of 92.0- 100, 58.0- 65.0 and 63.0- 100% after 24 h against *S. oryzae*, *R. dominica* and *T. castaneum*, respectively, on treated grains (Fig. 7.2; Table 7.2; Ogendo *et al.*, 2008). It implicitly emerges from the above arguments that volatile terpenoid constituents contribute to the repellence principle exhibited by essential oils against various insects. The fact that various plant products such as live plants, fresh and smoke (from Lamiaceae, Meliaceae, Myrtaceae, Mimosaceae) are successful cases of repellents against biting insects' makes their potential as repellents against insect pests of stored products more promising. Aromatic plants can be used to develop alternative technologies to reduce postharvest losses of staple foods in tropical areas. Since aromatic plants have long been used in daily life as insect repellents, they are generally regarded as safe and ecosocio-friendly than synthetic pesticides.

CHAPTER EIGHT

PHYTOTOXIC EFFECTS OF *Ocimum americanum* L. AND *Tephrosia vogelii* HOOK FRUIT ESSENTIAL OILS ON WHEAT SEED VIABILITY

Abstract

Laboratory bioassays were conducted in petri dishes to evaluate the phytotoxic effects of *O. americanum* and *T. vogelii* fruit essential oils on wheat seed viability. Each essential oil was evaluated at five rates (0, 0.05, 0.10, 0.15 and 0.20% v/w) with four replicates per concentration. Control treatments, acetone (0.05% v/w), Actellic 5EC (0.10% v/w) and soya oil (1.0% v/w) were included in the two bioassays and each replicated four times. Results showed that *O. americanum* and *T. vogelii* fruit essential oils had strong dose- and storage duration-dependent phytotoxic effects on seed viability. At the highest concentration (0.20% v/w) and 120 days of storage, *O. americanum* and *T. vogelii* fruit essential oils caused 25.0- 40.0 and 33.0- 51.0% inhibition of wheat seed germination with corresponding EC₅₀ values of 0.37- 6.22 and 0.19- 2.10% v/w, respectively. The two positive controls, Actellic 5EC (0.10% v/w) and soya oil (1.0% v/w), were strongly phytotoxic causing 27.5 and 56.8%, respectively, inhibition of wheat seed germination. Considering the value of traditional seeds in tropical agriculture, results of this study are discussed in the context of their relevance as grain protectants and implications for the informal seed industry. Further in-depth laboratory and field evaluations are recommended to determine the spectrum of phytotoxicity of *O. americanum* and *T. vogelii* essential oils and their major chemical constituents on the viability of cereal and legume seeds.

8.1 Introduction

Adoption of simple, low-cost and eco-friendly biological options based on natural crop protection approaches that make use of diversity in nature is a viable alternative to synthetic pesticides (Osorio, 2001). This natural protection approach has been particularly useful in weed control in which secondary metabolites produced by plants inhibit growth of other plant species in their vicinity (Dudai *et al.*, 2000; Mao *et al.*, 2004). Among the natural plant allelochemicals, essential oils and their constituents have attracted substantial scientific attention due to their phytotoxicity and inhibition of germination and growth. In particular, monoterpenes from a number of aromatic plants have inhibitory effects on germination of wheat (Dudai *et al.*, 1999).

Batish *et al.* (2004) reported that terpenoids, especially monoterpenes and sesquiterpenes, which are the main components of essential oils, are responsible for their inhibitory activity.

Aromatic plants, known to be rich in active principles, can play an important role in plant-plant interactions and constitute a potential source of allelochemicals. Essential oils, which are naturally occurring secondary metabolites, have been shown to possess broad-spectrum bioactivity such as insecticidal, repellent, antimicrobial, herbicidal, inhibition of germination and growth among other effects (Alves *et al.*, 2004; Boeke *et al.*, 2004c; Liu *et al.*, 2006). Recent local studies have revealed that $\geq 70\%$ of subsistence farmers in western Kenya use traditional methods, particularly plant-based products, to control insect pests of stored food grains (Ogendo *et al.*, 2003a; 2004a). In their earlier studies, Ogendo *et al.* (2004a) reported that crude powders of *L. camara* and *T. vogelii* had no effects on germination of maize seeds and also had no effect on quality (colour and odour) of treated maize grains over a 150-day storage. Although the potential of *O. americanum* and *T. vogelii* essential oils and other extracts as biopesticides have been documented (Shaaya *et al.*, 1997; Ogendo *et al.*, 2003b; Rozman *et al.*, 2007), no studies have focussed on their potential phytotoxic effects on seed germination and growth of seedlings. Hence, this study which evaluated the phytotoxic effects of *O. americanum* and *T. vogelii* fruit essential oil treatments on wheat seed viability, expressed as percent germination and percent germination inhibition, over a 120-day storage period.

8.2 Materials and Methods

8.2.1 Effect of essential oils on viability of treated seeds

Phytotoxic effects of *O. americanum* and *T. vogelii* fruit essential oils on wheat seed viability, expressed as percent germination and germination inhibition, were studied over a 120-day grain storage period according to Ogendo *et al.* (2004b). Each test essential oil, dissolved in acetone AR (99.8% GC), was applied to 25-g wheat seed samples in special self-sealing polythene bags (20 cm x 25 cm; 2 Litre) at four rates (0.05, 0.10, 0.15, 0.20%v/w). The negative controls consisted of untreated and acetone (0.05% v/w) treated seeds whereas Actellic 5EC (0.1% v/w) and crude soya oil (1.0% v/w) were the positive controls. All treated seeds were kept in fume chamber for 24 h to allow acetone to evaporate completely before being transferred into a room maintained at $30\pm 2^{\circ}\text{C}$, $68\pm 2\%$ RH and 12D: 12D (total darkness) for long term storage.

Unbiased seed sub-samples (4.5 g) were then drawn from each experimental unit containing treated seeds 0, 30, 60, 90 and 120 days after treatment. The seed sub-samples were germinated on moistened filter paper (Whatman No. 1) in plastic Petri dishes (diameter: 9.0 cm) arranged in a completely randomized design (CRD) with 4-8 replicates per treatment. The number of emerged seedlings (N_G) from each petri dish was counted and recorded after 7 days. The percent germination was computed according to the methods of Zibokere (1994) as follows:

$$\text{Germination (\%)} = \frac{(N_G)}{(T_G)} \times 100 \quad \text{Equation 4}$$

Where N_G and T_G represented the number of seedlings emerged and total number of seeds placed in Petri dish, respectively. A 4.5 g seed sub-samples (av. 50 seeds) was used in each germination replicate. The percent germination inhibition was calculated according to Mao *et al.* (2004) as follows:

$$\text{Germination Inhibition (\%)} = \frac{(G_C - G_T)}{G_C} \times 100 \quad \text{Equation 5}$$

Where G_C and G_T represented number of germinated seeds in untreated control and treated Petri dishes, respectively. Percent germination and inhibition data were processed, analyzed and interpreted as described in Chapter 3.

8.3 Results and Discussion

Seed viability, expressed as percent germination and germination inhibition caused thereof, were significantly influenced ($P < 0.0001$) by the plant species, duration of storage, concentration of essential oil and corresponding factor interactions. Comparisons with an initial seed viability of 86.5%, viability of untreated- and acetone-treated seeds were similar with only 5.5% reduction in germination. Acetone had no inhibitory effects on wheat seeds during the 120 days of storage. However, the two positive controls, Actellic 5EC (0.10% v/w) and soya oil (1.0%), were strongly phytotoxic causing 28.0 and 57.0%, respectively, inhibition of wheat seed germination after 120 days of storage.

The results showed species-, dose- and storage duration-dependent phytotoxic effects of *O. americanum* and *T. vogelii* essential oils on the percent germination of wheat seeds. At the highest concentration (0.20% v/w), the *O. americanum* and *T. vogelii* fruit essential oils caused 25.0- 40.0 and 33.0- 51.0% reductions (inhibition) in wheat seed germination over 120-day storage with corresponding EC₅₀ values of 0.37- 6.22 and 0.19- 2.10% v/w, respectively (Fig. 8.1; Table 8.1). In both bioassays, observed storage duration-dependent decrease in EC₅₀ values was a manifestation of increased wheat seed germination inhibition. The *T. vogelii* fruit oil (51.0% inhibition) had a higher inhibitory effect on wheat seed germination than *O. americanum* fruit oil (40.0% inhibition) after 120 days of storage. These results were in agreement with several past studies in which essential oils had inhibitory effects on germination and growth of both crop and weed species. In their search for bioherbicides, Mao *et al.* (2004) reported that vetiver essential oil (1.0 mg/ml) caused 90 and 72.0% germination inhibition of redroot pigweed and velvetleaf, respectively. In related studies *Artemisia princeps* and *Cinnamomum camphora* essential oils, at 500 µg ml⁻¹, significantly reduced wheat seed germination by 24.0 and 13.0%, respectively, compared against the negative control (Liu *et al.*, 2006). Recently, in separate investigations, dose-dependent allelopathic effects of *Cinnamomum zeylanicum*, *Lippia sidoides*, *Cymbopogon citratus*, *Ocimum gratissimum* and *Ruta graveolens* essential oils on lettuce and radish seed germination and radicle growth were reported (De Feo *et al.*, 2002; Alves *et al.*, 2004). Similarly, essential oil from leaves of *Eucalyptus citriodora* had LC₅₀ values of 0.32, 0.41 and 0.17 mg/L against *Triticum aestivum*, *Zea mays* and *Amaranthus viridis* in dose-response inhibition of germination and seedling growth and reduced chlorophyll contents (Batish *et al.*, 2004). However, in other studies involving treatment of stored grains, essential oils extracted from *Ocimum* spp., *Cymbopogon nardus*, *Momordica charantia* and *Ficus exasperata* reportedly had no inhibitory effects on germination of cowpea and bean seeds (Kéita *et al.*, 2001; Boeke *et al.*, 2004c; Ketoh *et al.*, 2005). Data comparisons showed that the two positive controls (Actellic 5EC and soya oil) and *O. americanum* and *T. vogelii* fruit essential oils equally inhibited wheat seed germination. The inhibitory principle of these essential oils could implicitly be attributed to the presence of terpenoids, especially monoterpenes and sesquiterpenes, known to have phytotoxic agents (Dudai *et al.*, 1999; Batish *et al.*, 2004; Mao *et al.*, 2004) and the biotransformation of terpenoid compounds by the germinating seeds (Dudai *et al.*, 2000). Hence, these essential oils if conclusively validated are potential alternatives to synthetic insecticides in

the protection of stored food grains but not seeds. Although plant-derived insecticides, such as essential oils, have broadspectrum bioactivity, safe to use and are ecosocio-friendly, their packaging for use in tropical agriculture should be handled in the context of compatibility with domestic food preservation and informal seed sector.

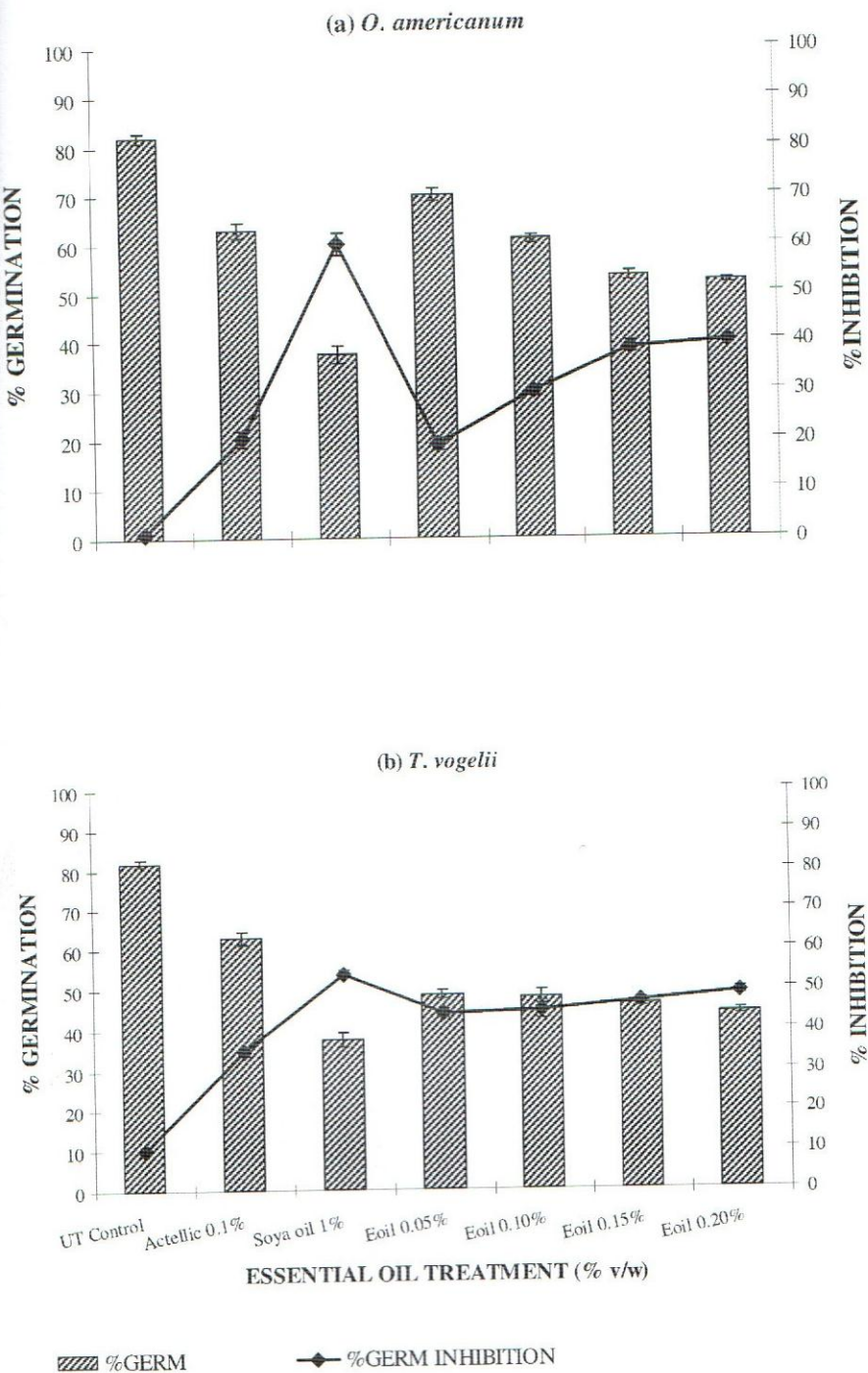


Fig. 8.1: Phytotoxic effects of (a) *O. americanum* and (b) *T. vogelii* fruit essential oils on mean percent germination ($\pm$ SE, $n=4$) of wheat seeds over a 4-month storage.

Table 8.1: Phytotoxic effects of *O. americanum* and *T. vogelii* fruit essential oils on percent germination of treated wheat seeds over a 4-month storage period

DURATION OF STORAGE (DAYS)											
Essential oil treatment (% v/w)		N	% Germination (Mean±SE, n=4)				% Germination Inhibition (Mean±SE, n=4)				
			30	60	90	120	30	60	90	120	
<i>O. americanum</i>:											
Actellic 5EC	0.10	20	74.5±1.1	69.2±2.7	60.6±1.6	62.8±1.6	13.9±1.6	20.1±3.6	30.0±2.2	27.5±2.7	
Soya oil	10.0	20	62.0±2.6	47.5±1.8	42.7±1.5	37.4±1.9	28.4±4.0	45.1±2.4	50.7±2.1	56.8±3.3	
E. oil	0.00	20	82.6±0.8	82.0±0.6	78.0±1.1	81.8±0.9	4.5±1.1	5.2±1.0	9.9±2.3	5.5±0.6	
	0.05	20	66.5±0.6	71.8±3.6	70.1±3.2	69.9±2.2	23.1±0.6	17.1±4.3	19.0±3.6	19.2±2.6	
	0.10	20	64.0±2.4	68.0±1.7	65.4±1.7	60.7±1.6	26.0±2.7	21.4±2.0	24.4±2.0	29.8±1.8	
	0.15	20	63.3±3.2	66.9±1.3	61.8±0.9	53.1±2.0	26.8±3.8	22.7±1.5	28.6±1.0	38.6±2.4	
	0.20	20	62.0±3.2	65.3±1.0	61.1±1.0	52.0±1.1	28.4±3.7	24.5±1.2	29.3±1.2	39.9±1.3	
LSD _{0.05}			6.2	5.9	4.8	6.1	6.9	6.6	5.7	8.8	
EC ₅₀ value			-	-	-	-	6.22	5.96	1.61	0.37	
95%FL			-	-	-	-	(3.76, 8.85)	(3.55, 7.90)	(0.95, 2.29)	(0.20, 1.05)	
<i>T. vogelii</i>:											
Actellic 5EC	0.10	20	74.5±1.1	69.2±2.7	60.6±1.6	62.8±1.6	13.9±1.6	20.1±3.6	30.0±2.2	27.5±2.7	
Soya oil	10.0	20	62.0±2.6	47.5±1.8	42.7±1.5	37.4±1.9	28.4±4.0	45.1±2.4	50.7±2.1	56.8±3.3	
E. oil	0.00	20	82.6±0.8	82.0±0.6	78.0±1.1	81.8±0.9	4.5±1.1	5.2±1.0	9.9±2.3	5.5±0.6	
	0.05	20	66.9±2.7	56.5±4.3	47.8±0.9	48.6±1.9	22.4±2.6	34.7±5.0	44.7±1.0	43.8±2.2	
	0.10	20	67.2±2.3	53.9±2.0	45.5±0.8	47.8±3.8	22.7±3.1	37.7±2.3	47.4±0.9	44.7±4.4	
	0.15	20	64.4±2.6	44.2±2.1	44.0±1.1	46.0±1.1	25.6±3.0	48.9±2.5	49.2±1.2	46.8±1.3	
	0.20	20	58.3±1.9	42.3±2.7	43.1±1.5	44.0±1.5	32.7±2.2	51.1±3.2	50.2±1.7	49.1±1.7	
LSD _{0.05}			4.9	7.9	4.3	6.2	5.3	8.5	4.7	7.1	
EC ₅₀ value			-	-	-	-	2.10	0.19	0.20	0.32	
95%FL			-	-	-	-	(1.04, 3.22)	(0.13, 1.14)	(0.15, 1.22)	(0.19, 1.43)	

For each test essential oil, any two means whose SE values overlap are not different at $\alpha = 0.05$ by Tukey's HSD test

CHAPTER NINE

GENERAL DISCUSSION

The coexistence of mankind and insects (pests and vectors) since our ancestors first evolved remains undisputed. Similarly the use of botanical pesticides in agriculture is as old as mankind. However, historical records show that the introduction of synthetic insecticides relegated the role of botanicals in agriculture to an essentially trivial position in the marketplace among crop protectants (Isman, 2006). Despite their phenomenal efficacies against insect pests, extensive use of synthetic chemical insecticides in agriculture is currently under threat owing to their negative effects such as toxicity to non-target organisms, ozone depletion, environmental destruction, disruption of natural biological balance, environmental contamination, health hazards and continued genetic resistance among pest populations among other effects (Isman, 2006; Shaaya and Kostyukovsky, 2006; Talukder, 2006; Kouninki *et al.*, 2005; Negahban *et al.*, 2007). Moreover, food production in the tropics remains at subsistence level which makes the use of modern insect pest management protocols largely incompatible and uneconomical. In recent years, there has been growing scientific search for methyl bromide and poshphine alternatives due to the ozone depletion and insect pest resistance by the former and latter fumigants, respectively (Shaaya *et al.*, 1997; Lee *et al.*, 2001; Rajendran and Sriranjini, 2008).

In conformity with international trade and biosafety standards, the scientific rationalization process must ensure that newly developed fumigants have high efficacy against target insects, leave no hazardous residues, have no adverse effects on the nutritional, flavour or processing quality of stored grains and are simple and convenient to use (Lee *et al.*, 2001; Shaaya and Kostyukovsky, 2006; Ogendo *et al.*, 2008). There has been a growing interest in the use of aromatic plant-based derivatives, particularly plant oils, for the protection of agricultural products due to their relative biosafety, inexpensiveness, low mammalian toxicity and eco-friendliness compared to the synthetic chemicals (Ketoh *et al.*, 2002; Papachristos and Stamopoulos, 2002; Talukder, 2006). Plant oils and their volatile constituents have demonstrated insecticidal (contact and fumigant), repellent and feeding deterrents (Garg and Siddiqui, 1992; Kéita *et al.*, 2001, Lee *et al.*, 2001; Moore and Lenglet, 2004; Talukder, 2006; Ogendo *et al.*, 2008; Rajendran and Sriranjini, 2008), growth and reproduction inhibition (Papachristos and

Stamopoulos, 2002b; Talukder, 2006), nematicidal and antimicrobial (Paster *et al.*, 1995; Qamar *et al.*, 2005; Matasyoh *et al.*, 2007) effects against storage insect pests and inhibition (Dudai *et al.*, 1999; Batish *et al.*, 2004) of treated seed grains.

The current study has, though full bioactivity spectrum not investigated, corroborated past findings on potential of plant essential oils as stored grain protectants. Results confirm that new scientific mileage has been covered in which clear intra-species and inter-plant variations in bioactivity of essential oils against major coleopteran pests of stored food grains was determined. Similarly, the fact that test essential oils produced significant instant and residual bioactivity (contact toxicity, repellence and seed viability) holds good promise for the development of alternative organic insect pest control protocols that needs to be carefully adapted for informal seed preservation. This was more evident when *O. americanum* leaf essential oil [50-70 $\mu\text{l/L}$ air ($\cong$ 50-70 g oil/ M^3 grain) and 7 d exposure] produced comparable efficacy to the recommended rates (30-50 g / M^3 grain) of methyl bromide, phosphine and Labiate sp. oil, ZP51 (Busvine, 1980; Kostyukovsky *et al.*, 2002a; Lee *et al.*, 2004). Additionally, temporal data comparisons revealed the possibility of long-term bioefficacy (toxic, repellent and reproduction inhibition) of plant essential oils against adult coleopteran species under hermetic conditions of grain storage. Appropriate recommendations have been generated to guide further investigation, validation and formulation studies for targeted exploitation of plant essential oils and their volatile constituents in the control of stored-product insect pests and preservation of seeds.

CHAPTER TEN

CONCLUSIONS AND RECOMMENDATIONS

10.1 Conclusions

The GC-MS analyses showed existence of marked intra-species and inter-plant variations in qualitative and quantitative composition of test essential oils. Fifty-six, 57 and 69 constituents were identified accounting for 81.8- 90.3, 60.7- 81.9 and 87.5- 94.1% of *O. americanum*, *T. vogelii* and *L. camara* essential oils, respectively. The *O. americanum* essential oils had a high percentage of monoterpenes with the five major ones accounting for 23.7- 64.6% compared to 21.7- 46.3% for the six major sesquiterpenes identified. The *T. vogelii* and *L. camara* essential oils, on the other hand, were dominated by sesquiterpenes with 12 and 6 major sesquiterpenes identified accounting for 31.0- 57.4 and 38.6- 56.5%, respectively. The existence of intra-species and inter-plant variability in essential oil compositions calls for strict chemical profiling and mapping of chemotypes in Kenya and judicious screening for bioactivity against major insect pests of stored durable agricultural products.

The study has confirmed the existence of moderate to strong concentration-, intra-species and inter-plant dependent instant and residual contact toxicity and reproduction inhibitory effects of *O. americanum*, *L. camara* and *T. vogelii* essential oils against adult *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis* insects. Results showed that *O. americanum*, *L. camara* and *T. vogelii* oils, at 2.0 $\mu\text{L/g}$, caused 13.0- 96.0, 5.0- 100 and 3.0- 83.0% kill, respectively, of the four test insects. The oils caused 28.0- 61.0, 35.0- 60.0 and 42.0- 68.0% reductions in adult F_1 progeny of all test insects. Temporal data comparisons showed depressed toxicity as manifested in increased LC_{50} values with storage duration and 19.0- 32.0 and 42.0- 54.0% reductions in adult F_1 progeny of *C. chinensis* by *O. americanum* and *T. vogelii* leaf oils, respectively.

The space fumigation studies have confirmed strong concentration-, intra-species and inter-plant-dependent fumigant toxicity of *O. americanum*, *L. camara* and *T. vogelii* essential oils against adult *S. oryzae*, *R. dominica*, *C. chinensis* and *T. castaneum*. The fairly low LC_{50} values of 0.38- 64.10 and 0.03- 135.8 $\mu\text{l/L}$ recorded for essential oils and selected terpenoids 24 h after treatment against all the test insects, except *T. castaneum*, makes the essential oils and their constituents potential fumigants for control of storage insects and as alternatives to synthetic

fumigants, methyl bromide and phosphine. The results of the wheat grain fumigation with *O. americanum* leaf essential oil has shown that a concentration of 50-70 $\mu\text{l/L}$ air ($\cong$ 50-70 g oil/ M^3 grain) and 7 d exposure were enough to obtain 80-100% kill of all test adult insects. The results of present study are comparable to the recommended rate of 30-50 g / M^3 grain for methyl bromide, phosphine and a Labiate sp. oil, ZP51. Hence, the *O. americanum* leaf essential oil is a potential alternative to synthetic fumigants in the control of insect pests of stored food grains.

Essential oils extracted from aerial parts of *O. americanum*, *L. camara* and *T. vogelii* and a monoterpene constituent, eugenol, had clear concentration-, exposure time-, species (plant and insect)- and plant part-dependent instant and residual repellent potency against adult *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis*. The intra-species and inter-plant variations in essential oil compositions provide the scientific principles for differential bioactivity responses elicited in the test insects. Although the PR values decreased marginally with grain storage duration, the self-sealing polythenes provided hermetic conditions that ensured efficacy was sustained over the 4-month storage period. The current study has demonstrated the potential of *O. americanum*, *L. camara* and *T. vogelii* essential oils and their volatile terpenoid constituents as repellents of coleopteran pests of stored food grains.

The *O. americanum* and *T. vogelii* fruit essential oils had strong concentration- and storage duration-dependent inhibition of wheat seed germination. The fact that essential oils, at 0.20% v/w ($\cong$ 200 ppm), reduced wheat seed viability by upto 51% within 4 months of storage has significant implications as grain protectants in tropical agriculture known to rely heavily on the informal seed sector.

10.2 Recommendations

Owing to resource limitations (time, cost, laboratory facilities and personnel), the full factorial combinations of plant parts, insect species (various developmental stages) and food grains (legume, cereal and other stored products) were not factored in the current bioactivity studies. Hence, it is highly recommended that further in-depth bioassay studies be conducted to evaluate:

- 1) The instant and residual contact toxicity spectrum of *O. americanum*, *L. camara* and *T. vogelii* essential oils, major constituents, blends and their aromatized and/ or encapsulated formulations against the major insect pests of stored food grains.
- 2) The fumigation (space and grain) potential of *O. americanum*, *L. camara* and *T. vogelii* essential oils, their terpenoid constituents and blends (oils and constituents) against major insect pests of stored food commodities.
- 3) The repellency (instant and residual) potential of *O. americanum*, *L. camara* and *T. vogelii* essential oils, their terpenoid constituents, blends (oils and constituents) and their slow release formulations against major storage insect pests.
- 4) The full phytotoxicity (germination inhibition, growth) and qualitative effects of *O. americanum*, *L. camara* and *T. vogelii* essential oils, their terpenoid constituents and blends (oils and constituents) on treated cereal and legume grains and vigour of emerging seedlings.
- 5) The spatial-temporal spectrum of intra-species and inter-plant variability in essential oil composition (chemical profiling), oil yields and mapping of *O. americanum*, *L. camara* and *T. vogelii* chemotypes in Kenya and accompanying bioactivity spectrum against major insect pests and pathogenic organisms of stored food commodities.
- 6) The socio-economic, biosafety and compatibility implications of plant essential oils, their constituents and blends as potential grain fumigants, toxicants and repellents in the context of tropical agriculture.
- 7) Additionally, domestication, agronomic and genetic manipulations of promising botanicals should be conducted for synchronized, optimized and cost-effective exploitation.
- 8) Finally, local industrial initiatives on cost-effective and socially acceptable essential oil extraction, packaging, application and agronomic protocols be developed.

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APPENDICES

Appendix 1: ANOVA- interspecies variation in oil toxicity (space fumigation)

Source of variation	DF	Sum of squares	F Ratio	Prob > F
<i>Sitophilus oryzae</i>:				
Plant Species	2	71571.03	98.0381**	<.0001
Plant Part	2	27321.08	37.4244**	<.0001
Plant Species*Part	4	64546.88	44.2082**	<.0001
Time	3	83026.22	75.8196**	<.0001
Plant Species*Time	6	4215.02	1.9246**	0.0751
Plant Part*Time	6	1192.78	0.5446NS	0.7743
Concentration	3	220928.22	201.7518**	<.0001
Plant Species*Conc.	6	23862.31	10.8955**	<.0001
Plant Part*Conc.	6	11983.36	5.4716**	<.0001
Time*Concentration	9	27574.33	8.3936**	<.0001
Error	507			
<i>Tribolium castaneum</i>:				
Plant Species	2	3189.2083	45.8190**	<.0001
Plant Part	2	1532.9590	22.0238**	<.0001
Plant Species*Part	4	3813.2938	27.3926**	<.0001
Time	3	3690.8411	35.3506**	<.0001
Plant Species*Time	6	626.8185	3.0018**	0.0068
Plant Part*Time	6	938.6605	4.4952**	0.0002
Concentration	3	8769.1912	83.9906**	<.0001
Plant Species*Conc.	6	2104.9507	10.0805**	<.0001
Plant Part*Conc.	6	539.0306	2.5814*	0.0179
Time*Concentration	9	2019.6164	6.4479**	<.0001
Error	507			
<i>Rhyzopertha dominica</i>:				
Plant Species	2	80386.21	124.9438**	<.0001
Plant Part	2	23385.22	36.3475**	<.0001
Plant Species*Plant Part	4	39059.54	30.3550**	<.0001
Time	3	33804.42	35.0280**	<.0001
Plant Species*Time	6	4304.48	2.2301*	0.0391
Plant Part*Time	6	1184.62	0.6138NS	0.7194
Concentration	3	145550.31	150.8186**	<.0001
Plant Species*Conc.	6	18114.65	9.3852**	<.0001
Plant Part*Conc.	6	11089.26	5.7453**	<.0001
Time*Concentration	9	9257.06	3.1974**	0.0009
Error	507			
<i>Callosobruchus chinensis</i>:				
Plant species	2	4476.389	35.998**	<.0001
Plant part	2	5684.3	32.0851**	<.0001
Plant species*Plant part	4	15865.4	22.3882**	<.0001
Time	2	19550.0	110.351**	<.0001
Plant species*Time	4	3470.9	9.7959**	<.0001
Plant part*Time	4	11502.9	21.6429**	<.0001
Concentration	3	120064.7	225.903**	<.0001
Plant species*Conc.	6	11917.1	11.2111**	<.0001
Plant part*Conc.	6	9120.7	8.5804**	<.0001
Time*Concentration	6	8307.0	7.8148**	<.0001
Error	226			

*Significant at $\alpha = 0.05$, ** Highly significant at $\alpha = 0.01$ and NS = non-significant at $\alpha = 0.05$.

Appendix 3: ANOVA- interspecies variation in *O. americanum* leaf essential oil fumigant toxicity (grain fumigation)

Source	DF	Sum of Squares	F Ratio	Prob > F
<i>S. oryzae</i>:				
Fumigation Duration (Days)	3	15862.24	22.0506**	<.0001
Post-Fumigation Time (Hours)	2	3663.79	7.6397**	0.0006
Fum.Duration*PostFum Time	6	14085.85	9.7906**	<.0001
Concentration (µl/L air)	4	286701.70	298.915**	<.0001
Fumigation Duration*Conc	12	9079.15	3.1553**	0.0004
PostFum Time*Conc	8	1828.39	0.9531NS	0.4738
Error	215			
<i>R. dominica</i>:				
Fumigation Duration (Days)	3	38709.22	198.697**	<.0001
Post-Fumigation Time (Hours)	2	7574.98	58.3243**	<.0001
Fum.Duration*PostFum Time	6	1665.56	4.2747**	0.0004
Concentration (µl/L air)	4	273906.34	1054.485**	<.0001
Fumigation Duration*Concen	12	14449.63	18.5427**	<.0001
PostFum Time*Conc	8	2912.64	5.6066**	<.0001
Error	215			
<i>T. castaneum</i>:				
Fumigation Duration (Days)	3	10653.93	36.9493**	<.0001
Post-Fumigation Time (Hours)	2	5337.49	27.7667**	<.0001
Fum.Duration*PostFum Time	6	4392.54	7.6170**	<.0001
Concentration (µl/L air)	4	213561.55	555.496**	<.0001
Fumigation Duration*Conc	12	5097.53	4.4197**	<.0001
PostFum Time*Conc	8	1753.19	2.2801**	0.0232
Error	215			

*Significant at $\alpha = 0.05$, ** Highly significant at $\alpha = 0.01$ and NS = non-significant at $\alpha = 0.05$.

Appendix 4a: ANOVA- interspecies variation in contact toxicity of plant essential oils against four stored-product insects

Source of variation	DF	Sum of Squares	F Ratio	Prob > F
<u>Sitophilus oryzae:</u>				
Plant Species	2	40567.225	75.776**	<.0001
Plant part	2	1332.547	22.859**	<.0001
Plant Species*Plant part	4	20700.3	81.534**	<.0001
Contact Time (Days)	5	23591.5	69.691**	<.0001
Plant Species*Cont.Time	10	12323.4	16.180**	<.0001
Plant part* Cont.Time	10	12424.0	14.681**	<.0001
Treatment (% v/w)	10	923336.4	3636.826**	0.0000
Plant Species*Treatment	20	12825.1	13.777**	<.0001
Plant part*Treatment	20	24205.4	15.890**	<.0001
Cont.Time *Treatment	50	137534.4	33.167**	<.0001
Error	1181			
<u>Rhyzopertha dominica:</u>				
Plant Species	2	55776.237	66.885**	<.0001
Plant part	2	2276.55	43.995**	<.0001
Plant Species*Plant part	4	10.68404	30.379**	<.0001
Contact Time (Days)	8	11.01530	15.660**	<.0001
Plant Species*Cont.Time	16	43.22097	27.310**	<.0001
Plant part* Cont.Time	16	54.94738	31.247**	<.0001
Treatment (% v/w)	10	628.59267	893.661**	0.0000
Plant Species*Treatment	20	29.43299	15.216**	<.0001
Plant part*Treatment	20	85.44466	28.583**	<.0001
Cont.Time *Treatment	80	230.44516	19.856**	<.0001
Error	1022			
<u>Tribolium castaneum:</u>				
Plant Species	2	30045.65	55.779**	<.0001
Plant part	2	1557.09	29.456**	<.0001
Plant Species*Plant part	4	6.0487	30.027**	<.0001
Contact Time (Days)	8	13.2518	32.892**	<.0001
Plant Species*Cont.Time	16	3.5014	3.863**	<.0001
Plant part* Cont.Time	16	1.0531	1.046**	0.4025
Treatment (% v/w)	10	244.5517	606.999**	<.0001
Plant Species*Treatment	20	116.2840	104.955**	<.0001
Plant part*Treatment	20	47.9120	27.982**	<.0001
Cont.Time *Treatment	80	50.9913	7.671**	<.0001
Error	1022			
<u>Callosobruchus chinensis:</u>				
Plant Species	2	73221.45	79.653**	<.0001
Plant part	2	2278.65	52.445**	<.0001
Plant Species*Plant part	4	0.085847	0.695 ^{NS}	0.4049
Contact Time (Days)	3	31.183018	252.553**	<.0001
Plant Species*Cont.Time	6	1.033471	4.185*	0.0160
Plant part* Cont.Time	6	8.886635	23.991**	<.0001
Treatment (% v/w)	10	556.342	1022.565**	<.0001
Plant Species*Treatment	20	10.813849	17.516**	<.0001
Plant part*Treatment	20	7.864657	4.550**	<.0001
Cont.Time *Treatment	30	70.476237	19.026**	<.0001
Error	365			

*Significant at $\alpha=0.05$, ** Highly significant at $\alpha=0.01$ and NS = non-significant at $\alpha=0.05$.

Appendix 4b: ANOVA- interspecies variation in adult F₁ progeny counts of four storage insects in contact with essential oil-treated grains

Source of variation	DF	Sum of Squares	F Ratio	Prob > F
<i>Sitophilus oryzae</i>:				
Plant Species	2	1005.780	19.986**	<.0001
Plant part	2	988.545	15.557**	<.0001
Plant Species*Plant part	4	10.233388	148.839**	<.0001
Contact Time (Days)	5	49.425670	239.624**	<.0001
Plant Species*Cont.Time	10	9.990215	20.758**	<.0001
Plant part* Cont.Time	10	6.583618	23.939**	<.0001
Treatment (% v/w)	6	32.014570	93.127**	<.0001
Plant Species*Treatment	12	0.887365	1.291 ^{NS}	0.2320
Plant part*Treatment	12	2.949418	5.362**	<.0001
Cont.Time *Treatment	30	82.429389	22.202**	<.0001
Error	585			
<i>Rhyzopertha dominica</i>:				
Plant Species	2	12334.008	27.787**	<.0001
Plant part	2	4559.43	20.973**	<.0001
Plant Species*Plant part	4	17.215	112.665**	<.0001
Contact Time (Days)	5	2747.6138	55.922**	<.0001
Plant Species*Contact Time	10	427.2097	3.726**	0.0006
Plant part* Contact Time	10	307.9221	2.686**	0.0095
Treatment (% v/w)	6	239.6328	2.439*	0.0244
Plant Species*Treatment	12	991.3019	5.044**	<.0001
Plant part*Treatment	12	105.5431	0.537 ^{NS}	0.8910
Contact Time *Treatment	30	1448.7181	2.949**	<.0001
Error	641			
<i>Tribolium castaneum</i>:				
Plant Species	2	3326.334	38.894**	<.0001
Plant part	2	1678.45	19.876**	<.0001
Plant Species*Plant part	4	2576.665	22.778**	<.0001
Contact Time (Days)	5	1187.030	24.612**	<.0001
Plant Species*Contact Time	10	119.324	1.060 ^{NS}	0.3876
Plant part* Contact Time	10	703.538	6.252**	<.0001
Treatment (% v/w)	6	66.148	0.686 ^{NS}	0.6612
Plant Species*Treatment	12	1047.573	5.430**	<.0001
Plant part*Treatment	12	611.504	3.170**	0.0002
Contact Time *Treatment	30	871.822	1.808**	0.0057
Error	641			
<i>Callosobruchus chinensis</i>:				
Plant Species	2	2277.234	28.854**	<.0001
Plant part	2	1033.78	13.975**	<.0001
Plant Species*Plant part	4	2655.235	17.665**	<.0001
Contact Time (Days)	2	5544.545	47.456**	<.0001
Plant Species*Contact Time	4	23.9251	59.141**	<.0001
Plant part* Contact Time	4	79.0496	117.242**	<.0001
Treatment (% v/w)	6	445.996	7.056*	<.0112
Plant Species*Treatment	12	26.4644	17.841**	<.0001
Plant part*Treatment	12	34.5611	32.037**	<.0001
Contact Time *Treatment	12	348.7052	27.805**	<.0001
Error	678			

*Significant at $\alpha = 0.05$, ** Highly significant at $\alpha = 0.01$ and NS = non-significant at $\alpha = 0.05$.

Appendix 5a: ANOVA- interspecies variation in *O. americanum* essential oils' residual contact toxicity against four storage insects

Source of variation	DF	Sum of Squares	F Ratio	Prob > F
<i>Ocimum americanum</i> fruit oil				
<i>S. oryzae</i>:				
Storage Duration (Days)	3	1.1163	21.2519**	<.0001
Contact Time (Days)	4	8.8981	127.045**	<.0001
Stor.Duration*Cont. Time	12	1.0949	5.2107**	<.0001
Treatment (% v/w)	6	1301.1044	12384.62**	0.0000
Stor.Duration*Treatment	18	1.3895	4.4086**	<.0001
Contact Time*Treatment	24	5.6742	13.5025**	<.0001
Error	489			
<i>R. dominica</i>:				
Storage Duration (Days)	2	35.64238	254.046**	<.0001
Contact Time (Days)	4	32.98893	117.566**	<.0001
Stor.Duration*Cont. Time	8	4.89317	8.7192**	<.0001
Treatment (% v/w)	6	643.82004	1529.64**	<.0001
Stor.Duration*Treatment	12	49.27412	58.5346**	<.0001
Contact Time*Treatment	24	16.67373	9.9037**	<.0001
Error	360			
<i>T. castaneum</i>:				
Storage Duration (Days)	2	37.49828	266.159**	<.0001
Contact Time (Days)	4	32.56518	115.572**	<.0001
Stor.Duration*Cont. Time	8	4.06285	7.2094**	<.0001
Treatment (% v/w)	6	740.24231	1751.39**	<.0001
Stor.Duration*Treatment	12	73.46731	86.9105**	<.0001
Contact Time*Treatment	24	17.54911	10.3801**	<.0001
Error	360			
<i>Ocimum americanum</i> leaf oil				
<i>C. chinensis</i>:				
Storage Duration (Days)	3	9.57372	37.7727**	<.0001
Contact Time (Days)	2	96.26859	569.736**	<.0001
Stor.Duration*Cont. Time	6	9.56044	18.8602**	<.0001
Treatment (% v/w)	6	483.59025	953.993**	<.0001
Stor.Duration*Treatment	18	8.67863	5.7069**	<.0001
Contact Time*Treatment	12	68.55226	67.6176**	<.0001
Error	285			

*Significant at $\alpha=0.05$, ** Highly significant at $\alpha=0.01$ and NS = non-significant at $\alpha=0.05$.

Appendix 5b: ANOVA- interspecies variation in *T. vogelii* essential oils' residual contact toxicity against four storage insects

Source of variation	DF	Sum of Squares	F Ratio	Prob > F
<i>Tephrosia vogelii</i> fruit oil				
<i>S. oryzae</i>:				
Storage Duration (Days)	3	5.17182	30.121**	<.0001
Contact Time (Days)	3	70.94834	413.20**	<.0001
Stor.Duration *Cont. Time	9	14.39059	27.937**	<.0001
Treatment (% v/w)	6	698.00944	2032.59**	<.0001
Stor.Duration *Treatment	18	9.28646	9.0140**	<.0001
Contact Time *Treatment	18	54.58894	52.987**	<.0001
Error	387			
<i>R. dominica</i>:				
Storage Duration (Days)	2	18.89615	110.188**	<.0001
Contact Time (Days)	3	52.16772	202.802**	<.0001
Stor.Duration *Contact Time	6	25.93197	50.4053**	<.0001
Treatment (% v/w)	6	470.06496	913.689**	<.0001
Stor.Duration *Treatment	12	9.83120	9.5547**	<.0001
Contact Time *Treatment	18	28.42608	18.418**	<.0001
Error	285			
<i>T. castaneum</i>:				
Storage Duration (Days)	1	1.04926	3.749**	0.0544
Contact Time (Days)	3	2.51857	2.9997**	0.0319
Stor.Duration *Contact Time	3	0.40902	0.4871 ^{NS}	0.6916
Treatment (% v/w)	6	498.11196	296.629**	<.0001
Stor.Duration *Treatment	6	11.79219	7.0223**	<.0001
Contact Time *Treatment	18	45.93937	9.1191**	<.0001
Error	183			
<i>Tephrosia vogelii</i> leaf oil				
<i>C. chinensis</i>:				
Storage Duration (Days)	2	3.46000	42.346**	<.0001
Contact Time (Days)	2	49.62139	607.307**	<.0001
Stor.Duration *Contact Time	4	0.86978	5.3225**	0.0004
Treatment (% v/w)	6	333.06679	1358.78**	<.0001
Stor.Duration *Treatment	12	3.12433	6.3730**	<.0001
Contact Time *Treatment	12	38.32840	78.182**	<.0001
Error	210			

*Significant at α= 0.05, ** Highly significant at α= 0.01 and NS = non-significant at α= 0.05.

Appendix 6: ANOVA- variation in residual effects of *O. americanum* and *T. vogelii* leaf essential oils against adult *C. chinensis* F₁ progeny over a 4 months storage period

Source of variation	DF	Sum of Squares	F Ratio	Prob > F
F₁ PROGENY COUNTS:				
<u>Ocimum americanum leaf oil:</u>				
Storage Duration (Days)	3	177.5024	173.192**	<.0001
Contact Time (Days)	4	881.9934	645.431**	<.0001
Stor.Duration*Cont. Time	12	107.2382	26.1585**	<.0001
Treatment (% v/w)	6	1926.1191	939.673**	<.0001
Stor.Duration*Treatment	18	133.1327	21.650**	<.0001
Cont. Time*Treatment	24	267.2944	32.600**	<.0001
Error	489			
<u>Tephrosia vogelii leaf oil:</u>				
Storage Duration (Days)	2	536.4757	1364.569**	<.0001
Contact Time (Days)	4	351.9765	447.640**	<.0001
Stor.Duration*Cont.Time	8	79.4404	50.516**	<.0001
Treatment (% v/w)	6	1090.3934	924.501**	<.0001
Stor.Duration*Treatment	12	218.7352	92.729**	<.0001
Cont.Time*Treatment	24	155.0950	32.875**	<.0001
Error	360			
REDUCTION IN F₁ PROGENY				
<u>Ocimum americanum leaf oil:</u>				
Storage Duration (Days)	3	10793.54	27.439**	<.0001
Contact Time (Days)	4	5028.49	9.588**	<.0001
Stor.Duration*Cont.Time	12	16252.47	10.329**	<.0001
Treatment (% v/w)	6	668360.97	849.543**	<.0001
Stor.Duration*Treatment	18	9254.20	3.9210**	<.0001
Cont.Time*Treatment	24	2702.84	0.859 ^{NS}	0.6598
Error	489			
<u>Tephrosia vogelii leaf oil:</u>				
Storage Duration (Days)	2	33934.78	251.328**	<.0001
Contact Time (Days)	4	9653.51	35.748**	<.0001
Stor.Duration*Cont.Time	8	1678.08	3.1071**	0.0021
Treatment (% v/w)	6	524752.12	1295.474**	<.0001
Stor.Duration*Treatment	12	28251.64	34.873**	<.0001
Cont.Time*Treatment	24	8400.48	5.185**	<.0001
Error	360			

*Significant at $\alpha=0.05$, ** Highly significant at $\alpha=0.01$ and NS = non-significant at $\alpha=0.05$.

Appendix 7: ANOVA- variation in residual effects of *O. americanum* and *T. vogelii* fruit essential oils on % germination of wheat seeds over a 4 months storage

Source	DF	Sum of Squares	F Ratio	Prob > F
<u>PERCENT GERMINATION:</u>				
Plant Species	1	4636.450	187.44**	<.0001
Storage Duration (Days)	3	4810.602	64.83**	<.0001
Plant Species*Storage Duration	3	2622.645	35.34**	<.0001
Treatment (% v/w)	6	22818.910	153.75**	<.0001
Plant Species*Treatment	6	1236.653	8.33**	<.0001
Storage Duration*Treatment	18	1432.013	3.22**	<.0001
Error	183	4526.544		

GERMINATION INHIBITION(%):

Plant Species	1	6196.599	187.44**	<.0001
Storage Duration (Days)	3	6429.353	64.83**	<.0001
Plant Species*Storage Duration	3	3505.156	35.34**	<.0001
Treatment (% v/w)	6	30497.390	153.75**	<.0001
Plant Species*Treatment	6	1652.783	8.33**	<.0001
Storage Duration*Treatment	18	1913.880	3.22**	<.0001
Error	183	6049.710		

*Significant at $\alpha=0.05$, ** Highly significant at $\alpha=0.01$ and NS = non-significant at $\alpha=0.05$.

Appendix 8: ANOVA- variation in instant repellence of *O. americanum*, *L. camara* and *T. vogelii* essential oils against adult stages of four storage insect pests

Source	DF	Sum of Squares	F Ratio	Prob > F
<i>Sitophilus oryzae</i>:				
Plant Species	2	3244.55	93.22**	<.0001
Plant Part	2	2998.41	65.76**	<.0001
Plant Species*Plant Part	4	54.911	0.32 ^{NS}	0.5721
Exposure Time (Hours)	3	4324.459	8.39**	<.0001
Plant Species*Exposure Time	6	2585.591	2.51*	0.0214
Plant Part*Exposure Time	6	625.985	0.61 ^{NS}	0.7246
Treatment (μL/g)	4	9304.763	13.54**	<.0001
Plant Species*Treatment	8	5712.657	4.16**	<.0001
Plant Part*Treatment	8	3125.453	2.27*	0.0217
Exposure Time *Treatment	12	2087.266	1.01 ^{NS}	0.4362
Error	437	75085.05		
<i>Rhyzopertha dominica</i>:				
Plant Species	2	9975.12	34.75**	<.0001
Plant Part	2	6553.55	29.65**	<.0001
Plant Species*Plant Part	4	2529.69	18.48**	<.0001
Exposure Time (Hours)	3	3832.10	9.33**	<.0001
Plant Species*Exposure Time	6	1348.67	1.64**	0.1338
Plant Part*Exposure Time	6	2003.72	2.44*	0.0248
Treatment (μL/g)	4	14019.59	25.61**	<.0001
Plant Species*Treatment	8	3763.49	3.44**	0.0008
Plant Part*Treatment	8	6434.82	5.88**	<.0001
Exposure Time *Treatment	12	1993.89	1.21 ^{NS}	0.2703
Error	437	59810.05		
<i>Tribolium castaneum</i>:				
Plant Species	2	11223.60	21.95**	<.0001
Plant Part	2	77894.22	18.90**	<.0001
Plant Species*Plant Part	4	3122.45	10.34**	<.0001
Exposure Time (Hours)	3	1033.7500	1.41 ^{NS}	0.2386
Plant Species*Exposure Time	6	5228.9486	3.57 ^{NS}	0.0019
Plant Part*Exposure Time (Hours)	6	978.9179	0.67 ^{NS}	0.6747
Treatment (μL/g)	4	8672.2917	8.89**	<.0001
Plant Species*Treatment	8	6613.6043	3.39**	0.0009
Plant Part*Treatment	8	3260.1740	1.67 ^{NS}	0.1040
Exposure Time *Treatment	12	666.3178	0.23 ^{NS}	0.9970
Error	358	87289.31		
<i>Callosobruchus chinensis</i>:				
Plant Species	2	7789.11	19.55	<.0001
Plant part	2	5677.23	9.97**	<.0001
Plant Species*Plant part	4	3997.34	6.77**	<.0001
Exposure Time (Hours)	3	1928.6458	9.19**	<.0001
Plant Species*Exposure Time	6	791.1209	1.89 ^{NS}	0.0824
Plant part*Exposure Time	6	358.0456	0.85 ^{NS}	0.5295
Treatment (μL/g)	4	6881.5417	24.60**	<.0001
Plant Species*Treatment	8	2131.7053	3.81**	0.0003
Plant part*Treatment	8	864.6778	1.55 ^{NS}	0.1401
Exposure Time *Treatment	12	791.9949	0.94 ^{NS}	0.5030
Error	358	25035.060		

Appendix 9: ANOVA- variation in residual repellence of *O. americanum*, *L. camara* and *T. vogelii* essential oils against adult stages of four storage insect pests

Source	DF	Sum of Squares	F Ratio	Prob > F
<i>Sitophilus oryzae</i>:				
Plant Species	1	0.930	0.013 ^{NS}	0.9092
Plant Part	0	0.000	.	.
Plant Species*Plant Part	0	0.000	.	.
Storage Duration (Days)	2	502.455	3.52*	0.0303
Plant Species*Storage Duration	2	473.735	3.31*	0.0370
Plant Part*Storage Duration	0	0.000	.	.
Exposure Time (Hours)	3	11587.612	54.04**	<.0001
Plant Species*Exposure Time	3	125.409	0.58 ^{NS}	0.6251
Plant Part*Exposure Time	0	0.000	.	.
Storage Duration *Exposure Time	6	912.723	2.13*	0.0484
Treatment (μL/g)	6	89027.827	207.61**	<.0001
Plant Species*Treatment	6	137.351	0.32 ^{NS}	0.9265
Plant Part*Treatment	0	0.000	.	.
Storage Duration *Treatment	12	6337.128	7.39**	<.0001
Exposure Time *Treatment	18	14452.232	11.23**	<.0001
Error	609	43524.89		
<i>Rhyzopertha dominica</i>:				
Plant Species	0	0.000	.	.
Plant Part	0	0.000	.	.
Plant Species*Plant Part	0	0.000	.	.
Storage Duration (Days)	0	0.000	.	.
Plant Species*Storage Duration	0	0.000	.	.
Plant Part*Storage Duration	0	0.000	.	.
Exposure Time (Hours)	3	938.728	5.84**	0.0007
Plant Species*Exposure Time	3	40.513	0.25 ^{NS}	0.8599
Plant Part*Exposure Time	0	0.000	.	.
Storage Duration *Exposure Time	3	152.679	0.95 ^{NS}	0.4170
Treatment (μL/g)	6	26435.714	82.20**	<.0001
Plant Species*Treatment	6	4828.125	15.01**	<.0001
Plant Part*Treatment	0	0.000	.	.
Storage Duration *Treatment	6	1432.366	4.45**	0.0003
Exposure Time *Treatment	18	5653.274	5.86**	<.0001
Error	285	15275.223		

***Tribolium castaneum*:**

Plant Species	0	0.000	.	.
Plant Part	0	0.000	.	.
Plant Species*Plant Part	0	0.000	.	.
Storage Duration (Days)	1	1464.509	14.97**	0.0001
Plant Species*Storage Duration	1	339.509	3.47 ^{NS}	0.0631
Plant Part*Storage Duration	0	0.000	.	.
Exposure Time (Hours)	3	348.859	1.19 ^{NS}	0.3136
Plant Species*Exposure Time	3	63.839	0.22 ^{NS}	0.8843
Plant Part*Exposure Time	0	0.000	.	.
Storage Duration *Exposure Time	6	868.824	1.48 ^{NS}	0.1830
Treatment ($\mu\text{L/g}$)	6	58198.165	99.13**	<.0001
Plant Species*Treatment	6	840.179	1.43 ^{NS}	0.2007
Plant Part*Treatment	0	0.000	.	.
Storage Duration *Treatment	12	1079.613	0.92 ^{NS}	0.5270
Exposure Time *Treatment	18	5346.339	3.04**	<.0001
Error	498	48728.93		

***Callosobruchus chinensis*:**

Plant Species	1	289.286	4.57*	0.0332
Plant Part	0	0.000	.	.
Plant Species*Plant Part	0	0.000	.	.
Storage Duration (Days)	1	393.750	6.22*	0.0130
Plant Species*Storage Duration	1	98.438	1.55 ^{NS}	0.2131
Plant Part*Storage Duration	0	0.000	.	.
Exposure Time (Hours)	3	2665.402	14.03**	<.0001
Plant Species*Exposure Time	3	476.339	2.51 ^{NS}	0.0585
Plant Part*Exposure Time	0	0.000	.	.
Storage Duration *Exposure Time	3	72.768	0.3832	0.7652
Treatment ($\mu\text{L/g}$)	6	52691.964	138.72**	<.0001
Plant Species*Treatment	6	404.464	1.06 ^{NS}	0.3831
Plant Part*Treatment	0	0.000	.	.
Storage Duration *Treatment	6	5045.313	13.28**	<.0001
Exposure Time *Treatment	18	5004.911	4.39**	<.0001
Error	396	25069.420		

Appendix 10: Author's Publications

10.1 Refereed journal publications

1. **Ogendo, J.O.**, Kostyukovsky, M., Ravid, U., Matasyoh, J.C., Deng, A.L., Omolo, E.O., Kariuki, S.T., Shaaya, E. (2008) Bioactivity of *Ocimum gratissimum* oil and two constituents against five insect pests attacking stored food products. *Journal of Stored Products Research* 44: 328-334.
2. **Ogendo, J. O.**, Deng, A. L., Tuey, R. K., Bett, P.K. (2008) Toxic effects of *Lantana camara* L. leaf extracts against the maize weevil, *Sitophilus zeamais* Motsch.(Coleoptera: Curculionidae) in stored maize grains. *Egerton Journal* (Accepted October 2008).
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