CHAPTER 1

GENERAL INTRODUCTION

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Maize is a staple food in Africa

Maize (*Zea mays* L.) belongs to the grass family Gramineae. It is an essential component of global food security. Maize is one of the most important cereal crops grown in the world and it forms one of the major diets of millions of people. In Africa, maize is primarily grown by small-scale farmers for use as both human food and animal feed. Its cob is consumed in different ways. For example, it could be grilled, boiled, roasted or made into various products (Polaszek & Khan, 1998). Industrially, maize is used to produce alcohol, starch, pulp abrasive, oil in the pharmaceutics and recently for fuel production (Morris, 2007; Acharya & Young, 2008; Tom, 2008). In Zimbabwe, maize is used for other non-food purposes such as beer brewing and in exchange for goods and services (Stanning, 1989). The principal producers of maize in sub-Saharan Africa are Kenya, South Africa and Tanzania, followed by Ethiopia and Nigeria (Kfir, 1998; Polaszek and Khan, 1998; Seshu Reddy, 1998).

Increased productivity in staple food, such as maize, is critical to raising rural incomes and stimulating broad-based economic growth (Eicher & Byerlee 1997). The demand for maize in developing countries, arguably, surpasses the demand for both wheat and rice. This is as a result of the growth in meat and poultry consumption, which consequently, have led to the rapid increase in the demand for maize as livestock feed. Thus, the exploding demand for maize presents an urgent challenge for most developing countries (Pingali & Pandey, 2000).

Constraints of maize production

Despite the worldwide increase in the demand for maize, its production is constrained by various biological, physical and environmental factors. These include the problems of insect attack, weeds and pathogen infestation, soil fertility and climate (Sanchez *et al*., 1997). In addition, the substitution of traditional cultivars by high-yielding varieties has raised the specter of massive maize-crop failure because of increased susceptibility of the latter to diseases and pests (Plucknett & Smith, 1982). Amidst other constraints of maize production, insects constitute a major threat. Insect pests destroy approximately 14% of all potential food production, including maize, despite the yearly application of more than 3000 million kilograms of pesticides (Pimentel, 2007). Losing crops to insect pests constitutes a great constraint to the realization of food security worldwide. Therefore, in order to meet the food demand for the ever increasing world population, it is necessary to address the issue of maize grain loss to insect pest damage (Berenbaum, 1995).

Insects are responsible for post harvest maize losses

Today, humans are waging an undeclared war against insects in the competitive struggle for existence and almost no crop in Africa is free from attack by insects, at least to some degree (Berenbaum, 1995).

Insect pest damage to stored grains including maize, results in major economic losses in Africa, where subsistence grain production supports the livelihoods of the majority of the population (Udo, 2005). Insect infestation of maize grains leads to the reduction in both quality and quantity of harvested crops and in most cases pre-disposes the stored grains to secondary attack by disease causing pathogens (Evans, 1987). Naturally, harvested grains such as maize are stored by small-scale farmers to provide food reserves as well as seed grains. Grain losses are severe among small-scale farmers who do not have access to modern storage techniques. In most cases maize grains are stored in sacks, plastic drums, hung on roofs or spread on the floor (Odeyemi *et al.,* 2006). Grain losses as high as 80% have been reported in developing countries (Pingali & Pandey, 2000; Tapondjou *et al.,* 2002). Post-harvest losses due to *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) have been recognized as an important constraint to grain storage in Africa (Markham *et al*., 1994; Oduor *et al.,* 2000).

Sitophilus zeamais (maize weevil) is a cosmopolitan pest of stored maize in Africa. Damage caused by this insect becomes obvious when the adult insect (Fig. 1) makes holes that reaches approximately 1mm in size in the grain and deposit its eggs within the hole. The insect then seals the hole with a gelatinous waxy secretion. The developmental stage of the insect takes place within the grain after which the adult weevil bores its way out, leaving a characteristic emergence hole on the grain (Hill, 1983; Rees, 2004).

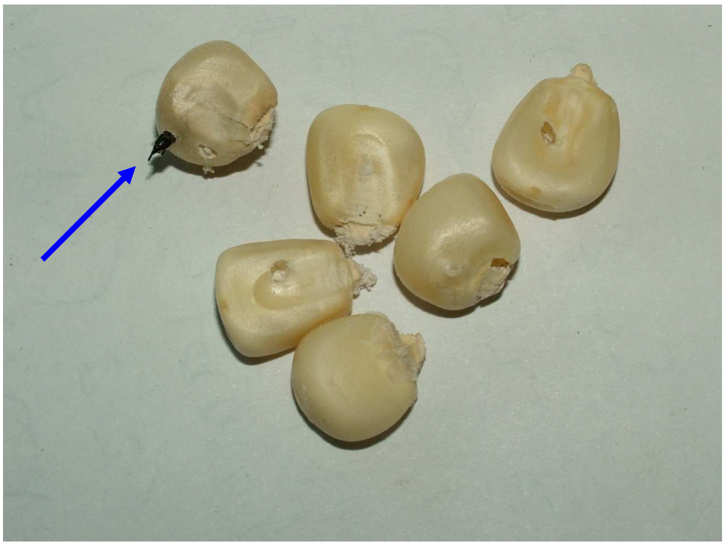

Fig. 1: Emerging adult of *S. zeamais* (arrowed) from infested grain

Protection of stored-grains against insect attack

The protection of stored-maize grains against insect attack is essential, especially for countries that have inadequate storage facilities and climatic conditions that favour deterioration of grains (Villalobos & Robledo, 1999). Currently, the control of insect pests is largely dependent on the use of synthetic chemicals in most parts of the world. However, the continuous use of synthetic insecticides is leading to problems such as pest resurgence, resistance and environmental hazards, including human poisoning and toxicity to other non-target organisms (Subramanyam & Hagstrum, 1995; Pascual-Villalobos & Robledo, 1999; Banwo & Adamu, 2003; Bughio & Wilkins, 2004). Furthermore, relatively few chemicals are registered for use on grains and in storage structures. Already, there are threats to the two widely used fumigants (methyl bromide and phosphine). In fact, there is progressive restriction on the use of phosphine because of its ozone depletion potential (Annis *et al*., 1999, EPA, 2001). There is, therefore, the need for the development of safe and environmentally friendly alternative agents for the control of insect pests of stored grains.

The search for alternative insect control agents.

The concern of the public on the problems associated with the continuous use of conventional insecticides have necessitated the search for possible alternatives. The use of plants in the protection of grains against insect infestation has been an age-long practice among small-scale farmers in Africa (Hassanali *et al*., 1990; Poswal and Akpa, 1991). Botanical insecticides comprise only a very small portion of the total volume of insecticides used annually, nonetheless, they remain important in insect pest management because they are believed to provide the most effective control against insect pests that have become resistant to other insecticides (Weinzierl, 2000). Plant-derived insecticides are short-lived in the environment, thus pose less risk to non-target organisms and are accepted by organic certification programs and certain consumer groups because they are naturally occurring (Isman, 2000; Weinzierl, 2000). Phytochemicals such as rotenone, nicotine and pyrethrum were all used as pesticides before the advent of synthetic insecticides (Jacobson 1989; Ohigash *et al*., 1991). Many members of families such as Myrtaceae, Asteraceae, Piperaceae, Meliaceae and Annonaceae are known to possess various chemical compounds which act as antifeedant, repellent insecticides or growth inhibitors to many insect species (Srivastava *et al*., 2001; Kouninki *et al.*, 2005; Formisano *et al*., 2008; Odeyemi *et al*., 2008). Certain plant essential oils and or their constituents have broad spectra of activity against insect and mite pests, fungi and nematodes (Isman, 2000).

Naturally, botanical insecticides are believed to possess certain attributes which put them at a higher advantage over conventional insecticides. These include low mammalian toxicity, less persistence in the environment, selectivity towards target pests and non-phytotoxicity (Rosenthal, 1986; Isman, 2006). These have led to the belief that plant-derived insecticides are safer than synthetic products. This, however, is not always the case. For instance, nicotine extracted from tobacco is one of the most widely-known botanicals. Although effective in pest control, nicotine is highly toxic to mammals and can readily be absorbed through the eyes, skin and mucous membranes (Carr *et al*., 1991). Rotenone, a polycyclic ketone widely used as a broad-spectrum insecticide extracted from the roots of *Lonchocarpus* spp, *Derris* spp and several other leguminous plant genera, is extremely toxic to aquatic life and also exhibits some level of toxicity to mammals (Carr *et al*., 1991; Cranshaw, 1992). The persistence of synthetic pesticides in the environment and in treated foods is a major constraint to their use. Conversely, the rapid degradation of phytochemical insecticides on exposure to light renders them unstable and less persistent (Jovetic, 1994), hence necessitating repeated applications. Although there is considerable potential for insecticides of plant origin, these products should be handled with caution. Detailed studies on their mammalian toxicity and residual effects are also required.

OBJECTIVES OF THIS STUDY

The overall aim of this project was to study the use of essential oils for the control of *Sitophilus zeamais*, a pest of stored maize grains; while the specific objectives were:

1) To obtain baseline information on the farmers' knowledge and experience of indigenous insect pest control in the Eastern Cape Province of South Africa

Indigenous Knowledge (IK) is unique to a particular culture and society. It is the basis for local decision-making in agriculture, health, natural resource management and other activities. IK is embedded in community practices, institutions, relationships and rituals (Woytek, 1998). It provides the basis for problem-solving strategies for local communities, especially the poor, and also represents an important component of global knowledge on development issues. In most cases, IK is an underutilized resource in the development processes (Woytek, 1998). Learning from IK, by investigating first, what local communities know and have, can improve the understanding of local conditions and provide a productive context for activities designed to help the communities. Traditional wisdom generated by centuries of trial and error often reveals the uses of plants that are related to microbial control, medicine and pest management (Dunkel & Sear, 1998).

Throughout history, many cultures have used natural products from plants to protect themselves, their crops and their livestock against insects (Dunkel & Sear, 1998).

Therefore, one of the objectives of this study was to understand the farmer's knowledge and experience of using indigenous methods in the control of insect pests of stored -

maize grain. The plants used by the small-scale farmers for insect pest control were identified and documented.

2) To screen some plant essential oils for their insecticidal activities using three bioassay methods

Problems associated with the use of conventional insecticides such as pest resistance and resurgence, negative impact on non-target organism, residues in food and the environment have generated some interest among researchers to search for possible environmental friendly alternatives (Duke, 1990; Shaaya *et al*., 1991).

Traditionally, essential oils obtained by steam distillation of plant foliage have been used to protect stored grains and legumes and to repel flying insects in the homes (Isman, 2000). Today, there is increasing interest in the use of natural products from plants to control pests.

Another objective of this study was to screen essential oils from *Mentha longifolia* L. subsp. *capensis*, *Tagetes minuta* L., *Helichrysum odoratissimum*, *Perlargonium graveolens* and *Rosmarinus officinalis* for their insecticidal potential against the common maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae).

3) To evaluate the effect of the essential oils (in 2 above) on some proximate and quality parameters of maize grains

The importance of cereal grains to the nutrition of millions of people around the world cannot be over-emphasized. Cereal grains form a major component of the diet of most people in developing countries, and are good sources of carbohydrate and protein (FAO, 1992). However, cases of changes in nutritional compositions of stored-grains, as a result of insect infestations have been reported (Jood *et al.,* 1996). These changes are influenced, to some extent, by factors such as the duration of storage, initial infestation level and storage conditions. It is therefore evident that there is a significant correlation between the degree of insect infestation and nutritional composition of stored grains (Jood *et al.*, 1996; Asawalam, 2006). However, information is lacking on the proximate composition of maize grains treated with essential oils.

Thus, one of the objectives of this study was to investigate the effect of the essential oils of *M. longifolia* and *T. minuta* on some chemical constituents and quality parameters such as colour and odour of maize grains treated with the above mentioned oils. These plants were chosen based on their readily and easily availability and their record for the production of good essential oils.

4) To investigate the toxicological effect of the essential oils using the rat model

The search for alternative insect pest control agents, have made the use of plant-derived insecticides to receive worldwide attention. Several plant species have been found to possess insecticidal properties against a wide range of insect pests. Protecting stored grains with plant products has been a common practice among some small-scale farmers. Unfortunately, the safety of most of these plants is often overlooked, simply because, most of them have been in use for a long time. However, it is very important to note that botanical insecticides are not safer than the synthetic ones, simply because the former are of natural origin (Weinzierl & Henn, 1994).

Therefore, another objective of this study was to evaluate the safety of the two essential oils (*M. longifolia* and *T. minuta*) tested in this study using rat models.

PLANTS USED FOR THIS STUDY

Prior to the commencement of this study, a baseline survey was conducted among the small-scale farmers in the Eastern Cape, South Africa, to obtain information on plants used for the protection of agricultural crops against insect pest attack. Information gathered from the farmers was augmented with that found in the literature and the following plants were found to be the commonest and most frequently used for protection against insect attacks; *Mentha longifolia* L. subspecies *capensis*, *Tagetes minuta* L., *Helichrysum oddoratissimum* L., *Pelargonium graveolens* L. and *Rosmarinus officinalis* L.

Mentha longifolia L.

M. longifolia L. (Wild mint) is a perennial herb with creeping rhizomes below the ground and erect flowering stems of up to 80 cm in height. There are several subspecies of *M. longifolia*, however in South Africa subspecies *capensis* is the most common. It is a fast growing plant that is widespread in the Eastern Cape, and a popular plant used for the treatment of different ailments such as cough, cold, asthma and other diseases (Van Wyk and Gericke, 2000; Van Wyk *et al*., 2002).

Figure 2: *Mentha longifolia* L.

Mentha is a genus of wide distribution and considerable economic importance. The shoots and leaves of several species are often used as a condiment. The essential oils, which are steam distilled from the herbage are processed into flavourings for food, medicine, mouthwash, toothpaste and powder, chewing gum and candy (Chamber and Hummer, 1994). Mint is the common name for approximately 25 perennial species from the genus *Mentha* in the Lamiaceae family. They are sources of essential oils which are used for flavouring, perfume production and medicinal purposes (Lange and Croteau, 1999).

The strong smell of the leaves is known to keep mosquitoes away when rubbed onto the body (Hutchings and Van Staden, 1994). The chief component of the essential oil from the leaves of *M. longifolia* appears to be the monoterpenes ketone and menthone (Oyedeji and Afolayan, 2005). The essential oil from the plant has been found to be an effective insecticide against several insect species (Cetin *et al*., 2006; Pascual-Villalobos and Robledo, 1999).

Pelargonium graveolens L.

Pelargonium graveolens (L.) belongs to the family Geraniaceae. The genus constitutes a large number of herbs, shrubs or undershrubs which are distributed mainly in South Africa and a few tropical Africa, Syria and Australia (Rana *et al*., 2002). *Pelargonium graveolens* is a fragrant bushy plant up to 100 cm high (Fig.3).

Figure 3: *Pelargonium graveolens* L.

The rose scented leaves of *P. graveolens* produces aromatic oil opon distillation which is referred to as geranium oil. The oil is one of the top 20 valued essential oils produced in the world. For instance, it is used in perfumery, cosmetics, food and pharmaceutical industries (Demarne and van der Walt, 1989; Narayana *et al*., 1986; Rao, 2002; Ravindra *et al*., 2004). Essential oil from this plant is obtained by hydrodistillation of the aerial parts of the plant.

Helichrysum oddoratissimum L.

Helichrysum oddoratissimum L. is an aromatic perennial herb or shrublet of the family Asteraceae. There are over 200 species of *Helichrysum* distributed all over South Africa, of which *H. oddoratissimum* is one of the best known and commonly used for medicinal purposes (Fig. 4).

Figure 4: *Helichrysum oddoratissimum* L.

H. oddoratissimum is used for the treatment of ailments such as coughs, colds, fever, infections, headache and menstrual pain. It is also a popular ingredient for wound dressings (Van Wyk *et al*., 2002). The plant is rubbed on bedding materials since it is known to be an effective insect repellent.

Tagetes minuta L.

Tagetes minuta L. is an important species of the family Asteraceae and is commonly known as wild Mexican marigold. *T. minuta* is an essential oil yielding plant. The oil is valued for its numerous uses. For example, it is used as flavoring in food, tea, perfumery, pharmaceutical industry and possesses medicinal values (Vasudevan *et al*., 1997; Kaul *et al.,* 2000; Ramesh & Virendra, 2008). Most importantly, the essential oil from *T. minuta* is well known for its insecticidal activities (Broussalis *et al*., 1999; Tomova *et al*., 2005).

Figure 5: *Tagetes minuta* L.

The plant is a branched and erect annual herb, approximately 1-2 m tall. The lower surface of the leaves possesses a number of small, punctuate, multicellular glands, which exude a liquorice-like aroma when ruptured. Glands are also found on the stems and bracts (Singh *et al*., 2003). *T. minuta* is native to temperate forests and mountain regions of most of the countries in the world. It originated in South America and has spread throughout the world as a weed. It has been reported from South Africa, Australia, Nigeria, India, Uruguay, East Africa (Kenya), Brazil, France, Chile, and the Chaco region of Paraguay (Singh *et al*., 2003).

***Rosmarinus officinalis* L.**

Rosmarinus officinalis L. (rosemary) is an evergreen, aromatic perennial shrub of the family Lamiaceae. The oil extracted from the plant is used in food products, perfumes and cosmetics.

Figure 6: *Rosmarinus officinalis* L.

Several authors have reported on the insecticidal capability of *R. officinalis* (Hori, 1998; Papachristos & Stamopoulos, 2002; Prajapati *et al*., 2005,).

THE INSECT USED IN THIS STUDY

The choice of the common maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) (Fig. 7) used in this study was based on the information gathered from the farmers, in a survey carried out in the Eastern Cape Pronvince, at the beginning of the study. The insect was found to be prevalent in almost all the farmer's stores visited. The majority of farmers reported that the insect is a deleterious pest on stored-maize grains.

Figure 7: *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae)

S. zeamais is a well known insect that attacks most cereals in storage, especially maize. There is a similar species (*Sitophilus oryzea),* which closely resembles *S. zeamais*. This could lead to the difficulty in differentiating between these two species physically. Therefore, a pure culture of S. *zeamais* used in this study was obtained from the ARC-Plant Protection Research Institute, Pretoria.

STRUCTURE OF THE THESIS

This thesis consists of published articles, manuscripts submitted for publication and those ready for submission for publication. The arrangement of the thesis is as follows:

'A review of the use of phytochemicals for insect pest control' and Farmers' knowledge and experience of indigenous insect pest control in the Eastern Cape Province of South Africa are presented in Chapters 2 and 3, respectively. The following articles are presented in Chapters 4 and 5: Insecticidal activities of essential oil from the leaves of *M. longifolia* against *S. zeamais* (Motschulsky) (Coleoptera: Curculionidae) and 'Evaluation of the activities of five essential oils against the stored maize weevil'. Chapter 6 presents the report on the 'Effect of the essential oils of *M. longifolia* and *T. minuta* on the proximate and nutritional parameters of maize grains treated with the oils'. In Chapters 7 and 8, the 'Toxicological evaluation of the essential oil from *M. longifolia* leaves in rats' and 'Safety evaluation of the essential oil of *T. minuta*' are presented. Chapter 9 is the 'General discussion and conclusions' of the study.

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CHAPTER 2

A REVIEW OF THE USE OF PLANT EXTRACTS FOR INSECT PEST CONTROL

CHAPTER 2

A review of the use of plant extracts for insect pest control

A review of the use of phytochemicals for insect pest control

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The search for safe and environmentally-friendly pest control options has led to the exploration of pesticidal plants for potential alternatives. Plants from several families have been reported to exhibit a wide range of biological activities against insect pests, some of which have been scientifically validated. Recently, attention has focused on the identification of the compounds present in plants that possess insecticidal properties. Although there is a wealth of information available on this field of science, the data are scattered in various journals and other publications. This paper presents a review of the literature on biological activities of pesticidal plant extracts, types of insecticidal phytochemicals, synergistic effects of pure compounds and crude extracts, role of botanical insecticides in integrated pest management, drawbacks of phytoinsecticides, and methods employed in the screening and validation of such products.

Key words: biological activities, botanical insecticides, integrated pest management.

Awareness of the environmental health hazards posed by synthetic pesticides, development of resistance to these chemicals leading to recurrent pest outbreaks, danger of misuse and presence of toxic residues in food, has led to a search for safe and environmentally-friendly alternatives (Gebreyesus & Chapya 1983; Singal & Singh 1990).

Plants are a rich source of natural products. Many species synthesise their own chemicals in defence against attack by herbivores, pests and pathogens. Some are well-known for their insecticidal properties, e.g. *Ocimum viride* Willd, *Piper mullesua* Buch. Ham., neem (*Azadirachta indica* A. Juss.) and tobacco (*Nicotiana tabacum* L.) (Jacobson 1989; Ohigashi et al. 1991; Owusu 2000; Srivastava et al. 2001; Boeke et al. 2004; Nathan et al. 2005). Phytochemicals such as rotenone, nicotine and pyrethrum have been used as pesticides by man before the advent of synthetic insecticides (Jacobson 1989; Ohigashi et al. 1991). Various members of the families Annonaceae, Asteraceae, Meliaceae, Myrtaceae and Piperaceae produce chemical compounds which act as antifeedants, repellents, biocides or growth inhibitors detrimental to many insect species (Srivastava et al. 2001). These insecticides of plant origin are commonly used in the form of aqueous/solvent extracts, powders, slurries, volatiles and oils, or as shredded segments (Keita et al. 2001; Srivastava et al. 2001; Tapondjou et al. 2002). The protection of stored agricultural products using plant materials is an age-old practice in Africa. In many parts of the continent, farmers mix grains with parts of other plants before storage to protect them against insects (Jacobson 1983; Poswal & Akpan 1991).

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Several workers have reported the screening of plant species for insecticidal properties. Unfortunately, these data are scattered in various journals and other publications. This paper presents a review of the information available on biological activities of phytoinsecticides, insecticidal constituents of various plant families, synergistic effects of pure compounds and crude extracts, role of botanical insecticides in integrated pest management, and the extraction and bioassay methods employed in testing botanical insecticides.

Biological activities of phytoinsecticides

Larvicidal phytochemicals

Toxicity of plant extracts towards larvae of insects has been reported by several authors. Myristicin extracted from *P. mullesua* effectively killed fourth-instar larvae of *Spilarctia obliqua* (Walker) (Lepidoptera: Arctiidae) after 24 hours following topical application (Srivastava et al. 2001). In an experiment to assess the efficacy of crude seed extract of sugar-apple (*Annona squamosa* L.) against larvae of the diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae), 84 % larval mortality was achieved at 0.5 % concentration. Activity of the sugar-apple aqueous extract compared favourably with 1 % rotenone and pyrethrum, two well-known commercial insecticides of botanical origin (Leatemia & Isman 2004).

The insecticidal activities of plants from 14 families in southeastern Spain were screened by

Pascual-Villalobos & Robledo (1999). Extracts from species in the Brassicaceae, Asteraceae and Clusiaceae showed appreciable levels of larval toxicity. Similarly, infestation and deadheart injury caused by larvae of the maize stalk borer, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae), were reduced in maize (*Zea mays* L.) plants treated with extracts of China-berry (*Melia azedarach* L.), endod (*Phytolacca dodecandra* L.) and Peruvian pepper (*Schinus molle* L.) (Gebre-Amlak & Azerefegne 1999). The high insecticidal potential of neem has been attributed to the presence of azadirachtin (a complex limonoid) in the plant. The antifeedant and growth-inhibitory effect of neem limonoids on fifth-instar larvae of the rice leafroller, *Cnaphalocrosis medinalis* (Guenee) (Lepidoptera: Pyralidae), has been documented by Nathan et al. (2005).

Phytochemicals used as antifeedants, repellents and growth inhibitors

The presence of certain chemicals in plants prevents insects from feeding on them. This leads to starvation of the insects and, in some cases, eventual mortality. *O.viride* proved to be an effective insect repellent that compares very well with the widely explored *A. indica.* It exhibited strong repellency towards *Tribolium casteneum* (Herbst) (Coleoptera: Tenebrionidae) and the rice weevil, *Sitophilus orzyae* (Linnaeus) (Coleoptera: Curculionidae), which are both serious pests of maize and rice (*Oryza sativa* L.). Treatment of rice grain with leaf extract of *O. viride* resulted in less than 25 % survival of the two insects after 10 days (Owusu 2000). Ethanol extracts of *M. azedarach* fruit were investigated at various concentrations for their effect on the elm leaf beetle, *Pyrrhalta luteola* (Muller) (Coleoptera: Chrysomelidae). A concentration of 2 % of the extract deterred feeding by larvae and adult insects by as much as 86.4 % and 73.8 %, respectively (Valladares et al. 1997).

Mixing the diet (brown rice powder) of the maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae), with ground seed of Malabar nightshade (*Basella alba* L.) or leaves of turpeth (*Operculina turpethum* (L.) Silva Manso) and *Calotropis gigantea* R.Br., at concentrations of 5 % prolonged the developmental phase and reduced the number of adult insects that emerged by 62 %, 95 % and 70 %, respectively (Haque et al. 2000).

Tuncer & Aliniazee (1998) studied the effects of neem extract on *Myzocallis coryli* (Goeze) (Homoptera: Aphididae). The extract caused instant mortality of young nymphs and also restricted fecundity, prolonged the development period and reduced survival of subsequent generations. Similarly, oviposition was impeded in cowpea weevil, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae), present in cowpea (*Vigna unguiculata* (L.) Walp.) seed when the seed was mixed with powder obtained from tobacco, *Erythrophleum suaveolens* (Guill. & Perr.) Brenan and *Ocimum gratissimum* L. (Ofuya 1990). Powder derived from tobacco, *Tephrosia vogelii* Hook. f. and *Securidaca longepedunculata* Fresen. also reduced weevil emergence in infested cowpea seed, whereas bitter gourd (*Momordica charantia* L.), tree dracaena (*Dracaena arborea* Hort. Angl. ex Link), *T. vogelii, Blumea aurita* DC. and horsewood (*Clausena anisata* (Willd.) Hook. f. ex Benth.) exhibited repellency towards the weevil (Ofuya 1990; Boeke et al. 2004).

Wood ash is another plant product which has been found useful in the control of insect pests. It is generally believed that inert substances cause a loss of body moisture in insects. The use of ash is one of the traditional ways of protecting grains from storage pests among low-resource farmers in some parts of the world, including Africa, China, Japan and India (Wolfson et al. 1991; McIsaac & Edwards 1994; Dennis 2002). A minimum ratio of three parts of ash to four parts of cowpea seed (by mass) prevented growth in *C. maculatus* adult populations (Wolfson et al. 1991). A similar study by Katanga-Apuuli & Villet (1996) indicated that one part of ash to five parts of cowpea seed (by mass) provided adequate protection to the seed against damage by the above weevil.

Phytochemicals used as fumigants

Fumigation remains one of the most effective methods for protecting grains, feedstuffs and other agricultural commodities from insect infestation. However, complications such as pest resistance and ozone depletion associated with the fumigants currently widely in use, have led to a decrease in the number of products available. This has necessitated a search for natural alternatives. A large number of plant extracts from various herbs have been assessed for fumigant toxicity against insects. Essential oils from some members of the Lamiaceae induced 90 % mortality in adult populations of the maize weevil, rice weevil, cowpea weevil and

Sitotroga cereallela (Linnaeus) (Lepidoptera: Gelechidae) after 24 hours of exposure to a concentration of 1.4–4.5 µl l^{-1} (Shaaya et al. 1997). Similarly, 70 to 80 % mortality was recorded in populations of *C. maculatus* after 12 hours exposure to essential oils from basil (*Ocimum basilicum* L.) and *O. gratissimum* (Keita et al. 2001). Oils from cinnamon (*Cinnamomum zeylandicum* Blume), mustard (*Brassica juncea* (L.) Czerniak), *Cocholeria aroracia* Linne and common sweet flag against *S. orzyae* were effective when used as fumigants in closed containers (Kim et al. 2003). Varma & Dubey (2001) reported the efficacy of essential oils from *Caesulia axillaris* (Roxb.) and corn mint (*Mentha arvensis* L.) as fumigants for protecting wheat grain against biodeterioration. Benzaldehyde occurring in peach (*Prunus persica* (L.) Batsch) and 1,8-cineole extracted from eucalyptus was shown by Lee et al. (2001a) to be effective in the control of rice weevil, thus providing further evidence that natural volatiles could be used as fumigants for the control of stored-grain insect pests.

Types of insecticidal phytochemicals

Several groups of insecticidal chemicals have been identified in plants, including terpenoids, alkaloids, glycosides, phenols and tannins. These compounds have different behavioural and physiological effects on insects (Varma & Dubey 1999). Eugenol, isoeugenol and methyleugenol (benzene derivatives) exhibited contact toxicity towards maize weevil (Huang et al. 2002). Efficacy of the meliacarpin derivatives, 1,3-dicinnamoyl-11-hydroxymeliacarpin, 1-cinnamoyl-3-methacrylyl-11-hydroxymeliacarpin and 1-cinnamoyl-3-acetyl-11-hydroxymeliacarpin extracted from China-berry leaves, compared very well with that of the well-known azadirachtin. When larvae of *Spodoptera littorallis* (Boisduval) (Lepidoptera: Noctuidae) were fed a diet containing meliacarpin derivatives their growth was seriously impeded (Bohnenstergel et al. 1999).

Oil extracted from rosemary (*Rosmarinus officinalis* L.) exhibited pronounced insecticidal activity against *Acanthoscelides obtectus* (Say) (Coleoptera: Curculionidae) adults (Papachristos et al. 2004). Chemical analysis of the oil indicated that it contained some diterpenoids, diterpenoid quinones, triterpenoids and flavonoids (Papachristos et al. 2004; Mahmoud et al. 2005). Similarly, chemical analysis of essential oil from corn mint that exhibited toxicity towards the rice weevil, showed it to be rich in menthol, menthone, limonene, alpha-pinene, beta-pinene and linalool (Lee et al. 2001b). Other phytoinsecticides included cymol, ascaridole, carvacrol, cis-beta-farnesen, alpha-terpinen and estragol (Tapondjou et al. 2002; Ketoh et al. 2005).

Two piperidine alkaloids, piperonaline and piperoctadecalidine isolated from long pepper (*Piper longum* L.), exhibited toxicity towards *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) (Park et al. 2002)

Synergistic effects of pure compounds and crude extracts

The presence of different chemical constituents in a plant enhances its resistance to attack by herbivores. This is believed to be due to additive or synergistic action between the constituents, rather than the effect of the individual compounds per se. Bekele & Hassanali (2001) studied the blend effects of essential oil constituents extracted from *Ocimum kilimandscharicum* Gürke and *Ocimum kenyense* Ayob. ex A J Paton on maize weevil and *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae). Although camphor, one of the major components of the oils, was largely responsible for the toxic action, results indicated that overall toxicity was due to the combined effect of the various components. The bioactivity of cymol and essential oils from Mediterranean cypress (*Cupressus sempervirens* L.) and Sydney blue gum (*Eucalyptus saligna* Sm.) was investigated on maize weevil and *Tribolium confusum* du Val (Coleoptera: Tenebrionidae) by Tapondjou et al. (2005). Crude oil extracts from both plants exhibited greater repellency and toxicity towards the test insects than cymol, indicating that they contained other components which enhanced their repellent and toxic activity. Scott et al. (2002) reported the synergistic interaction of extracts from *Piper tuberculatum* Jacq. against *Aedes atropalpus* (Coquillett) (Diptera: Culicidae). Tertiary and quaternary mixtures of extracted piperamides had greater-than-additive toxicity than the single compounds or binary mixtures. Further studies on some botanical insecticides against green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), revealed that capsaicin, a cyclic amide responsible for the pungency of capsicums (*Capsicum* spp.), acted synergistically in mixtures with insecticides such as pyrethrins and insecticidal soap to produce higher levels of mortality than when acting alone (Edelson et al. 2002). One of the major advantages of the synergistic interaction of plant extracts is that it restricts the development of resistance to phytoinsecticides by insects because of the involvement of diverse chemical compounds.

Role of botanical insecticides in integrated pest management

Integrated pest management (IPM) is essentially a holistic approach to pest control, aimed at optimising the use of two or more methods for the management of pests within a particular cropping system (Dent 2000). Research has shown that botanical insecticides can play a role in IPM (Gahukar 2000; Scott et al. 2002). Recent research has focused on the possibility of incorporating botanical insecticides into IPM systems. This included the impact of botanicals on natural enemies, i.e. beneficial organisms that attack or kill pest organisms. They could be parasites, predators or pathogens.

Although biological pesticides in most cases create problems such as groundwater pollution and vertebrate toxicity, their potential interactions with non-target organisms need to be carefully evaluated (Wood & Hughes 1993). Joint research by the International Foundation of Science, Wageningen University, the Netherlands, and the ARC-Plant Protection Research Institute in South Africa, indicated that neem and China-berry plant extracts effectively controlled diamondback moth, with no negative effect on two of its natural parasitoid species (Charleston et al. 2005). This confirmed the report by Goudegnon et al. (2000), who studied the effect of deltamethrin and neem kernel solution on diamondback moth and *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae) parasitoid populations. Neem products therefore appear to have the potential to be incorporated in IPM for some crops.

Treatment of rice moth (*Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae)) eggs with neem seed oil had no adverse effect on the development of the parasitoid, *Trichogramma chilonis* (Ishii) (Hymenoptera: Trichogrammatidae). However, the oil prevented oviposition and exhibited mild toxic effects on the adult parasitoids, thus suggesting that pre-treatment of rice with neem extracts should be avoided (Raguraman & Singh 1999). Similarly, parasitism of *C. maculatus* by *Dinarmus basalis* (Rondani) (Hymenoptera: Pteromalidae) was negatively affected by powders derived from tobacco and *T. vogelii,* and essential oil extracted from turpentine grass (*Cymbopogon schoenanthus* Spreng.) (Ketoh et al. 2002, 2005; Boeke et al. 2003). From the above it is evident that insecticides of plant origin have a future role to play in the development of safe and environmentally-friendly pest control options, but further studies are required to elucidate their effects on natural enemies of pests.

Drawbacks of phytoinsecticides

Low mammalian toxicity, less persistence, selectivity towards target pests and non-phytotoxicity are among the qualities attributed to insecticides of plant origin. There is often a misconception that these insecticides are safer than synthetic products. This, however, is not always the case. For instance, nicotine extracted from tobacco is one of the most widely-known botanicals. Although effective in pest control, it is highly toxic to mammals and can readily be absorbed through the eyes, skin and mucous membranes (Carr et al. 1991).

Rotenone, a polycyclic ketone extracted from the roots of *Lonchocarpus, Derris* and several other leguminous plant genera, is another example. It is widely used as broad-spectrum insecticide, but is extremely toxic to aquatic life and also exhibits some level of toxicity to mammals (Carr et al. 1991; Cranshaw 1992). Persistence of synthetic pesticides in the environment and in treated foods is a major constraint to their use. Conversely, the rapid degradation of phytochemical insecticides on exposure to light renders them unstable and less persistent, hence necessitating repeated applications.

Although there is considerable potential for insecticides of plant origin, these products should be handled with caution. Detailed studies on their mammalian toxicity and residual effects are also required.

Methods employed in the extraction and bioassay of phytoinsecticides

A lack of uniformity in screening methods for the evaluation of botanical insecticides was apparent in the articles reviewed. Other discrepancies observed were in the sources and methodologies employed for the processing of plant materials. In most cases, plant materials were sourced from either the forest, local medicinal herb shops or purchased from commercial industries (Lamiri et al. 2001; Lee et al. 2001b). There are various methods for screening plants for activity. The type of solvent used for extraction and the method of extraction are of great importance as it will determine the chemical constituents in the extract. Also, the choice of solvent depends on the ultimate purpose of the extract (George et al. 2001). The most widely used extraction techniques include aqueous or solvent extraction, Soxhlet extraction and steam distillation (Bouda et al. 2001; Kawuki et al. 2005; Scott et al. 2005). Haque et al. (2002) reported some variation in the efficacy of different solvent extracts from water-pepper (*Polygonum hydropiper* L.) against rice hispa beetle, *Dicladispa armiger* (Olivier) (Coleoptera: Chrysomelidae). Similarly, Lapornik et al. (2005) found ethanol and methanol to be more effective than water for extracting antioxidants from plants. It is therefore essential that solvents be carefully selected before commencing any extraction procedure.

Testing of plant extracts for efficacy as insecticide has been attempted through various techniques in the past. The most common methods comprised contact, fumigation, growth inhibition, antifeedant, repellent and nutritional bioassays (Bouda et al. 2001; Huang et al. 2002; Kim et al. 2003). There is great variation in the age, number of insects tested, concentration and exposure period between bioassays with the same insect species. Specific protocols could be developed for certain insect groups, which may be modified when required.

It is obvious that there is a need for the development of standard testing protocols in identifying potential insecticidal phytochemicals. It will prevent duplication of research efforts and make research findings more readily comparable. Studies on insecticidal activities of many plant species, whether indigenous or exotic, in different geographic areas should be encouraged, since there may be variation in the chemical composition of plants from different locations.

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CHAPTER 3

FARMERS' KNOWLEDGE AND EXPERIENCE OF INDIGENOUS INSECT PEST CONTROL IN THE EASTERN CAPE, SOUTH AFRICA

CHAPTER 3

Farmers' Knowledge and Experience of Indigenous Insect Pest Control in the Eastern Cape, South Africa

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FARMERS' KNOWLEDGE AND EXPERIENCE OF INDIGENOUS INSECT PEST CONTROL IN THE EASTERN CAPE PROVINCE OF SOUTH AFRICA

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ABSTRACT

This study was conducted to document the knowledge of small-scale farmers regarding indigenous insect pest control methods in the Eastern Cape, South Africa. A survey was carried out using a combination of questionnaires and Focus Group Discussions. More than 70% of the farmers were literate, with both males (46%) and females (54%) actively involved in farming. 58% of the farmers were pensioners. Crops cultivated included maize and vegetables. The level of pest awareness among the farmers was high (92%), with over 70% of farmers relying on synthetic insecticides for pest control. 63% percent of the farmers were, however, were aware of indigenous methods of insect pest control. Unfortunately, such methods are currently being neglected and knowledge of their application was found to be eroding. It is necessary to re-popularise the indigenous methods of insect control given that they are mostly safer and cheaper than synthetic insecticides.

Keywords: farmers' knowledge; indigenous insect control

INTRODUCTION

Cereals such as maize, wheat and rice are the world's most important food crops. They are the dietary mainstay of many people, providing three-quarters of the world's energy and more than half of its protein needs, (Johnson 1984). Maize is not only a major cereal crop for human consumption but is also used in animal feeds and other industrial raw materials (Guy 1987). In South Africa, it is one of the staple foods and is consumed by more than 90% of the rural population. Unfortunately, annual maize production has declined mainly due to insect attack, especially in the developing world. According to the International Maize and Wheat Improvement Centre, insects are a major cause of the deterioration of maize produced in tropical and sub-tropical regions, with the damage occurring either before or after the harvest. Consequently, the control of insect infestation in maize has become an important aspect of both its production and storage.

Sitophilus zeamais Motsch., commonly known as the maize weevil, is one of the most destructive pests in tropical and sub-tropical regions and post-harvest losses due to this insect have been recognized as an increasingly important constraint in Africa (Markham *et al* 1994). Other maize pests include root worms, wire worms, aphids, armyworms, grasshoppers, stem borers, termites and earworms. The grain moth (*Sitotroga cereallela* [Oliver]) may also become a problem during storage. In many storage systems, the control of stored grain pests has largely been dependent upon chemicals such as methyl bromide and phosphine. However, due to the ozone-depleting effect of methyl bromide, its use might soon be restricted. Also, many cases of resistance to phosphine have been reported in some stored product insects across the world (World Meteorological Organization 1991; Zettler 1993). These and other problems (such as the high cost of chemicals, their availability and the health hazards posed by their repeated use) have sparked the need to search for possible alternatives.

Plants from several families exhibit a wide range of biological activities against insect pests (Sekou *et al* 2001; Bouda *et al* 2001). Natural pesticides from plant extracts such as rotenone, nicotine and pyrethrum have been in existence long the advent of synthetic organic chemicals (Jacobson 1989; Hajime 1991). Compared to synthetic organic chemicals, the natural products are biodegradable, have low mammalian toxicity and pose little or no danger to the environment if used in small amounts (Sekou *et al* 2001). The objective of this study was to document methods used by small-scale farmers in the control of maize pests and their knowledge of indigenous plants which can be used for insect control.

STUDY AREA

The study was conducted in the Eastern Cape province of South Africa in the following locations: Indwe, Lady Frere, Queenstown, Umtata, Whittle Sea, King Williams Town, Engcobo, Bazia and Alice. The province consists of the old East Cape, Ciskei and Transkei homelands and came into existence in 1994 after the subdivision of South Africa into nine provinces. It lies within latitude 31.36 S - 32.48 S and longitude 26'52 E -28'49 E with a population density of 90 people per square kilometre. The area receives a mean annual rainfall of 700 mm and a range of temperatures from 18 to 25°C. It has a great diversity of vegetation and is reported to have the highest number of vegetation types in South Africa (Low & Rebelo 1998). Farming can be described as a mixed system, involving the production of livestock and crops at home gardens and arable fields (Trollope & Coetzee 1975).

METHODOLOGY

The study protocol was approved by the Govan Mbeki Research and Development Centre in accordance with the University of Fort Hare research and ethical policy. A survey was conducted to assess the knowledge of small-scale rural farmers regarding pests and their experience in controlling crop pests using indigenous methods. A total of 24 respondents were randomly selected in each location and asked to complete a questionnaire which was prepared and tested beforehand. In addition, Focus Group Discussions were conducted comprising five groups of no less than 10 people per group. Before the commencement of any interviews or discussions, the purpose of the study was clearly explained to the farmers.

RESULTS

The majority of the farmers were above 56 years old and both males and females were actively involved in farming (Table 1). The respondents were mostly pensioners and over 50% had some level of formal education. As shown in Table 2, more than 50% of the farmers had 10 years of farming experience. Few grew only maize and vegetables, while over half produced a combination of maize, potatoes, onions, garlic, beetroot and cabbage. The majority of the farmers were aware of plant pests (Table 3), citing insect damage as one of the major problems encountered, especially in maize production. Incidences of insect infestation were reported to occur mostly during the summer period, while fewer attacks were experienced during winter. According to the respondents, the stem borer (for example, *Chilo partellus* [Swinhoe]) is one of the pests which pose a major threat to maize growers in the Eastern Cape. It attacks the growing maize by penetrating the whorl, which may lead to the complete death of the plant if the attack is severe. Other pests listed by the farmers include grasshoppers, cut worms, millipedes, moles and birds. The maize storage weevil (*Sitophilus zeamais*) was identified as the cause of a significant loss of harvested maize. The infestation begins right in field and continues in the store. This study revealed that chemical control was the predominant method used in the study area (Table 3). However, at least 50% of the farmers were aware of indigenous pest control methods. A list of plants used for insect pest control is shown in Table 4. According to the farmers, maize grains can be mixed with wood ash or paraffin to prevent insect attack. *Aloe ferox* ash is sprinkled on vegetables to repel insects, while some farmers store their grains in specially prepared bunkers. In other cases, baboon hooves are bought from traditional medical practitioners and put in drums or tanks containing the grains. It was also noted that in the past, a special dance called "Ingolongo" was performed around the field by girls and this was believed to be effective for insect control.

DISCUSSION

The high level of pest awareness observed among the farmers may be attributed to their many years of farming experience as well as their literacy. Over 70% of them had formal education and more than 10 years of farming experience. The fact that a high percentage of these farmers are pensioners may account for why they are involved mainly in subsistence farming. Some of the farmers, however, do grow crops for home consumption as well as animal feeding, while only a few grow them for sale. A similar observation was made by Perret *et al* (2000) who reported that a large majority of rural farmers in the Eastern Cape do not grow crops for profit.

Insects and other pests were reported to be a major threat to most of the cultivated crops. One way to overcome this problem is intercropping, which is a common practice in many parts of Africa. Crops like onion and garlic, which are among the crops planted by the interviewed farmers, are known to have characteristic pungent smells that repel insects. In fact, other workers have reported on the insecticidal properties of garlic and onion (Jacques, *et al* 1999; Park & Sang 2005). Generally, conditions are favourable for insects during summer which explains the high level of insect infestation reported by the farmers at that time of the year. As for *Sitophilus zeamais,* the level of infestation is often determined by the time of harvest and storage conditions. In most of the visited farms, maize grains were either spread on the ground inside an open room, stored in plastic containers and synthetic bags or spread on top of the roof. Some of these storage conditions may encourage rapid insect development and cross infestation of grains that were originally not infested. A great reliance on synthetic insecticides was reported – as in other parts of the world – despite the fact that most of the farmers criticized their use. The continuous use of these chemicals may be due to a lack of reliable alternatives. The use of indigenous methods of pest control is, however, still a viable option. Interestingly, quite a large number of the farmers were aware of these techniques, even though they are currently neglected. Indeed, it was observed during this study that the knowledge of the application of these indigenous methods was being eroded, especially among the young farmers.

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Table 1: Socio-economic characteristics of the Eastern Cape rural farmers.

Sources	Frequency n=24	Percentage
Ages		
26–35	1	4.2
36–45	2	8.3
46–55	7	29.2
56 and above	14	58.3
Sex		
Male	11	45.8
Female	13	54.2
Education		
No formal education	1	4.2
Junior school	14	58.3
High school	6	25.0
Tertiary education	3	12.5
Sources of income		
Pension	14	58.3
Employed	6	25.0
Unemployed	4	16.7

Table 2: Crops grown and farming experience of the Eastern Cape rural farmers.

Sources	Frequency n=24	Percentage
Crops grown		
Maize only	2	8.3
Vegetables* only	2	8.3
Maize and vegetables	20	83.4
Years of farming		
≤ 1 Year	1	4.7
2–5 years	2	8.3
6–10 years	1	4.7
≥ 10 years	20	83.3

*Vegetables: beet root, cabbage, onions, lettuce.

Table 3: Knowledge of pests and control methods among the Eastern Cape rural farmers.

Sources	Frequency n=24	Percentage
Pest awareness		
Aware of pest	22	91.7
No awareness of pest	2	8.3
Effect of pest damage		
Low yield	7	29.2
Reduce both yield and quality	12	50.0
Affect neither yield nor quality	5	20.8
Methods of pest control		
Chemical control	18	75.0
Non-chemical control	3	25.0
Knowledge of indigenous control		
Aware of indigenous control	15	62.5
Not aware of indigenous control	9	37.5

Table 4: Plants used for the control of crop insect pests in the Eastern Cape.

Scientific name	Common name	Local name	Method of Preparation and use
Nicotiana tabacum L.	tobacco	Icuba iesixhosa	Aqueous extract is prepared from the leaves and sprayed on the crops. In some cases, leaves are burnt near infested field in order to repel insects.
Roella glomerata A.DC.	–	ibosisi	The leaves are placed on the growing crop to repel insects.
Lantana camara L.	sage	utywala bentake	A mixture made from the water extract of the plant and the hair of the pig is sprayed on infested crops.
Tagetes minuta L.	wild marigold	unukanuke	Aqueous extract is prepared and sprayed on crops.
Aloe ferox Mill.	cape aloe	umhlaba	Aqueous extract is prepared and sprayed on crops.

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CHAPTER 4

INSECTICIDAL ACTIVITIES OF ESSENTIAL OIL FROM THE LEAVES OF *MENTHA LONGIFOLIA* L. SUBSP. *CAPENSIS* AGAINST *SITOPHILUS ZEAMAIS* (MOTSCHULSKY) (COLEOPTERA: CURCULIONIDAE)

CHAPTER 4

Insecticidal activities of essential oil from the leaves of *Mentha longifolia* L. subsp. *capensis* on *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae)

Insecticidal activities of essential oil from the leaves of *Mentha longifolia* L. subsp. *capensis* against *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae)

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Toxicity of the essential oil from the leaves of *Mentha longifolia* (Lamiaceae) was evaluated against *Sitophilus zeamais*, an insect that is the major cause of deterioration in stored grains, including maize. The contact, fumigation and repellency bioassays revealed that the oil was toxic to the insect. Increases in oil concentration and time of exposure resulted in a progressive increase in insect mortality. The dose of essential oil at 0.50 μl/g of maize grains in the contact bioassay caused 100 % mortality of *S. zeamais*, compared to less than 10 % mortality recorded at 0.125 μl/g. The essential oil demonstrated moderate fumigation toxicity against the test insects at concentrations of 24 and 32 μl of oil per litre of air, respectively. A high repellency was recorded for the oil at all the concentrations tested. Repellency values of as high as 100 % were recorded for most concentrations. *Mentha longifolia* could be a potential agent for the protection of agricultural stored products against *S. zeamais*.

Key words: wild mint, Lamiaceae, post harvest losses, insect mortality, oil toxicity.

INTRODUCTION

Insects are the major cause of deterioration of maize produced in tropical and subtropical regions. *Sitophilus zeamais* (Coleoptera: Curculionidae) commonly known as the maize weevil, is a field-to-store pest and one of the most serious pests of stored grain worldwide. Post-harvest losses due to *S. zeamais* have been recognized as an increasingly important constraint to maize production in Africa (Markham *et al.* 1994).

There is a need for adequate protection of maize grains from insect attack in order to meet food demand world wide. For several years, the control of stored product pests including *S. zeamais* has been a major part of storage management. Methyl bromide and phosphine are the most commonly used fumigants for the control of stored product pests, including *S. zeamais*. However, because of the ozone-depleting effect of methyl bromide, the U.S. Environmental Protection Agency (EPA 2001) has proposed it be banned. Also, cases of resistance to phosphine have been reported in some stored product insects (Subramanyam & Hagstrum 1995). The recent awareness of the environmental and health hazards of synthetic insecticides, such as methyl bromide and phosphine, has led to the search for safe and environmental friendly alternatives (Gebreyesus & Chapya 1983; Singal & Singh 1990).

Secondary plant metabolites are a large reservoir of chemicals with biological activities (Duke 1990). Phytochemicals such as rotenone, nicotine and pyrethrum were all used as pesticides before the advent of synthetic insecticides (Jacobson 1989; Ohigash *et al.* 1991). Many members of the families Myrtaceae, Asteraceae, Piperaceae, Meliaceae and Annonaceae are known to possess various chemical compounds which act as antifeedant, repellent insecticides or growth inhibitors to many insect species (Srivastava *et al.* 2001). Investigating plant materials for pest control properties may therefore offer the opportunity to find alternative insecticides.

Mentha longifolia L. subsp. *capensis*, wild mint, is an aromatic herb of the family Lamiaceae. It is a fast-growing plant that is widespread in the Eastern Cape, South Africa, and a popular traditional medicine that is mainly used for the treatment of respiratory and other diseases (Van Wyk & Gericke 2000). The strongly aromatic leaves from this plant are rubbed on bedding and the body to repel insects (Hutchings & Van Staden 1994).

In this study, we evaluated the repellent, contact and fumigation effects of the essential oil extracted from the leaves of *M. longifolia* on *S. zeamais*.

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MATERIAL AND METHODS

Adults of *S. zeamais* were obtained from a colony maintained by the Plant Protection Research Institute, Pretoria. South Africa. These were mass-reared on whole maize grains in 5-l plastic containers in a controlled chamber, at 28 ± 2 °C and 50-65 % RH in the Department of Botany, University of Fort Hare. Newly emerged, one-week-old insects were used in the bioassay (Keita *et al.* 2001; Tapondjou *et al.* 2002; Negahban *et al.* 2007).

The leaves of *M. longifolia* were collected from Nkonkobe municipality in the Eastern Cape, South Africa. A voucher specimen was deposited in the herbarium at the University of Fort Hare (Kem 01/2006). Fresh leaves from the plant were subjected to hydrodistillation for 3 h, using a Clevenger-type apparatus (British Pharmacopoeia, 1980). The composition of the oil obtained was determined by gas chromatography–mass spectrometry (GC–MS). A single extract of the oil was used for all the experiments.

The oil was analysed on a Hewlett- Packard HP 5973 mass spectrometer interfaced with an HP-6890 gas chromatograph. The following column and temperature conditions were used: initial temperature 70 °C, maximum temperature 325 °C, equilibrium time 1 min, ramp 5 °C/min, final temperature 240 °C; inlet mode: split less, initial temperature 220 °C, pressure 60.7 kPa, purge flow 26.3 ml/min, purge time 0.50 min, gas type helium; column: capillary, 30 m × 250 μm i.d, film thickness 0.25 μm, initial flow 1.0 ml/min, average velocity 37 cm/s; MS: EI method at 70 eV.

Fumigation bioassay

The fumigation chambers consisted of 500 ml glass jars with screw on lids. For the bioassay, solutions of 0, 4, 8, 12 and 16 μl of the oil were each diluted in 1 ml acetone, to correspond to concentrations of 0, 8, 16, 24 and 32 μl/l of air. One ml of each concentration was then separately applied to 7 mm discs of Whatman No. 1 filter paper, air-dried for 10 min and placed at the bottom of the jars. Twenty one-week-old adult insects were placed on muslin cloths (21 × 29 mm) each with 40 g whole maize grains. The cloths were tied closed with rubber bands and hung at the centre of the jars, which were then sealed with air-tight lids. There were four replicates for each concentration. Fumigation was carried out for 24 h after which the insects were transferred from the fumigation chamber onto clean maize, and mortality was checked daily for a further six days. Percentage mortality was calculated based on control mortality accounted for by Abbott's formula (Abbott 1925).

Repellency bioassay

The repellent effect of *M. longifolia* essential oil against *S. zeamais* was studied using a modified area preference method of Tapondjou *et al.* (2005). The test area consisted of 9 cm Whatman No. 1 filter paper cut into two halves. Different concentrations of the essential oil were prepared by diluting 1, 2, 4, 6, 8 and 10 μl of the oil in 1 ml acetone. Concentration was calculated by dividing the oil quantity (μl) by the surface area of half the filter paper (31.81 cm^2). Using a pipette, 0.5 ml of the above solution was applied uniformly to a half filter paper corresponding to concentrations of 0.03, 0.06, 0.13, 0.19, 0.25, and 0.31 μl of oil/cm^2 of the filter paper, respectively. The other half was treated with 0.5 ml acetone alone and this served as the control. Both essential oil-treated and acetone-treated filter paper halves were air-dried for 10 min to evaporate the solvent. With the aid of a clear adhesive tape, both halves were later joined together into full discs and placed in 9 cm glass Petri dishes. Twenty one-week-old, unsexed adult insects were released at the centre of the rejoined filter paper disc and the Petri dish was covered. Each treatment was replicated four times. The numbers of insects present on the control (Nc) and treated (Nt) areas of the paper were recorded for 2 h.

Percentage repellency (PR) values were calculated as follows:

$$PR = ((Nc - Nt)/(Nc + Nt)) \times 100$$

PR data were arcsine-transformed before they were analysed using ANOVA.

Contact toxicity

The contact effect of the essential oil of *M. longifolia* on the adults of *S. zeamais* was investigated as described by Tapondjou *et al.* (2005). Maize grains were treated with concentrations of 0, 5, 10, 15 and 20 μl of essential oil in 1 ml acetone. The different concentrations of the oil were mixed with 40 g samples of grain in 500 ml glass jars, corresponding to 0, 0.125, 0.25, 0.37 and 0.5 μl/g of maize grain, respectively. These were thoroughly stirred to allow for homogeneity of the oil on the treated grains. Treated samples were air-dried for an hour in order to get rid of the solvent. The grains were

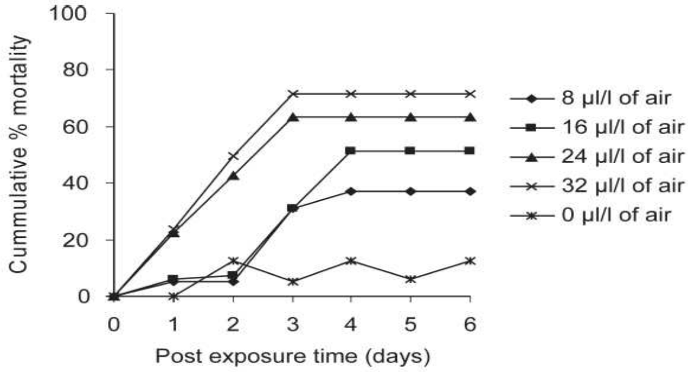

Fig 1. Effect of fumigation with essential oil of *Mentha longifolia* on the adults of *Sitophilus zeamais*.

thereafter infested with twenty one-week-old *S. zeamais* adults per jar and each jar was covered with a cotton mesh held in place by cover rims. There were four replicates per treatment. Dead insects in each jar were counted daily. Percentage mortality was calculated based on control mortality accounted for using Abbott's formula (Abbott 1925). The LC_{50} value was calculated by probit analysis using SAS programme (SAS 2000).

RESULTS

Chemical constituents of *M. longifolia* oil

Chemical analysis of the oil revealed the presence of 15 compounds (Table 1) with the most important being 1-8-cineole (25.46 %), menthone (17.85 %) and pulegone (29.93 %).Other compounds present were mainly mono- and sesquiterpenoids.

Fumigation bioassay

The fumigation effect of the essential oil of *M. longifolia* on the adult *S. zeamais* is shown in Figs 1 and 2. There was a gradual increase in the percentage insect mortality as the concentration of the oil increased, with the highest mortality recorded at the concentration of 32 μl/l air. There was, however, no further increase in insect mortality as from the third day after exposure.

Contact toxicity

In this bioassay, an increase in oil concentration and time of exposure resulted in a progressive increase in insect mortality. At 0.5 μl/g, the oil had a rapid effect on the insect, resulting in 100 % mortality within two days of treatment (Fig. 3).

Table 1. Chemical constituents of the essential oil of *Mentha longifolia* from Eastern Cape, South Africa.

No.	Constituents	% Composition
1	Alpha-pinene	2.51
2	Beta-pinene	3.49
3	1,8-Cineole	25.46
4	Trans sabinene hydrate	2.28
5	Menthone	17.85
6	Cyclohexanone	2.89
7	Cis-isopulegone	2.38
8	Alpha-terpineol	1.81
9	Pulegone	29.93
10	Piperitone	0.34
11	2,Cyclohexan-1-one	0.59
12	Cis-jasmone	0.11
13	Beta-caryophyllene	1.59
14	Germacrene-D	1.61
15	Bicyclogermacrene	0.20

This concentration was found to be more toxic to *S. zeamais* in comparison to less than 10 % mortality recorded at 0.125 μl/g. Concentrations 0.25 μl, 0.37 μl and 0.50 μl/g of grains achieved 100 % insect mortality six days after treatment, but the effect of the oil at 0.25 μl/g of grains was delayed for the first two days. The effect of the different concentrations on mortality is shown in Fig. 4. An LD_{50} value of 0.14 μl/g grain was obtained.

Repellency bioassay

The essential oil of *M. longifolia* exhibited 90–100 % repellency against adult *S. zeamais* at all concentrations. There were no significant differences in the repellency action of the oil against

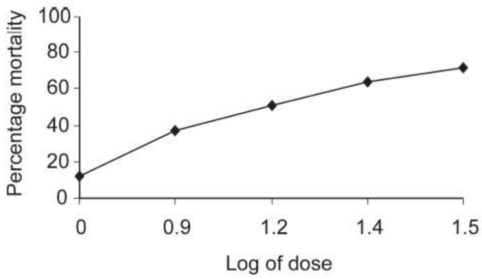

Fig. 2. Dose response curve on the fumigant effect of the essential oil of *Mentha longifolia* on *Sitophilus zeamais*.

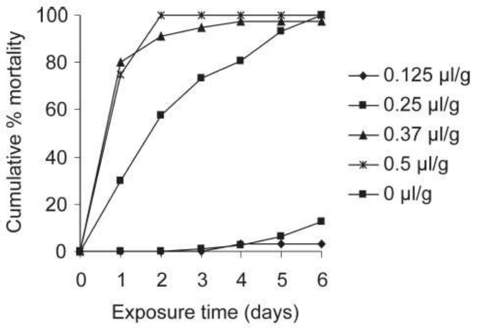

Fig. 3. Contact effect of the essential oil of *Mentha longifolia* on the adults of *Sitophilus zeamais*.

S. zeamais at the various concentrations tested. A PR value greater than 90 % from the four replicates was recorded one hour after treatment.

DISCUSSION

The results from this study show that the essential oil of *M. longifolia* exhibited different levels of toxicity against *S. zeamais* at the various concentrations. In previous studies, the insecticidal activities of plant oils have been attributed to some of their major chemical constituents (Coats *et al.*1991; Lee *et al.* 2001a; Tapondjou *et al.* 2002). The oil of this herb is rich in compounds such as, 1,8-cineole, menthone, and pulegone. Previous studies have demonstrated the insecticidal activities of menthone, pulegone, alpha-pinene and other terpenes (Lee *et al.* 2001a,b).

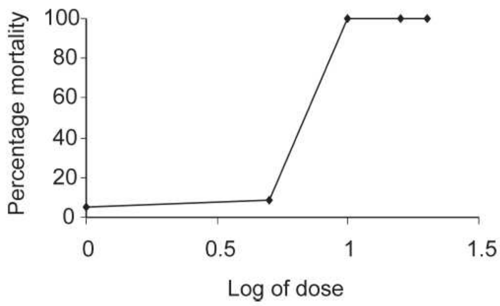

Fig. 4. Dose response curve on the contact effect of the essential oil of *Mentha longifolia* on *Sitophilus zeamais*.

The presence of more than one compound in plant oils has been known to be an advantage in pest control. Compounds present in essential oils are known to act synergistically against the physiology of many insects (Bekele & Hassanali 2001; Scott *et al.* 2002; Tapondjou *et al.* 2005). Bekele & Hassanali (2001) studied the blend effects of some essential oil constituents extracted from *Ocimum kilimandscharicum* and *Ocimum kenyense* on two postharvest pests. Although one major component of the oils was found to be largely responsible for the toxic action, other treatments indicated that the toxicity was due to the combined effect of the different components. The toxic effect of the essential oil of *M. longifolia* on *S. zeamais* in this study may likely be as a result of cumulative effects of the various constituents present in the oil.

From the Probit analyses of the oil, an LD_{50} value of 0.14 μl/g grain was obtained for the contact bioassays. This value compares favourably with an LD_{50} value of 38.05 μl/40 g grain that was obtained by Tapondjou *et al.* (2005), when the effect of the oils of *Cupressus sempervirens* and *Eucalyptus saligna* was investigated against *S. zeamais* and *Tribolium confusum*. The rapid action of the oil in inducing as much as 100 % mortality within a 48-hour exposure period indicated that it could be a candidate for future insecticide. Considering the results of the various bioassays in this study, the essential oil of *M. longifolia* has the potential to be employed in the control of *S. zeamais*.

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CHAPTER 5

EVALUATION OF THE ACTIVITIES OF FIVE ESSENTIAL OILS AGAINST THE STORED MAIZE WEEVIL

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Evaluation of the Activities of Five Essential Oils against the Stored Maize Weevil

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The contact, repellent and fumigation effects of essential oils from *Tagetes minuta* L., *Mentha longifolia* L., *Rosmarinus officinalis* L., *Helichrysum odoratissimum* L. and *Pelargonium graveolens* L. were investigated against maize weevil, *S. zeamais* (Motschulsky) (Colepotera: Curculionidae). The effects of the oils were dose-dependent with gradual increases in insect mortality as the oil concentrations increased. For the contact bioassay, the oils of *T. minuta* and *M. longifolia* acted rapidly, causing 100% mortality within two days after treatment at 0.375 and 0.50 μL/g grain concentrations, respectively. The repellent effect of these two oils against *S. zeamais* was also well pronounced. A Percentage Repellency (PR) value of more than 90% was obtained for the two oils, which puts them in the highest repellency class V. The level of repellency caused by the essential oils of *R. officinalis*, *H. odoratissimum* and *P. graveolens* were 51.1%, 49.4% and 51.7%, respectively. However, most of the oils demonstrated very low fumigation activity against the weevil. For the fumigation bioassay, all the oil concentrations tested did not achieve insect mortality more than 12.5%, even six days after treatment, except for the oil of *M. longifolia* that exhibited over 70% mortality at 32 μL/L of air. The present study revealed that the essential oils of *R. officinalis*, *H. odoratissimum* and *P. graveolens* had weak contact and fumigation effects against *S. zeamais*.

Keywords: Wild mint, Lamiaceae, contact bioassay, repellency, fumigation, *Sitophilus zeamais*.

Insect pest damage to stored grains results in major economic losses in Africa, where subsistence grain production supports livelihoods of the majority of the population [1]. Post harvest losses due to *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) have been recognized as an increasingly important constraint to grain storage in Africa [2].

Currently, the control of insect pests is largely dependent on the use of synthetic chemicals in most parts of the world. However, the continuous use of synthetic insecticides is leading to problems such as pest resurgence, resistance and environmental hazards [3a,3b]. Furthermore, the range of chemicals registered for use on grains and in storage structures is small. Already, there are threats to the two widely used fumigants: methyl bromide and phosphine. In fact, there is progressive restriction on the use of phosphine because of its ozone depletion potential [3c,3d]. Therefore, there is the need for the development of safe and environmental friendly alternative chemicals for the control of insect pests of stored grains. Traditionally, essential oils obtained by steam distillation of plant foliage have been used to protect stored grains and legumes and to repel flying insects in the homes [4a]. Today, there is an increasing interest in the use of natural products from plants to control pests.

Mentha longifolia L. (wild mint) is an aromatic herb of the family Lamiaceae. It is a fast growing plant that is widespread in the Eastern Cape, South Africa, and a popular medicinal plant that is mainly used for the treatment of respiratory and other diseases [4b]. The strongly aromatic leaves of this plant are rubbed on beddings and the body to keep off insects [4c]. Similarly, *Rosmarinus officinalis* L. (rosemary) is an evergreen, perennial shrub of the family Lamiaceae. The oil extracted from the plant is used in food products, perfumes and cosmetics. Species of *Helichrysum* are aromatic perennial herbs or shrublets with densely hairy leaves. Pain relieving, anti-infective and anti-inflammatory activities have been reported for several *Helichrysum* species.

H. odoratissimum is one of the commonly used medicinal plants in South Africa [5a].

Tagetes minuta L., popularly known as Mexican marigold, is a member of the family Asteraceae species of which are well known for their insecticidal activities [5b,5c]. Commercially, the oils are used in perfumery and as food flavors [5d]. The present study was undertaken to investigate the insecticidal potential of essential oils of *T. minuta*, *M. longifolia*, *R. officinalis*, *H. odoratissimum* and *P. graveolens* against the common maize weevil, *Sitophilus zeamais*.

The analysis of the essential oil of *T. minuta* showed the presence of 11 compounds among which were limonene (12.3%), *cis*-β-ocimene (12.8%), butanoic acid (20.7%), α-terpinolene (3.4%) and allo-ocimene (0.6%) (Table 1). Pulegone (29.9%), 1,8-cineole (25.5%) and menthone (17.8%) were the main compounds found in *M. longifolia* oil. The predominant chemical constituents present in the oil of *P. graveolens* were citronellol (17.9%), geraniol (15.0%) and citronellyl formate (13.9%), while *R. officinalis* oil contained α-pinene (7.9%), camphene (5.5%), 1, 8-cineole (10.3%) and camphor (13.7%) as the main constituents.

The essential oil of *M. longifolia* showed the most fumigant toxicity against *S. zeamais,* achieving 71.7% mortality three days after treatment (Figure 1a). This was followed by 43.7% mortality recorded for the oil of *P. graveolens* (Figure 1b). The oils of *T. minuta* and *R. officinalis* demonstrated very low fumigation activities against adult *S. zeamais*. All the oil concentrations tested did not achieve insect mortality more than 12.5% six days after treatment (Figure 1c and 1d). However, no activity was observed for *H. odoratissimum.* The essential oil concentrations used in this study resulted in minimal insect mortality, with no significant differences among the various concentrations.

The mortality effects of the essential oils of *T. minuta* and *M. longifolia* on S. *zeamais* using the contact method were dose-dependent (Figures 2a and 2b). A gradual increase in insect mortality was observed as the oil concentrations increased. The oils acted rapidly, causing 100% mortality within two days of treatment at 0.25 and 0.50 μL/g grain, respectively. Although, the effect of *T. minuta* oil on *S. zeamais* was slower at 0.125μL/g grain, more than 55% mortality was recorded six days after the treatment. The oils of *P. graveolens* and *R. officinalis* demonstrated very weak contact effects against the insect (Figures 2c and 2d) and *H. odoratissimum* showed no activity.

The repellent effect of the essential oils of *T. minuta* and *M. longifolia* against *S. zeamais* was well pronounced in this study. All the concentrations tested evoked a high level of repellency against the insect. A PR value higher than 90% was obtained for the oils of *T. minuta* and *M. longifolia,* which put them in the highest repellency class V. However, the essential oils of *R. officinalis*, *H. odoratissimum* and *P. graveolens* exhibited the same level of repellency against the weevil (Table 2).

The present study has shown that the oils of *T. minuta* and *M. longifolia* are effective as both contact and repellent agents against *S. zeamais*. The higher insect mortality recorded in the contact and repellent bioassays of the oils of these plants was likely due to the higher concentration of the oil in the inter-granular air of the treated grains, which made it possible for the oil to penetrate the cuticular membrane of the insect, subsequently causing abrasion and dehydration of the insect [6a]. The presence of terpenoids, such as limonene, menthone and pulegone, could have contributed to the insecticidal activity of the oil. According to Weaver *et al.* [6b], the insecticidal activity of *Tagetes erecta* was attributed to the presence of terpenoids in the plant.

The contact and repellent activities of the essential oil of *T. minuta* and *M. longifolia* against *S. zeamais* may, therefore, be attributed to the interaction of the various chemical compounds present in the oil. As shown in the oil analysis, 1,8-cineole, menthone, limonene and some other chemical constituents found in these oils have been reported to possess some insecticidal activities [6c,6d]. Such synergistic biological effects have been reported by several authors [6e-6g]. Other factors that may affect the insecticidal activity of essential oils include the susceptibility of the test insect [7a] and the degree of absorption in the treated commodity [6a]. According to Shaaya & Kostyukovysky [6a], the less the oil absorption the higher the concentration of the oil in the inter-granular air, and the route of entry of the oil, (inhalation, ingestion or skin absorption). The low fumigation activities demonstrated by the oils of *T. minuta, R. officinalis*, *H. odoratissimum* and *P. graveolens*, against *S. zeamais* in this study could

Table 1: Chemical constituents (%) of the essential oils of *T. minuta, M. longifolia, H. odoratissimum, P. graveolens* and *R. officinalis.*

No	Constituents	Kovat index	*Tagetes minuta*	*Mentha longifolia*	*Helichrysum odoratissimum*	*Pelargonium graveolens*	*Rosmarinus officinalis*
1	α-Pinene	940		2.51	4.20	0.38	7.88
2	Camphene	957					5.55
3	β-Pinene	970		3.49	1.27		1.81
4	Myrcene	984			2.20	2.65	1.40
5	α-Terpinene	1010					0.75
6	Limonene	1022	12.33		14.55	0.40	
7	1,8-Cineole	1025		25.46	6.56		10.33
8	*Trans*-Sabinene hydrate	1029		2.28			
9	*trans*-β-Ocimene	1032				1.45	0.12
10	γ-Terpinene	1051			1.30		1.36
11	Sabinene	1052	1.76				
12	Terpinolene	1083			0.76		1.40
13	Decane	1084	2.47				
14	Allo-ocimene	1186	0.60		0.41		
15	Linalool	1092			0.69	5.67	1.74
16	(*Z*)-3-hexenyl acetate	1093	0.94				
17	*cis*-Rose oxide	1104				0.78	
18	*cis*-β-Ocimene	1130	12.85			0.60	
19	Isomenthone	1136		2.89			
20	Butanoic acid	1142	20.68				
21	*cis*- Isopulegone	1149		2.38			
22	Menthone	1150		17.85	1.44	7.09	
23	Camphor	1151			0.43		13.72
24	Borneol	1177					8.51
25	Terpinen-4-ol	1186			1.04		3.56
26	α-Terpinolene	1210	3.36				
27	Pulegone	1231		29.93			
28	Verbenone	1236					15.74
29	*cis*-1,2,3,4-tetramethyl-1-cyclobutene	1243	1.87				
30	Citronellol	1245			2.88	17.92	
31	Citronellyl formate	1275			2.34	13.94	
32	Geraniol	1276			1.60	15.05	0.92
33	*cis*-Tagetone	1283	5.64				
34	Bornyl acetate	1323					10.73
35	α-Copaene	1366				0.49	
36	Geranyl acetate	1376				0.50	
37	*cis*-Ocimenone	1388	4.28				
38	β-Caryophyllene	1422		1.59		1.32	1.75
39	α-Guaiene	1432				1.04	
40	Germacrene-**D**	1459		1.61		1.09	
41	γ-Curcumene	1472			2.15		
42	δ-Guaiene	1478			1.01		
43	δ-Cadinene	1519			1.27	1.01	
44	Caryophyllene oxide	1581			2.42		
45	Viridiflorol	1590			.35		

be as a result of the short exposure period (24 h) and the concentrations used. Longer exposure periods and higher concentrations should, therefore, be considered in future experiments.

The members of the genus *Tagetes* are known to possess large quantities of active substances that are detrimental to insect survival [7b]. Similarly, an early study had shown menthone to possess fumigant toxicity towards *Sitophilus oryzae* [7c]. The findings from this study suggest that essential oil from the leaves of *T. minuta* and *M. longifolia* could be potential protective agents against *S. zeamais*.

Experimental

Insects: Adult *S. zeamais* were obtained from a colony maintained by the Plant Protection Research Institute, Pretoria, South Africa. They were mass reared on whole maize grains in 5 L plastic containers in a controlled chamber at 28±2°C and 50-65% RH in the Department of Botany, University of Fort Hare. Newly emerged adult insects, one week old were used in the bioassays.

Plant materials and oil distillation: The leaves of *T. minuta, M. longifolia, R.. officinalis, H. odoratissimum* and *P. graveolens* were collected

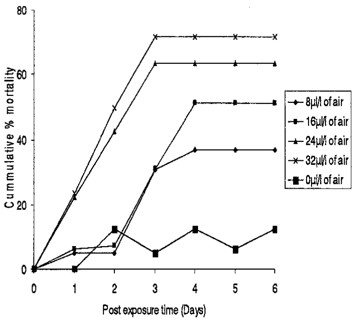

Figure 1a. *Mentha longifolia*

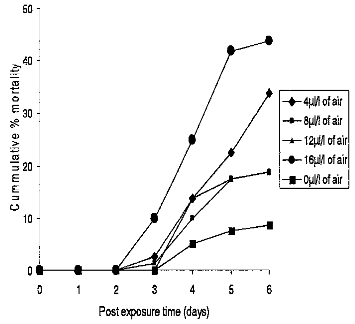

Figure 1b. *Pelargonium graveolens*

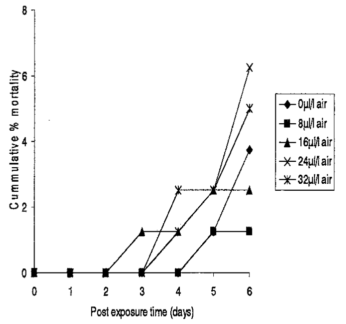

Figure 1c. *T. minuta*

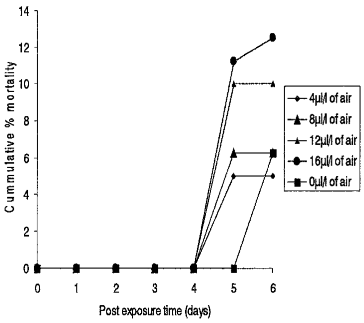

Figure 1d. *Rosmarinus officinalis*

Figure 1: Fumigation effect of essential oils on adult mortality of *S. zeamais*.

from the Eastern Cape Province, South Africa. These were authenticated by Prof DS Grierson at the Deparment of Botany, University of Fort Hare. Voucher specimens were deposited in the herbarium of the University. Fresh leaves from each plant were subjected to hydrodistillation for 3 h, using a Clevenger- type apparatus [8a]. A single extract of each oil was used for the experiments.

Oil analysis: The oils were analyzed on a Hewlett-Packard gas chromatograph, Model HP 5973, interfaced with a VG analytical 70-280s double-focusing mass spectrometer, mass range 35-425 *m/z*, operating at electron impact of 70 eV with an ion source temperature of 240°C. An HP-column was used, 30 m x 0.25 mm i.d., similar to DB 5, film thickness 0.25 µm; helium was the carrier gas at a flow rate of 1mL /min with a split ratio of 1 : 25. The oven temperature was programmed from 70°C to 240°C at 5°C/ min [8b].

Oil identification: The components of the oils were identified by matching their spectra and retention indices with those present in the Wiley 275 library (Wiley New York) in the computer library.

Fumigation bioassay: The fumigation chambers were made up of 500 mL glass jars with screw lids. For the bioassay, the 5 essential oils were separately diluted in 1 mL acetone, to final concentrations of 0, 8, 16, 24 and 32 uL/L of air. One mL of each concentration was then separately applied to 7 mm Whatman No. 1 filter paper, dried in the air for 10 min and placed at the bottom of a glass jar. Twenty (one week old) adult insects were placed in muslin cloth (21 x 29 mm) containing 40 g whole maize grains. The cloth was tied with rubber bands and hung at the centre of the jars, which were then sealed with air-tight lids. Each concentration was replicated 4 times. Fumigation was carried out for 24 h after which the insects were transferred from the chamber into clean maize, and mortality was checked daily for a further 6 days. Percentage mortality was calculated using the following formula: $P_T = (P_o - P_c) / (100 - P_c)$; where, P_T= Corrected mortality (%), P_o= Observed mortality (%), P_c= Control mortality (%) [8c].

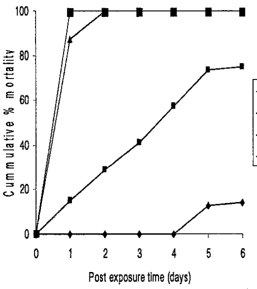

Figure 2a. *T. minuta*

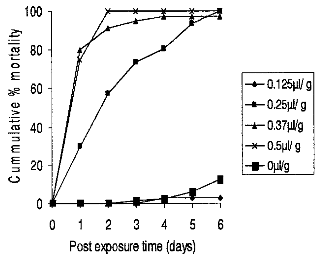

Figure 2b. *M. longifolia*

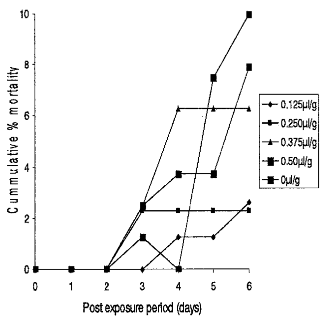

Figure 2c. *Perlagonium graveolens*

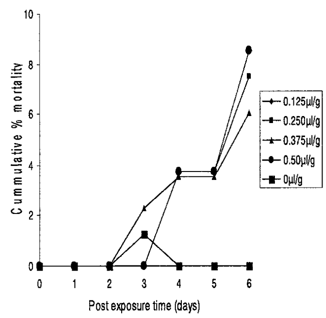

Figure 2d. *Rosmarinus officinalis*

Figure 2: Contact effect of essential oils on adult mortality of *S. zeamais*.

Contact bioassay: The contact effect of the 5 essential oils on adult *S. zeamais* was investigated, as described by Tapondjou [8d]. Maize grains were treated with concentrations of 0, 5, 10, 15 and 20 µL of essential oil in 1 mL acetone. Each concentration was mixed with 40 g samples of grains in a 500 mL glass jar, corresponding to 0, 0.125, 0.250, 0.375 and 0.5 µL/ g of grain, respectively. Each treatment was thoroughly stirred to allow for homogeneity of the oil on the treated grains. Treated samples were air dried for 60 min in order to remove the solvent [8d]. The grains were thereafter infested with 20 one week old adults of *S. zeamais* per jar and each jar was covered with a cotton mesh held in place by cover rims. There were 4 replicates per concentration. Dead insects in each jar were counted daily. Percentage mortality was calculated as in 2.4 above.

Repellency bioassay: The repellent effect of the 5 essential oils against *S. zeamais* was investigated using the modified area preference method of Tapondjou [8d]. The test area consisted of 9 cm (diameter) Whatman No. 1 filter paper cut into 2 halves. Different concentrations of the essential oils were prepared by diluting 1, 2, 4, 6, 8 and 10 µL of the oil in 1 mL acetone. Using a pipette, 0.5 mL of each sample was applied uniformly to a half filter paper, corresponding to concentrations of 0.03, 0.06, 0.13, 0.19, 0.25, and 0.31 µL of oil / cm^2 of the filter paper, respectively. The other half was treated with 0.5 mL acetone alone and served as the control. Both essential oil treated and acetone treated halves were air-dried for 10 min to evaporate the solvent [8d]. With the aid of a clear adhesive tape, both halves were later joined together into a full disc and placed in a 9 cm glass Petri dish. Twenty unsexed adult insects (one week old) were released at the centre of the remade filter paper disc and the Petri dish was covered. Each treatment was replicated 4 times. The number of insects present on the control (Nc) and

CHAPTER 6

PROXIMATE COMPOSITION AND QUALITY PARAMETER ASSESSMENT OF MAIZE GRAINS TREATED WITH THE ESSENTIAL OILS FROM *MENTHA LONGIFOLIA* L. AND *TAGETES MINUTA* L.

CHAPTER 6

Proximate composition and quality parameter assessment of maize grains treated with the essential oils from *Mentha longifolia* L. and *Tagetes minuta* L.

* This chapter has been submitted for publication in this format in the International Journal of Food and Nutrition

Proximate composition and quality parameter assessment of maize grains treated with the essential oil of *Mentha longifolia* L. and *Tagetes minuta* L.

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Abstract

The effect of the essential oil of *M. longifolia* was investigated on some quality parameters of maize grains stored over a period of three months. No major adverse effect of the oil was observed on the grains after the storage periods. The composition values of the grains were : ash, 7.05-8.58 %; crude fat, 11.25-11.42%; crude fibre, 9.77-10.65%; crude protein, 17.83-19.50% and total carbohydrates, 52.26- 52.4%. A gradual decrease on the effect of the oil on grain odour was observed with increase in the time of storage. However, a slight change was observed in the colour of the stored grains.

Introduction

Maize (*Zea mays* L.) is a member of the grass family Gramineae, to which all the major cereals belong. It forms one of the main cereal crop grown in the world today. It is one of the staple foods in Sub-Saharan Africa, where three quarters of maize produced is consumed as human food.

Insects are a major cause of deterioration of stored produce in tropical and sub-tropical regions. They develop successfully in stored produce because of their adaptation to diverse habitat and diets, thus, their control is a major part of storage management (FAO 1985; Guy, 1987). Protection of agricultural products in storage against insect attack is essential. Insect pest damage to stored grains results in major economic losses in Africa, where subsistence grain production supports livelihoods of the majority of the population (Udo, 2005).

Recent concern on the detrimental effect of agricultural chemicals on health and the environment has led to interest in reducing chemical input for crop production. Intensive use of synthetic insecticides for insect control has been reported to have led to problems such as pest resurgence, resistance, negative impact on non-target organism, health as well as environmental hazards. These pressures have accelerated the search for more environmentally and toxicologically safe as well as more selective and effective pesticides (Duke, 1990; Perry *et al*., 1998, cited in Leatemia and Murray, 2004).

Natural products from plants have being receiving worldwide attention and plants from several families have been screened for their insecticidal potentials. However, there is dearth information on the effect of the essential oil of *Mentha longifolia* and *Tagetes minuta* on nutritional composition and some quality parameters of maize stored with these oils. Therefore, this study was undertaken to evaluate the effect of the essential oils of *Mentha longifolia* L. and *Tagetes minuta* L. on nutritional composition and some quality parameters of stored maize grains.

Materials and Methods

Plant preparation

The leaves of *M. longifolia* and *T. minuta* were collected from Nkonkobe municipality in the Eastern Cape, South Africa. A voucher specimen was deposited in the herbarium at the University of Fort Hare. Fresh leaves from the plant were subjected to hydrodistillation for 3 h, using a Clevenger-type apparatus (British Pharmacopoeia, 1980). The oil collected was stored in a dark bottle and kept in the refrigerator at 5^{o}C until needed for the experiment.

Preparation of maize grains

Maize grains free from insecticide were collected from a farmer in Melani Village, Eastern Cape, South Africa and clean maize which showed no sign of insect infestation was further treated in an oven at 40^{o}C for two hours and used for the experiments.

Proximate analysis

The effects of the essential oils of *M. longifolia* and *T. minuta* on maize grain were investigated using a modified method of Jood *et al*., 1996. Jars containing 240 g maize grains were separately mixed with essential oil dissolved in acetone corresponding to concentrations of 0, 0.125, 0.25, 0.375 and 0.5 µl/g of maize grain respectively, and each concentration was replicated four times. These were thoroughly stirred to allow for homogeneity of the oil on the treated grains. At zero, one, two and three months after treatment, the grains were analyzed for moisture, ash, total protein, crude fat, total carbohydrates and crude fibre by employing the standard methods of analysis of the Association of Analytical Chemists (AOAC 1984).

Ash content was determined by incineration of samples in a muffle furnace (Naber Industrieofenbau, Bremen, Germany) at 550^{o}C for eight hours. Crude fat was determined by successive extraction of the sample with diethyl ether. For determination of crude fibre, the samples were extracted with 1.25% sulphuric acid for 30 min and further extracted with 1.25% sodium hydroxide. The obtained solid was ashed in a furnace and crude fibre determined by computing the difference in weight of the crucible and ash content. Protein content was determined following the microkjeldal procedure. Samples (0.5 g) were digested with sulphuric acid and a catalyst, followed by calorimetric determination of nitrogen. To obtain the crude protein percentage, nitrogen value was multiplied by 6.25.

Grain quality evaluations

Samples were taken from grains (in section above) after 0, 30, 60, and 90 days of storage. The samples were used to determine the grain quality parameters: grain colour and odour.

The effect of the essential oil of *M. longifolia* on the odour and colour of treated and untreated maize grains was determined for three months adopting the method of Ogendo *et al.*, 2004. Using a modified scale, grain samples were assessed for changes in odour and colour. A scoring scale of 1-5 was defined separately for each of the two parameters. Initial scores were taken at day 0, which represented the values just before grain treatment.

The following scale was used in scoring for changes in grain odour.

1. No difference in grain odour
2. Grain has little offensive odour
3. Grain has moderately offensive odour

4. Grain has offensive odour
5. Grain has very offensive odour

The following scale was used in scoring for changes in grain colour:

1. No detectable change i.e natural white with a few yellow/ red grains
2. Slight changes (≤ 5%) from natural white/yellow/red to light brown
3. Moderate change (> 5 to 30%) from natural white/yellow/red to light brown
4. Great change (30 to 50%) from natural white/yellow/red to dark brown
5. Highly significant change (>50%) making grain unacceptable for human consumption

Each grain sample was coded and presented to individual panelists in a well-lit and ventilated room for assessment. A total of six panelists were used to score for change in grain odour and colour. Not more than one assessor was allowed into the assessment room at a time, to ensure that scores were independent from each other. The whole exercise was repeated on a monthly basis for three months using the same set of panelists. In order to remove bias, blank scoring sheets were used on each assessment dates.

Results and Discussion

The nutritional composition of maize grains treated with the essential oil of *M. longifolia* was not affected even after three months of storage. There were no significant differences in the parameters measured. The composition values were: ash, 7.05-8.58 %; crude fat, 11.25-11.42%; crude fibre, 9.77-10.65%; crude protein, 17.83-19.50% and total carbohydrates, 52.26- 52.40% (Table 1). *M. longifolia* oil did not significantly change the colour of treated grains for the 90 days storage period (Fig. 1). In Contrast, significant differences were observed in the odour of treated grains, with the strongest odour observed in the earlier storage period, but with decreases with later storage duration (Fig. 2).

T. minuta oil significantly affected the colour of treated grains earlier in the storage period. This effect was observed to decrease as the storage duration progressed (Fig. 3). However, significant decreases in the effect of oil on the odour was observed later during storage (Fig. 4).

Earlier studies have shown that the nutritional composition of grains treated with plant products are not adversely affected in the absence of insect infestation. In a similar study by Jood *et al.*, (1996) nutritional composition of grains treated with powder from neem, citrus, garlic, podina and neem oil remained unaffected one month after storage. However, in their study, changes were observed later due to insect infestation. Asawalam (2006) found *Piper guineense* seed oil not to have any adverse effect on treated maize grain after three months of storage. It does imply that it is possible to get rid of the essential oil odour on treated grains with longer storage period.

Therefore, it may be concluded from the present study that the essential oils from *M. longifolia* and *T. minuta* had no major adverse effect on maize grain quality.

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Table 1. Proximate composition of maize grains treated with the essential oil from *M. longifolia*. Data is mean (transformed) ± SEM; n= 5.

	Composition (%)		
Parameter	30 days	60 days	90 days
Ash	7.05 ± 0.74^{a}	8.36 ± 0.23^{a}	8.58 ± 0.31^{a}
Crude fat	11.42 ± 0.15^{a}	11.25 ± 0.08^{a}	11.25 ± 0.13^{a}
Crude fibre	9.77 ± 0.16^{a}	10.65 ± 0.15^{a}	9.94 ± 0.09^{a}
Crude protein	19.50 ± 0.31^{a}	18.48 ± 0.33^{ab}	17.83 ± 0.53^{a}
Carbohydrate	63.89 ± 0.25^{a}	64.16 ± 0.31^{a}	64.54 ± 0.45^{a}

Means followed by the same letters along the rows are not significantly different at $p \leq 0.05$.

Table 2. Proximate composition of maize grains treated with the essential oil from *T. minuta*

Data is mean (transformed) ± SEM; n= 5.

Parameter	Composition (%)		
	30 days	60 days	90 days
Ash	7.40 ± 0.26[a]	7.06 ± 0.20[a]	7.01 ± 0.22[a]
Crude fat	10.86 ± 0.13 [a]	7.06 ± 0.20 [b]	6.88 ± 0.22 [b]
Crude fibre	9.63 ± 0.21[a]	9.61 ± 0.20[a]	9.60 ± 0.19[a]
Crude protein	18.98 ± 0.28[a]	18.56 ± 0.30[a]	18.77 ± 0.35[a]
Carbohydrate	64.42 ± 0.26[a]	64.93 ± 0.26[a]	64.59 ± 0.31[a]

Means followed by the same letter along the rows are not significantly different at $p \leq 0.05$.

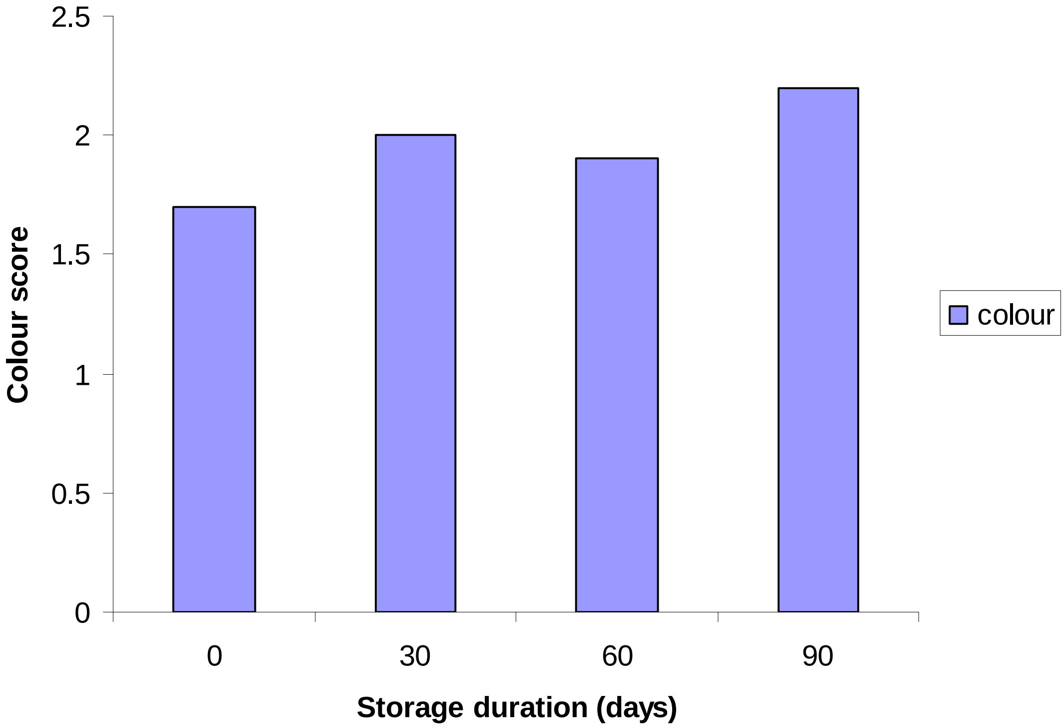

Figure 1. Effect of the essential oil of *M. longifolia* on the colour of stored-maize grains.

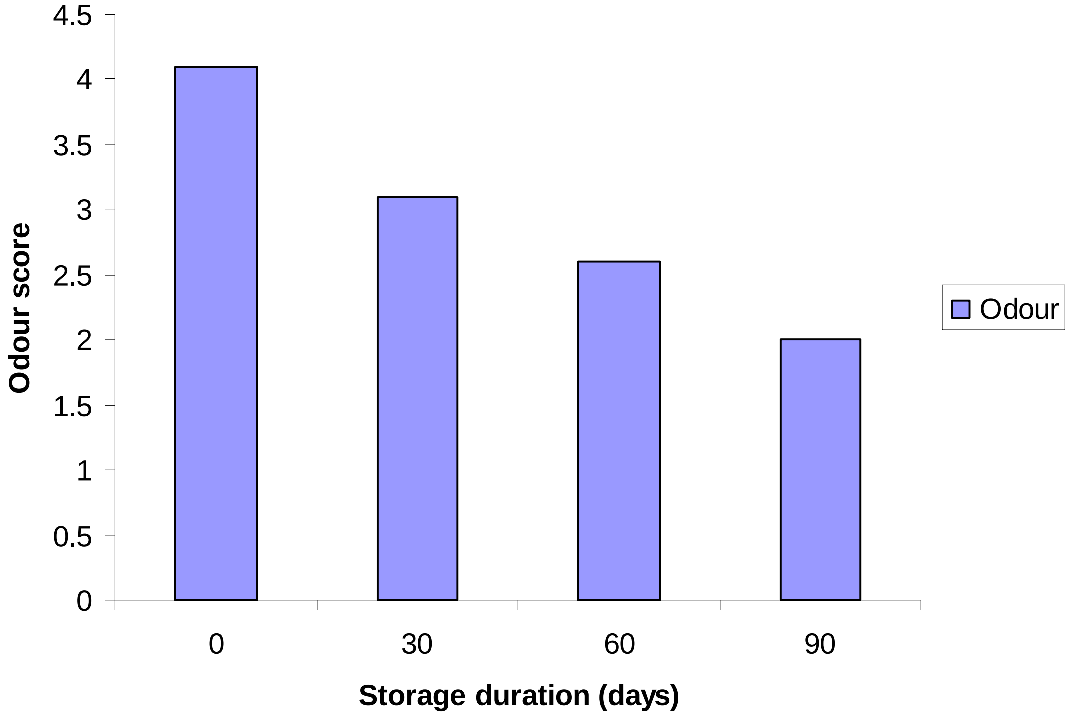

Figure 2. Effect of the essential oil of *M. longifolia* on the odour of stored-maize grains.

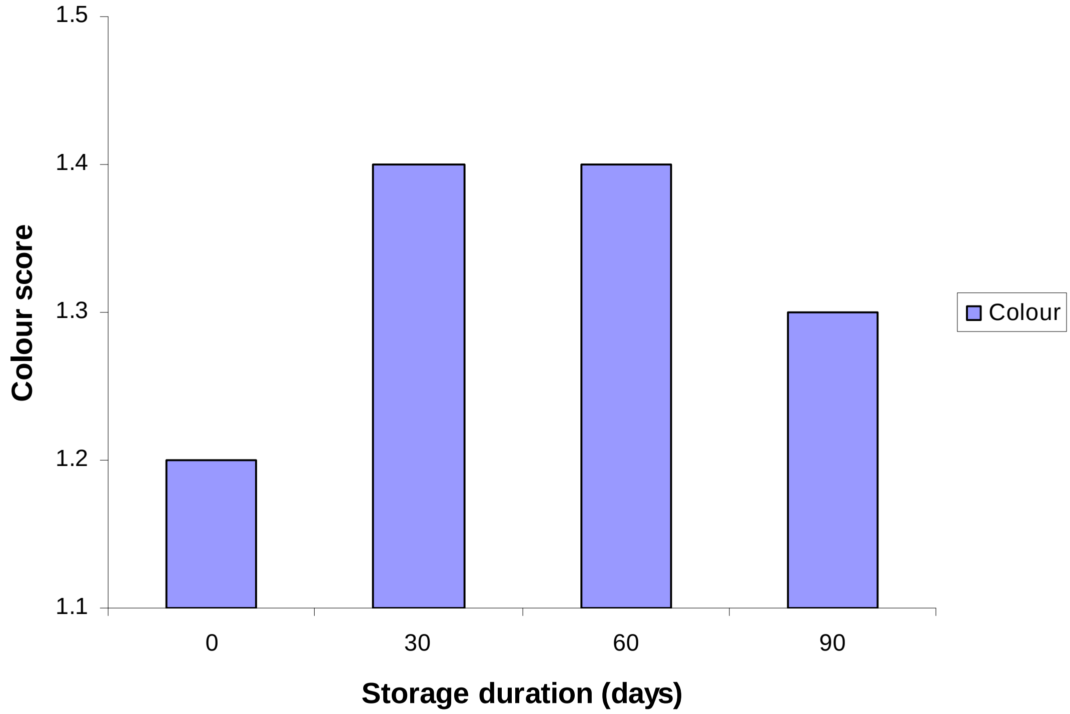

Figure 3. Effect of the essential oil of *T. minuta* on the colour of stored-maize grains.

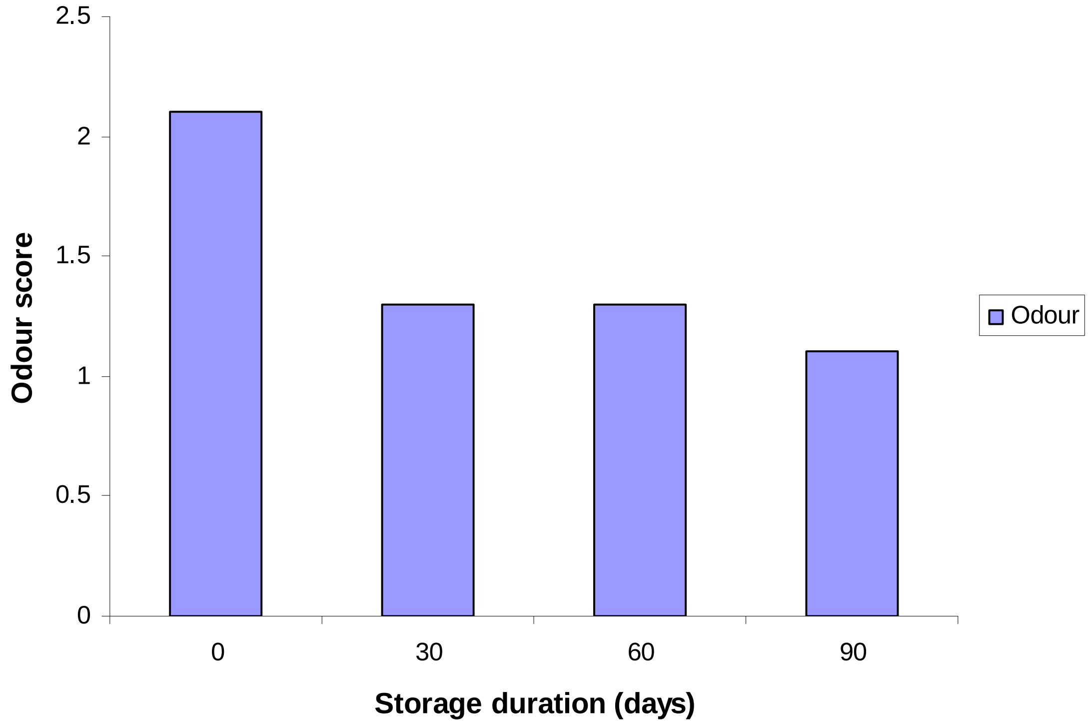

Figure 4. Effect of the essential oil of *T. minuta* on the odour of stored-maize grains.

CHAPTER 7

EFFECT OF ADMINISTRATION OF THE ESSENTIAL OIL FROM *TAGETES MINUTA* L. LEAVES IN WISTAR RATS

CHAPTER 7

Effect of administration of the essential oil from *Tagetes minuta* L. leaves in Wister rat.

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Effect of Administration of the Essential Oil from *Tagetes minuta* L. Leaves in Wistar Rats

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Abstract: The effects of the essential oil from *Tagetes minuta* leaves at 125, 250, 375 and 500 μL kg^{-1} b.wt. on some biochemical parameters of Wistar rats were studied. There was no significant difference in packed cell volume, mean corpuscular hemoglobin concentration, monocytes, lymphocytes, eosinophils, basophils, serum alanine transaminase, liver and kidney body weight ratios. However, the 125 μL kg^{-1} b.wt. resulted in significant decrease in red blood cell and hemoglobin, whereas the same dose produced increase in mean corpuscular volume, mean corpuscular hemoglobin, neutrophils and large unstained cell. Sodium, inorganic phosphorus, conjugated bilirubin, albumin, globulin and total protein at all the doses were not affected while potassium, chloride, urea, creatinine and total bilirubin concentration were increased at certain doses. Whereas the activity of serum gamma glutamyl transferase was significantly increased at all the doses, those of serum aspartate transaminase were increased only at 125 and 500 μL kg^{-1} b.wt. The kidney-body weight ratio was increased only at 375 and 500 μL kg^{-1} b.wt. The result showed that the oil of *T. minuta* had a mild effect on the parameters investigated at certain doses. This dose and parameter specific effects may influence the use of the essential oil from *T. minuta* as an insecticide against *Sitophilus zeamais* in maize grains meant for human and animal consumption.

Key words: *Tagetes minuta*, essential oil, biochemical parameters, insecticide, *Sitophilus zeamais*

INTRODUCTION

In sub-Saharan Africa, maize (*Zea mays* L.) is one of the most nutritional crops; however, proper storage of grains continues to be a challenge for subsistence farmers (Govender *et al*., 2008). Insects are one of the major causes of qualitative and quantitative losses in agricultural stored products. Poor storage techniques and some environmental factors encourage the growth of insect population in farm stores. Farmers and grain traders in sub-Saharan Africa are then forced to sell stored produce prematurely because of deterioration due mostly to insect damage. Producers have expressed the need for a relatively cheap and safe method of insect control (Stathers *et al*., 2002).

The heavy reliance on the use of conventional insecticides has led to problems of insect resurgence, resistance, negative impact on non-target organisms, health and environmental hazards. These have raised concern among the public for the need to search for a safe and environmentally friendly pest control options (Duke *et al*., 2003).

There is now a growing interest in the exploration of the natural vegetation for possible alternatives (Jovetic, 1994). Botanicals are important in insect management. They are known to provide effective control against insects that have become resistant to other insecticides (Weinzierl, 2000). Several plants have been found to possess insecticidal activities against a wide range of agricultural pests. *Tagetes minuta* (L.) (Mexican marigold) is a member of the family Asteraceae, which are well known for their insecticidal activities (Broussalis *et al*., 1999; Tomova *et al*., 2005). Previous study from our laboratory revealed that the oil from *Tagetes* plant had a strong contact effect against the stored maize weevil. However, there is dearth of toxicological information on the essential oil. Therefore, the present study was conducted to evaluate the toxicological effect of the essential oil from *T. minuta* leaves in rats.

MATERIALS AND METHODS

Plant material and authentication: Samples of the plant, collected from Nkonkobe municipality, Eastern Cape, South Africa in March, 2008, were authenticated by

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Professor DS Grierson of the Department of Botany, University of Fort Hare. A voucher specimen (Kem 02/2008) was deposited at Giffen herbarium of the University. This study was conducted between April and May, 2008.

Experimental animals: Twenty Wistar rats of both sexes weighing between 200 and 230 g were obtained from the Animal House of the Agricultural and Rural Development Research Institute (ARDRI), University of Fort Hare, South Africa. The rats were housed in polypropylene cages placed in well-ventilated house conditions. They were maintained on EPOL brand pelleted feed (EPOL feeds, South Africa Ltd.) and tap water *ad libitum*. The study was carried out following approval from ethical committee on animal use and care of University of Fort Hare, Alice.

Assay kits and chemical reagents: The assay kits for creatinine, urea, calcium, phosphorus, albumin, bilirubin, alkaline phosphatase, gamma glutamyl transferase, alanine and aspartate transaminases were obtained from Roche Diagnostic GmbH, Mannhein, Germany.

Preparation of essential oil: The procedure described by Asekun *et al.* (2007) was used. Briefly, fresh leaves from *T. minuta* were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus. The oil was stored in air-tight container at room temperature before administration.

Animal grouping and administration of essential oil: The rats were randomly grouped into five consisting of 4 rats each. Group A (control) received orally, 1 μL kg^{-1} b.wt. of distilled water for 14 days while Groups B, C, D and E received 125, 250, 375 and 500 μL kg^{-1} b.wt. of the essential oil, respectively, for same number of days. The oil and the distilled water were administered daily at 0900-1000 h.

Preparation of serum: The method described by Yakubu *et al.* (2005) was used. Briefly, under ether anaesthesia, the neck area of the rats was quickly shaved to expose the jugular veins. The veins after being slightly displaced (to avoid contamination with interstitial fluid) were sharply cut with a sterile scapel blade and an aliquot of the blood was collected into BD vacutainer sample bottles for the haematological analysis. The remainder was allowed to clot for 10 min at room temperature and then centrifuged at 1282 xg for 5 min using Hermle Bench Top Centrifuge (Model Hermle, Z300, Hamburg, Germany). The sera were stored frozen overnight before being used for the assay. The heart, kidney and liver were thereafter removed and weighed to determine their organ body weight ratio.

Determination of biochemical parameters: Adopting the method of Tietz *et al.* (1994), the levels of creatinine, uric acid, calcium, chloride, sodium and potassium ions, phosphorus and urea were determined. Total cholesterol, LDL-C, HDL-C, triglyceride, albumin, bilirubin, protein, alkaline phosphatase, gamma glutamyl transferase, alanine and aspartate transaminase were determined in the serum using assay kits from Roche Diagnostics, GmbH, Germany on Roche modular (model P800) Mannhein, Germany. The Advia 2120 (Bayer, Germany) was used for the haematological parameters.

Statistical analysis: Data obtained were subjected to one way Analysis of Variance (ANOVA) and means were separated by the Duncan Multiple Range Test. Percentage data were transformed to arcsine before analysis. Significant levels were tested at 5%.

RESULTS

The result on the haematological parameters, function indices of the kidney and liver as well as the serum enzymes showed that the essential oil has mild effect at some specific doses. Whereas the administration of *T. minuta* essential oil did not produce any significant difference in packed cell volume, mean corpuscular hemoglobin concentration, monocytes, lymphocytes, eosinophils and basophils at all the doses investigated, there were alterations in the remaining haematological parameters at specific doses. While the 125 μL kg^{-1} b.wt. resulted in significant decrease in red blood cell and hemoglobin, the same dose also produced increase in the concentration of mean corpuscular volume, mean corpuscular hemoglobin, neutrophils and large unstained cell. On the other hand, the 250 μL kg^{-1} b.wt. significantly decreased the red blood cell and haemoglobin whereas the platelet and large unstained cells were increased. While the 375 μL kg^{-1} b.wt. only increased the white blood cell count, the highest dose (500 μL kg^{-1} b.wt.) produced increase in large unstained cells (Table 1).

Although, the essential oil did not produce any significant effect on sodium, inorganic phosphorus conjugated bilirubin, albumin, globulin and total protein content of the animals at all the doses investigated, there were significant increases at certain doses in the concentrations of potassium, chloride, urea, creatinine and total bilirubin (Table 2).

While there was no significant difference in the liver and heart body weight ratios at all the doses investigated, the 500 μL kg^{-1} b.wt. produced increase in kidney-body weight ratio (Fig. 1).

Table 1: Effect of the essential oil of *Tagetes minuta* L. on haematological parameters of rats (N = 4)

Haematological parameters	Control	Doses (µL kg⁻¹ b.wt.) 125	250	375	500
WBC ($\times 10^9$ L^{-1})	5.24±1.05^a	4.25±0.27^a	5.24±0.47^a	7.82±1.18^b	5.24±0.42^a
RBC ($\times 10^{12}$ L^{-1})	9.14±0.15^a	8.32±0.29^c	8.40±0.16^{bc}	8.98±0.25^{abc}	9.04±0.20^{ab}
Hb (g dL^{-1})	16.13±0.25^a	15.35±0.48^{ab}	15.20±0.04^b	16.03±0.31^{ab}	15.25±0.10^{ab}
PCV (l L^{-1})	0.54±0.01^a	0.51±0.02^a	0.51±0.01^a	0.53±0.01^a	0.52±0.01^a
MCV (fl)	58.43±0.49^{bc}	61.40±0.62^a	60.23±0.85^{ab}	59.20±0.93^{abc}	57.23±0.78^c
MCH (pg)	17.68±0.11^b	18.48±0.17^a	18.08±0.32^{ab}	17.90±0.20^{ab}	16.90±0.28^c
MCHC (g dL^{-1})	30.20±0.25^a	30.05±0.24^a	30.00±0.40^a	30.18±0.30^a	29.55±0.13^a
Platelet ($\times 10^9$ L^{-1})	955.25±32.23^b	876.50±22.82^b	1052.75±7.15^a	879.25±32.84^b	937.50±32.53^b
Neutrophils (%)	12.31±1.38^{bc}	16.39±0.66^a	14.60±1.45^{ab}	10.23±0.73^c	14.28±0.43^{ab}
Monocytes (%)	28.97±2.33^a	37.27±2.57^a	32.61±2.82^a	29.77±5.60^a	36.33±3.23^a
Lymphocytes (%)	54.17±3.17^a	42.18±2.09^a	48.74±4.62^a	53.75±6.12^a	42.88±4.17^a
LUC (%)	14.02±0.59^b	17.73±0.81^a	17.36±0.12^a	16.09±0.72^{ab}	18.46±1.77^a
Eosinophils (%)	6.25±0.67^{ab}	6.27±0.30^{ab}	5.70±0.85^b	4.86±0.31^b	7.15±0.53^a
Basophils (%)	3.41±0.49^a	2.71±0.14^a	2.66±0.31^a	2.71±0.14^a	3.00±0.49^a

Means with the same superscripts as control across the rows are not significantly different ($p>0.05$). WBC: White Blood Cell, RBC: Red Blood Cell. PCV: Packed Cell Volume, Hb: Hemoglobin, MCV: Mean Corpuscular Volume, MCHC: Mean Corpuscular Hemoglobin Concentration, and LUC: Large Unstained Cell

Table 2: Effect of the essential oil of *Tagetes minuta* L. on the liver and kidney function indices of rats (N = 4)

Parameters	Control	Doses (µL kg⁻¹ b.wt.) 125	250	375	500
Sodium (mmol L^{-1})	138.25±0.65^a	140.00±0.71^a	138.00±0.71^a	138.20±1.81^a	139.500±1.71^a
Potassium (mmol L^{-1})	5.23±0.18^b	5.73±0.11^a	5.23±0.13^b	5.58±0.09^{ab}	5.450±0.14^{ab}
Chloride (mmol L^{-1})	107.50±1.19^b	109.50±0.87^{ab}	110.50±0.50^a	109.25±0.85^{ab}	110.750±0.85^{ab}
Inorganic phosphorus (mmol L^{-1})	3.15±0.06^a	3.18±0.17^a	3.03±0.16^a	3.13±0.05^a	3.150±0.06^a
Urea (mmol L^{-1})	5.88±0.05^{bc}	5.80±0.21^c	6.60±0.25^a	5.90±0.28^{bc}	6.500±0.11^{ab}
Creatinine (mmol L^{-1})	53.75±1.03^b	51.00±1.35^b	51.50±1.50^b	51.50±1.85^b	60.250±2.72^a
Total bilirubin (µmol L^{-1})	4.00±0.41^{ab}	6.00±1.47^a	4.50±0.50^{ab}	2.75±0.48^b	5.250±1.31^{ab}
Conjugated bilirubin (µmol L^{-1})	0.95±0.03^a	1.75±0.25^a	1.25±0.25^a	0.98±0.03^a	1.480±0.50^a
Albumin (mmol L^{-1})	18.00±0.41^a	18.50±0.50^a	19.25±0.48^a	18.50±0.29^a	18.750±0.48^a
Globulin (mmol L^{-1})	41.25±0.48^a	39.25±0.85^{ab}	37.50±0.50^b	40.50±0.6^a	39.250±0.63^{ab}
Total protein (g L^{-1})	59.25±0.25^a	57.75±1.31^a	56.75±0.85^a	59.00±0.71^a	58.000±1.08^a

Means with the same superscripts as control across the rows are not significantly different ($p>0.05$)

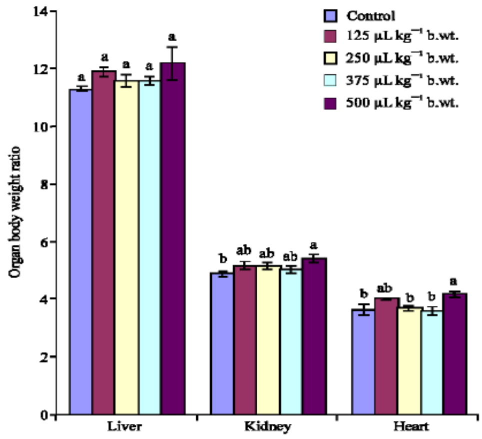

Fig. 1: Effect of the essential oil of *Tagetes minuta* L. on some organ body weight ratio of rats. Bars with the same letter(s) as control for same organ are not significantly different ($p>0.05$)

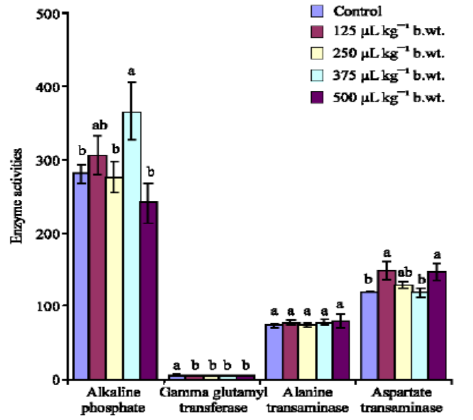

Fig. 2: Effect of the essential oil from *Tagetes minuta* L. on some enzyme activities of rat serum. Bars with the same letter(s) as control for each enzyme activity are not significantly different ($p>0.05$)

Administration of the essential oil did not produce any significant effect on the serum alanine transaminase activity at all the doses, but increased the activity of γ-glutamyl transferase. The essential oil also resulted in increase in aspartate transaminase activity only at 125 and 500 μL kg^{-1} b.wt. and alkaline phosphatase activity at 375 μL kg^{-1} b.wt. only (Fig. 2).

DISCUSSION

Assessment of haematological parameters can be used to determine the extent of deleterious effect of a foreign compound including plant extracts on the blood. It can also be used to explain blood relating functions of a plant extract or its products (Yakubu *et al*., 2007). The non-significant effect on packed cell volume, mean corpuscular haemoglobin concentration, monocytes, lymphocytes, eosinophils and basophils is an indication that the percentage of blood volume taken up by the red blood cell was not altered (Ganong, 2001). It may also be that the concentration of haemoglobin in the cell was not altered or that the oil did not interfere with the effector cells of the immune system. The alterations in the red blood cells, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin, neutrophils and large unstained cells at 125 and 250 μL kg^{-1} b.wt. may suggest destruction of matured red blood cells and this may affect the oxygen carrying capacity of the blood at this dose (McLellan *et al*., 2003). Mean corpuscular volume and mean corpuscular haemoglobin have particular importance in the diagnosis of anaemia in most animals (Coles, 1986). The increase in these parameters at this dose may imply that the oil is capable of inducing anaemia. However, since the higher doses did not bring about alteration in these parameters, it may be logical to infer that the animals were only trying to adapt to the effect of the oil at the lower doses.

The serum proteins and electrolytes evaluated in this study are useful parameters to indicate impairment in the functional capacity of the liver and kidney. Bilirubin, an index of liver damage is the major breakdown product of red blood cells. The increase in total bilirubin might affect the ability of the liver to transform bilirubin to the bile pigment-bilirubin glucoronide (Naganna, 1989). Such elevated level of bilirubin is an indication of impairment in the liver functional capacity (Moudgil and Narang, 1989). The fact that there was no alteration in the levels of albumin, globulin and total protein indicate mild, selective and localized toxicity of the essential oil on the liver.

Potassium is a major component of cardiac function. The increase in serum potassium ion observed with 125 μL dose suggests a possible adverse effect on the sodium pump that maintains the constancy of the extracellular concentration of potassium. Similarly, the increase in chloride ions at 250 and 500 μL doses suggests tubular dysfunction (Chawla, 1999). Urea is the major nitrogen-containing metabolic product of protein catabolism. The increase in serum urea content at 125 and 250 μL doses may be attributed to impairment in the urea cycle (Yakubu *et al*., 2003). Such increase in serum urea concentration indicates renal dysfunction. Creatinine is a metabolic by-product of muscle metabolism. The increase in serum creatinine content at the highest dose of the essential oil suggests glomerular and tubular dysfunction (Chawla, 1999). Therefore, the dose specific effect produced by the essential oil on the indices of kidney damage investigated in this study suggests selective toxicity.

Organ body weight ratio is a marker of cell constriction and inflammation (Moore and Dalley, 1999). The non-significant effect on the liver and heart-body weight ratios suggests that the essential oil did not cause inflammation or constriction of the hepatocyte and cardiac cells. However, the increase in the kidney-body weight ratio at 500 μL kg^{-1} b.wt. only suggests inflammation of the nephron. This further show the mild and selective toxicity of the oil.

There are many enzymes found in the serum that did not originate from the serum. During tissue damage, some of these enzymes find their way into the serum, probably by leakage (Wills, 1985). Serum enzyme measurements are therefore a valuable tool in clinical diagnosis, providing information on the effect and nature of pathological damage to any tissue. Therefore, the increase in serum γ-glutamyl transferase activity at all the doses, aspartate transaminase activity at 125 and 500 μL kg^{-1} b.wt. and alkaline phosphatase activity at 375 μL kg^{-1} b.wt. may indicate tissue damage leading to leakage of tissue enzymes to the serum. Such leakage from the tissue may adversely affect the normal functioning of these enzymes such as adequate transportation of required ion or molecules across the cell membrane (Akanji *et al*., 1993), transamination reaction involving the transaminases, glutathione metabolism and resorption of amino acids by γ-glutamyl transferase (Kaplan and Pesce, 1996).

The result of the present study has shown that the oil of *Tagetes minuta* is of mild toxicity. The alterations produced at certain doses on some of the indices of liver and kidney function, haematological parameters and kidney-body weight ratio suggest that the effect of the essential oil from *Tagetes minuta* leaves is dose and tissue index selective.

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CHAPTER 8

TOXICOLOGICAL EVALUATION OF THE ESSENTIAL OIL FROM *MENTHA LONGIFOLIA* L. SUBSP. CAPENSIS LEAVES IN RATS

CHAPTER 8

Toxicological evaluation of the essential oil from *Mentha longifolia* L. subsp. *capensis* leaves in rat.

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Toxicological evaluation of the essential oil from *Mentha longifolia* L. subsp. *capensis* leaves in rats

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Running title: Toxicity of *Mentha longifolia* essential oil

Abstract: The effects of the essential oil from the leaves of *Mentha longifolia* L. subsp. *capensis* on some biochemical parameters of Wistar rats were studied. The oil at 125, 250, 375 and 500 µl/kg body weight reduced ($P<0.05$) the red blood corpuscles (RBC) and lymphocytes with no definite pattern on the white blood corpuscles (WBC) and mean corpuscular volume (MCV). The doses significantly increased the neutrophils, monocytes, large unstained cells (LUC), liver-body weight ratio, serum concentrations of cholesterol, triglyceride, HDL-C and inorganic phosphate but no effect on the heart body weight ratio, serum LDL-C, Na^+, Ca^{2+}, Cl^-, K^+, creatinine and uric acid. The 500µl/kg body weight also increased the kidney-body weight ratio. In contrast, the oil reduced the serum urea and atherogenic index. The total and conjugated bilirubin, together with the total protein and albumin in the serum increased only at 125 µl/kg body weight. The serum alkaline phosphatase activity also increased with no significant change in gamma-glutamyl transferase, alanine and aspartate aminotransaminases. The results indicate dose and parameter specific effect of the essential oil. Although, the essential oil from *M. longifolia* leaves may not predispose to atherosclerosis, it may increase the functional activity of the rat liver at the least dose investigated. Therefore, the essential oil from *Mentha longifolia* may not be completely 'safe' at the doses investigated.

Keywords: essential oil, functional activity, haematological parameters, *Mentha longifolia*, selective toxicity, serum lipids.

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Introduction

The search for environmentally safe pesticides has fostered research on the use of plant materials for the protection of crops against pests, both on the field and in storage.[1] Secondary plant metabolites are a large reservoir of chemicals with biological activities.[2] Phytochemicals such as rotenone, nicotine and pyrethrin were all used as pesticides before the advent of synthetic insecticides.[3,4] Many members of Myrtaceae, Asteraceae, Piperaceae, Meliaceae and Annonaceae are known to possess various chemical compounds which act as antifeedants, repellents or growth inhibitors to many insect species.[5] One of such plant from Lamiaceae that has been well studied for its antibacterial properties is *Mentha longifolia* L. subsp. *capensis*.[6,]

Mentha longifolia L. subsp. *capensis* (Lamiaceae), is a fast growing plant that is widespread in the Eastern Cape of South Africa. It is a popular plant used for the treatment of respiratory ailments and other diseases.[7] The strong smell of the leaves keep mosquitoes away when rubbed onto the body.[8] The main constituents of the essential oil from *Mentha longifolia* leaves obtained from Eastern Cape of South Africa include 1,8-cineole (25.46%), menthone (17.85%) and pulegone (29.93%). Other minor components include β-pinene (3.49%), cyclohexanone (2.89%), α-pinene (2.51%), cis-isopulegone (2.38%), trans sabinene hydrate (2.28%), α-terpineol (1.81%), germacrene-D (1.61%), β-caryophylene (1.59%) and a trace amount of 4 other constituents.[9] The essential oil from the plant has been found to be effective as an insecticide against several insect species.[9,10]

Several toxicological effects have been associated with the oil components of *Mentha arvensis* and *Mentha spicata*. In particular, menthol has been found to interact with cytosolic Ca^{2+}, probably through an intracellular Ca^{2+} store release and Ca^{2+} channel blocking.[11, 12] Menthone has been reported to be a growth inhibitor[13], whereas pulegone, a potent abortifacient, is metabolized by hepatic microsomal mono-oxygenases to a series of hepatotoxins that cause liver cancer.[14] Similarly, Spindler and Madsen[15] reported that all haematological and biochemical parameters as well as absolute and relative weights of the organs were within normal range whereas the histopathological examination revealed alteration in the rat brain following the administration of peppermint oil (oil from *Mentha piperita*) for 86 days experimental period. Unfortunately however, there is dearth of information on the toxicological effect of the essential oil from *Mentha longifolia* leaves growing in Eastern Cape of South Africa.

This study was, therefore, undertaken to evaluate the possible toxicological effect of the essential oil from *Mentha longifolia* in rats. This was evaluated on haematological parameters, serum enzymes and lipid profile as well as organ-body weight ratios and function indices.

Materials and Methods

Plant material and authentication

Samples of the plant, collected from Nkonkobe municipality in the Eastern Cape, South Africa, at full maturity (flowering stage) were authenticated by Professor DS Grierson of the Department of Botany, University of Fort Hare. A voucher specimen (Kem 01/2006) was deposited at the Giffen Herbarium of the University.

Experimental animals

Twenty- five Wistar rats of both sexes, weighing between 200 and 230 g, were obtained from the Animal House of the Agricultural and Rural Development Research Institute (ARDRI), of the University of Fort Hare. The rats were housed in polypropylene cages placed in well-ventilated house conditions (temperature 23 ± 1˚C; photoperiod: 12 h natural light and 12 h dark; humidity: 45-50%). They were fed with EPOL brand pelleted feed (EPOL feeds Ltd, South Africa) and tap water *ad libitum.* The study was carried out following approval from the Ethical Committee on the use and care of laboratory animals of the University of Fort Hare, South Africa.

Assay kits

The assay kits for creatinine, urea, uric acid, calcium, phosphorus, cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol, albumin, bilirubin, alkaline phosphatase, gamma-glutamyl transferase, alanine and aspartate aminotransferase were obtained from Roche Diagnostic GmbH, Mannhein, Germany.

Preparation of essential oil

Fresh leaves (256.90 g) of *M. longifolia* (flowering) were subjected to hydrodistillation in an all glass Clevenger-type apparatus for 3 h according to the method described in the British Pharmacopoeia.[16] This gave 9.24 g of the oil corresponding to a percent yield of 3.6 (w/w). The oil was stored in an air-tight container at 4˚C.

Animal grouping and administration of essential oil

The rats were completely randomized into five groups consisting of five rats each. Group A (control) received orally, 1µl/kg body weight of distilled water on a daily basis for 14 days, while Groups B, C, D and E were treated like the control except that they also received 125, 250, 375 and 500 µl/kg body weight of the essential oil, respectively. The oil and the distilled water were administered daily at 0900 – 1000 hr.

Preparation of serum

The method described by Yakubu *et al.*[17] was used in the preparation of the serum. The rats were initially weighed before the sacrifice. Under ether anaesthesia, the neck area of the rats was quickly shaved to expose the jugular veins. The veins after being slightly displaced (to avoid contamination with interstitial fluid) were sharply cut with a sterile scalpel blade and an aliquot (2 ml) collected into EDTA sample bottles (BD Diagnostics, Preanalytical Systems, Midrand, US) for the haematological analysis while the remainder (5 ml) was allowed to clot for 10 min at room temperature. The clotted blood was centrifuged at 1282 *g* x 5 min using Hermle Bench Top Centrifuge (Model Hermle, Z300, Hamburg, Germany).

The serum was stored frozen overnight before being used for the assay. The heart, liver and kidney samples were, thereafter, excised from the animals, blotted in tissue paper and then weighed for the determination of the organ-body weight ratio.

Determination of biochemical parameters

Adopting the method of Tietz *et al.*[18], the levels of creatinine, uric acid, calcium and chloride ions, urea, sodium, potassium ions and phosphorus were determined. Total cholesterol, LDL-C, HDL-C, triglyceride, albumin, bilirubin, protein, alkaline phosphatase, gamma-glutamyl transferase, alanine and aspartate aminotransferase were determined using assay kits from Roche Diagnostics, GmbH, Germany, on a Roche modular (model P800), Mannhein, Germany. The Advia 2120 (Bayer, Germany) was used for the determination of haematological parameters.

Statistical analysis

Data obtained were subjected to one way analysis of variance (ANOVA) and means were separated by Duncan's Multiple Range Test.[19] Percentage data were transformed to arcsine before analysis. Significant levels were tested at $P<0.05$.

Results

The essential oil from *Mentha longifolia* leaves produced no definite pattern on the white blood cells (WBC) at all the doses investigated whereas the neutrophils, monocytes, large unstained cells (LUC) and mean corpuscular volume (MCV) increased significantly ($P<0.05$). While there was significant decrease ($P<0.05$) in the red blood cells (RBC) and lymphocytes, the eosinophils, basophils, packed cell volume (PCV), haemoglobin (Hb), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were not significantly altered (Table 1).

The essential oil at all the doses increased ($P<0.05$) the liver-body weight ratio whereas the heart-body weight ratio compared favourably with the control. The highest dose (500 µl/kg body weight) also increased the kidney-body weight ratio (Figure 1).

Apart from the increase in serum inorganic phosphorus as well as reduction in serum urea concentration, the essential oil did not produce any appreciable change in the serum concentrations of creatinine, uric acid, sodium, calcium, chloride and potassium ions (Table 2). Similarly, aside from the 125 µl/kg body weight that increased the serum concentration of total protein, albumin, total bilirubin as well as conjugated bilirubin, the values of these indices were not significantly different at other dose levels (Table 2).

The serum alkaline phosphatase activity increased at all the doses whereas the activities of gamma-glutamyl transferase, alanine and aspartate aminotransferases in the serum compared favourably ($P>0.05$) with their controls (Figure 2).

Administration of the essential oil from *Mentha longifolia* leaves increased the serum cholesterol, triglyceride and high density lipoprotein-cholesterol (HDL-C) concentrations. The serum low density lipoprotein-cholesterol (LDL-C) content was not significantly different from the control. The computed atherogenic index was also reduced by the essential oil (Figure 3).

Discussion

Assessment of haematological parameters can be used to determine the extent of deleterious effect of a foreign compound including plant extracts on the blood. It can also be used to explain blood relating functions of a plant extract or its products.[20] In this study, the significant decrease in RBC may be an indication that the extract impaired the production of the blood cells or the rate of destruction of the matured red blood cells was greater than the rate of its production from the bone marrow. However, the non-significant effect of the essential oil of *Mentha longifolia* on the Hb implied that the blood parameter was not adversely affected. The MCV, MCH and MCHC relate to individual red blood cells and are of particular importance in the diagnosis of anaemia.[21, 22] The non-effects on these indices imply that the extract did not affect the incorporation of haemoglobin into red blood cells.[22] It also indicates that the average size of RBC (microcytes) and haemoglobin weight per RBC were unaffected by the oil. Similarly, the non-significant effect of the oil on PCV implies that the percentage of blood volume taken up by red blood cells was not altered. All these are indications of selective toxicity of the oil on the red blood cells and their related parameters.

The reduction in WBC only at the least dose of the essential oil (125 µl/kg body weight) may be due to an attempt by the animals to adapt to the effect of the extract since the other dose levels did not produce any significant change on the parameter. The non-significant effect on the platelets may imply that thrombopoietin was not affected by the oil.[23] Neutrophils are matured cells that attack and destroy bacteria in the blood.[24] The significant increase in the neutrophils by the oil may imply enhancement in the ability of the blood component to phagocytose. Lymphocytes are the main effector cells of the immune system.[25]

The administration of the essential oil appears to exhibit stimulatory effect on the effector cells of the immune system. The fact that the eosinophils and basophils were not altered in this study implies the selective toxicity of the oil on the haematological parameters. This contrasts with findings of Spindler and Madsen[15] which showed that the haematological parameters following the administration of the oil from *Mentha piperita* and *Mentha arvensis* were within normal range.

The biochemical indices evaluated in this study are useful parameters to assess the functional capacity of the liver and kidney.[26] In this study, the significant increase in the liver and kidney-body weight ratios may indicate a toxic effect of the essential oil. This agreed with the findings of Madsen *et al.*[27] after dosing rats with menthone, a component of peppermint oil. Therefore, the effect of the essential oil on the organ-body weight ratio in this study may be attributed to its menthone constituent. In contrast, the non-significant effect on the heart might be that the essential oil did not adversely affect the organ.

Inorganic electrolytes occur in large quantities in both extracellular and intracellular fluids. They comprise the most single important factor in the transfer and movement of electrolytes between the extracellular and intracellular compartments.[28] Phosphate normally released during cell breakdown is used in building nucleic acid of cells. The observed significant increase in phosphorus suggests adverse effect on the normal tubular reabsorption or excretion of this ion by the kidney. On the other hand, the non-effect of the oil on sodium, calcium, chloride and potassium ion may be an indication that these ion-dependent processes were not adversely affected. Urea is the major nitrogen-containing metabolic product of protein catabolism. The reduction in the serum urea concentration observed in this study suggests abnormality in the physiological excretion of urea caused by a non-renal factor which is the essential oil in this study. The effect produced by the oil on the uric acid and creatinine indicate non-impairment in the glomerular, tubular and renal functions. This further shows that the oil is selective in its effect on the kidney function indices.

Bilirubin, a metabolic breakdown product of heme derived from senescent red blood cells, is also one of the most commonly used liver function tests. The increase in total protein, albumin, total bilirubin and conjugated bilirubin observed with the least dose may be adduced to increase in the functional activity of the liver.[26] This selective dose specific increase in the serum proteins may be an adaptation attempt to the effect of the oil. This adaptation became evident when higher doses did not produce any significant change in the liver function parameters (Table 2).

Alkaline phosphatase (ALP) is a ‘marker’ enzyme of damage for the plasma membrane and endoplasmic reticulum.[29,30] It is frequently used to assess the integrity of the plasma membrane.[31] Enzymes from diseased or damaged tissues may become recognizable in the serum presumably by leakage through altered cell membrane of the rat organs.[32] The increase in serum ALP activity in this study confirmed that damage was inflicted on the plasma membrane.[26] Such increase in the activity of the enzyme may be due to disruption of the ordered lipid-bilayer of the membrane structure of the affected organ. Therefore, the oil might have labialized the plasma membrane of the rat tissues.

Γ-glutamyl transferase is a membrane bound enzyme which catalyses the transfer of γ-glutamyl group between peptides and amino acids.[33] Similarly, aminotransferases are cytosolic enzymes that can be used to assess damage to the liver and heart.[30,34] The non-significant effect of the oil on these serum enzymes suggest that their levels were not altered.

Alterations in the concentration of major lipids like cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol and triglycerides can give useful information on the predisposition of the heart to atherosclerosis and its associated coronary heart disease.[35, 36] High blood cholesterol concentrations is an important risk factor for cardiovascular disease.[37, 38] Therefore, the elevated serum cholesterol concentration observed with the oil may be due to an increase in the concentration of acetyl CoA arising probably from increased β-oxidation of fatty acids, since acetyl CoA is a key substrate in the biosynthesis of cholesterol.[39] Although acetyl CoA was not measured in this study, nevertheless, the increase in serum cholesterol concentration may not be beneficial to the animals as it may enhance the incidence of atherosclerosis and hypertension.[40] The increase in serum triacylglycerol concentration observed in this study may be attributed to accelerated lipolysis which may deplete the store of fatty acids.[38]

High-density lipoprotein cholesterol (HDL-C) is considered to have anti-atherogenic properties, since there is a negative correlation between HDL and risk of cardiovascular disease. The increase in HDL-C may be clinically beneficial to the animal. It has been demonstrated that an increase in the concentration of HDL-C correlates inversely with coronary heart disease.[41] The biochemical importance of HDL-C lies in the fact that it removes cellular cholesterol, transferring it for excretion. Therefore, the increase in serum cholesterol concentration as observed in this study may not constitute health hazards since there was a corresponding increase in HDL-C which may assist in the excretion of cholesterol.

Low-density lipoprotein cholesterol (LDL-C) is a primary carrier of plasma cholesterol and has been implicated in atherosclerosis.[42] The non-significant effect of the oil on LDL-C coupled with the reduced atherogenic index further lend credence to the non-predisposition to atherosclerosis and coronary heart disease by the essential oil from *Mentha longifolia* leaves. Despite increasing the cholesterol and triacylglycerol contents of the serum, it is unlikely that the risk of atherosclerosis and coronary artery diseases will be enhanced by the essential oil, since the reduction in atherogenic index was accompanied by an increase in HDL-C.

Our study has shown that the essential oil from *Mentha longifolia* leaves may not predispose the rats to atherosclerosis despite the alterations produced in the serum lipids. The study also showed that the essential oil from the leaves of *Mentha longifolia* exhibited dose and parameter specific effects on the biochemical parameters investigated. Therefore, the essential oil from *Mentha longifolia* may not be completely 'safe'to use as an insecticide in stored maize grains.

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Authors' disclosure statement

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Table 1: Effect of the essential oil of *Mentha longifolia* L. on haematological parameters of rats.

		Amount of essential oil administered per kg body weight			
Haematological Parameters	Control	125µl	250µl	375 µl	500 µl
WBC (X 10^9/l)	8.10±0.36[ab]	6.07 ±0.36[c]	8.69±0.81[bc]	7.25±0.28[a]	8.98 ±0.60[ab]
RBC (X 10^{12}/l)	9.09±0.07[a]	8.28±0.17 [b]	8.81±0.17[ab]	8.63±0.14[ab]	8.41±0.19[b]
PCV (l/l)	0.51± 0.01[a]	0.51± 0.01[a]	0.50± 0.01[a]	0.50± 0.01[a]	0.51± 0.01[a]
Hb (g/dl)	16.20±0.06[a]	15.75±0.38 [a]	15.75±0.28[a]	15.70±0.25[a]	15.98±0.20[a]
MCV (fl)	56.65±0.20 [b]	60.45±1.11[a]	57.03±0.93[ab]	57.53±0.70[ab]	60.00±1.80[ab]
MCH (pg)	17.80±0.23[a]	18.93± 0.46[a]	17.88±0.36[a]	18.20±0.40[a]	19.00±0.23[a]
MCHC (g/dL)	31.45±0.49[a]	31.15±0.22[a]	31.38±0.22[a]	31.65±0.37[a]	31.68±0.31[a]
Platelet (X10^9/l)	887.50± 41.86[a]	996.75±37.26[a]	970.00±50.83[a]	913.50±32.84[a]	1026.75±53.42[a]
Neutrophils (%)	8.10±0.12[a]	10.43±0.37[b]	11.08±1.96[b]	11.48±1.20[b]	10.83±0.80[b]
Monocytes (%)	20.15± 0.49[c]	24.88±0.33[bc]	26.70±1.54[b]	25.00±0.83[bc]	33.93±0.49[a]
Lymphocytes (%)	61.30±0.82[a]	54.68±0.51[b]	54.73±1.24[b]	54.65±1.30[b]	46.15±3.22[c]
LUC (%)	16.30±0.86[c]	18.28±1.04[ab]	18.98±0.71[b]	18.15±0.93[ab]	19.68±0.19[a]
Eosinophils (%)	5.40±0.35[a]	5.33±0.20[a]	5.40±0.63[a]	6.30±0.76[a]	5.48±0.62[a]
Basophils (%)	3.85±0.12[a]	4.23±1.00[a]	4.05±0.17[a]	4.00±0.24[a]	4.50±0.22[a]

Data are mean ± SE values.
Means with the same letter across the rows are not significantly different ($P < 0.05$). WBC, white blood cell; RBC, red blood cell; PCV, packed cell volume; Hb, hemoglobin; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; LUC, large unstained cell.

Table 2: Effect of the essential oil of *Mentha longifolia* L. on the liver and kidney function indices of rats.

	Amount of essential oil administered per kg body weight				
Parameters	Control	125µl	250µl	375 µl	500 µl
Sodium (mmol/l)	144.67 ± 1.50[a]	163.33 ± 5.10[a]	143.33 ± 0.90 [a]	144.67 ± 0.9 [0a]	143.67 ± 0.90 [a]
Potassium (mmol/l)	5.87 ± 0.27[b]	8.10 ± 1.9[a]	6.18 ± 0.43[ab]	5.73 ± 0.29[b]	6.03 ± 0.24[b]
Chloride (mmol/ l)	110.67 ± 0.70[ab]	112.00 ± 2.00[a]	107.00 ±1.20[b]	109.33 ± 0.90[ab]	107.67 ± 1.20[b]
Phosporus Inorganic (mmol /l)	2.73 ± 0.28[b]	3.60 ± 0.30[a]	3.23 ± 0.22[ab]	3.03 ± 0.09[ab]	3.20 ± 0.06[ab]
Calcium (mmol/l)	2.33 ± 0.05[a]	2.32 ± 0.10[a]	2.33 ± 0.02[a]	2.36 ± 0.01[a]	2.38 ± 0.03[a]
Urea (mmol/l)	7.60 ± 0.30[a]	7.23 ± 0.33[ab]	6.27 ± 0.13[b]	6.33 ± 0.55[b]	6.03 ± 0.35[b]
Creatinine (mmol/l)	46.67 ± 3.67[a]	47.33 ± 4.33[a]	48.67 ± 2.03[a]	51.67 ± 3.53[a]	48.00 ± 2.08[a]
Uric acid (mmol/l)	0.10 ± 0.02[a]	0.17 ± 0.02[b]	0.12 ± 0.03[a]	0.11 ± 0.02[a]	0.13 ± 0.02[a]
Bilirubin total (µmol/l)	10.33 ± 2.85[a]	28.67 ± 1.67[a]	11.00 ± 1.53[a]	8.00 ± 0.58[a]	10.00 ± 1.00[a]
Bilirubin Con- jugated (µmol/l)	4.33 ± 0.67[a]	10.33 ± 0.33[b]	5.33 ± 0.88[a]	5.00 ± 0.58[a]	4.33 ± 0.33[a]
Albumin (mmol/l)	50.20±0.14[a]	52.66±0.11[b]	50.41±0.07[a]	50.39±0.08[a]	50.20±0.17[a]

Total protein (g/l)	60.67 ± 2.85[a]	81.33 ± 1.33[a]	61. 67 ± 0.88[a]	60.00 ± 0.58[a]	61.00 ± 2.08[a]

Data are mean ± SE values.
Means with the same letter across the rows are not significantly different (P <0.05).

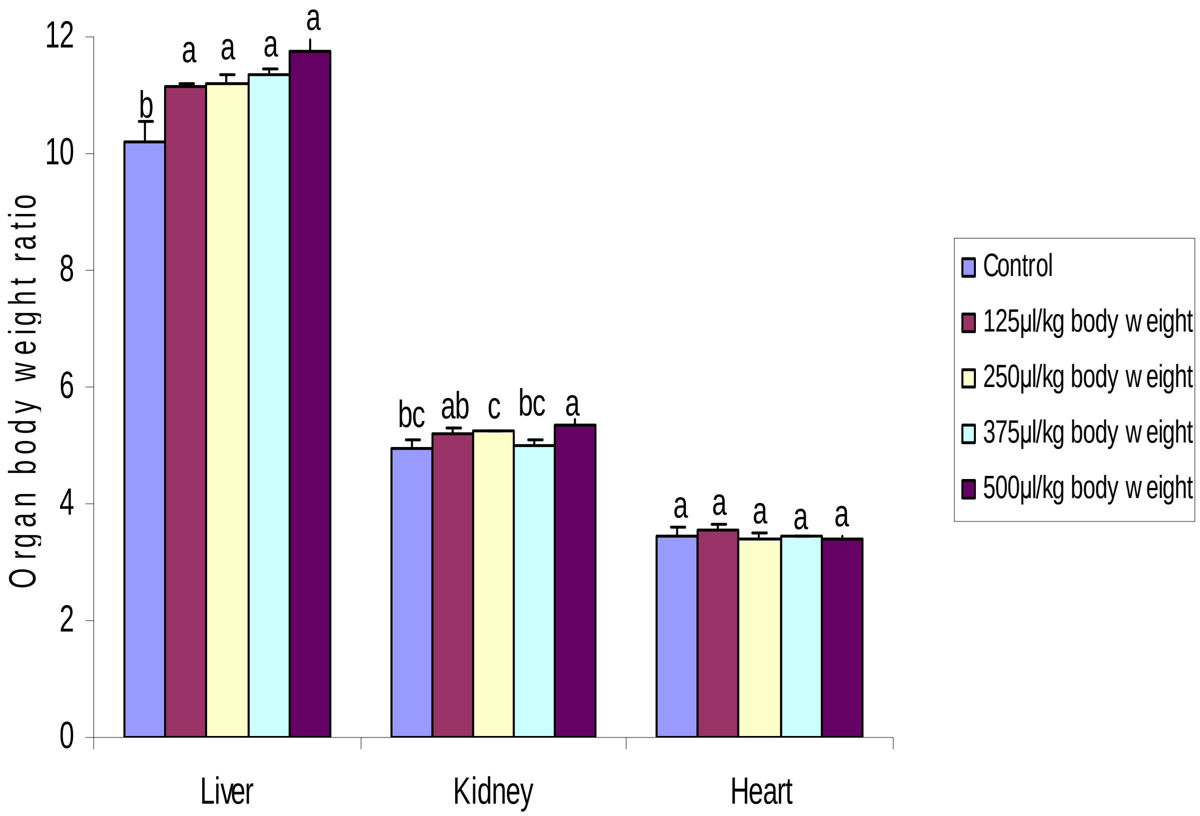

Figure 1: Effect of the essential oil of *Mentha longifolia* L. on organ body weight ratio of rats. Histograms of an organ with the same letter are not significantly different ($P < 0.05$).

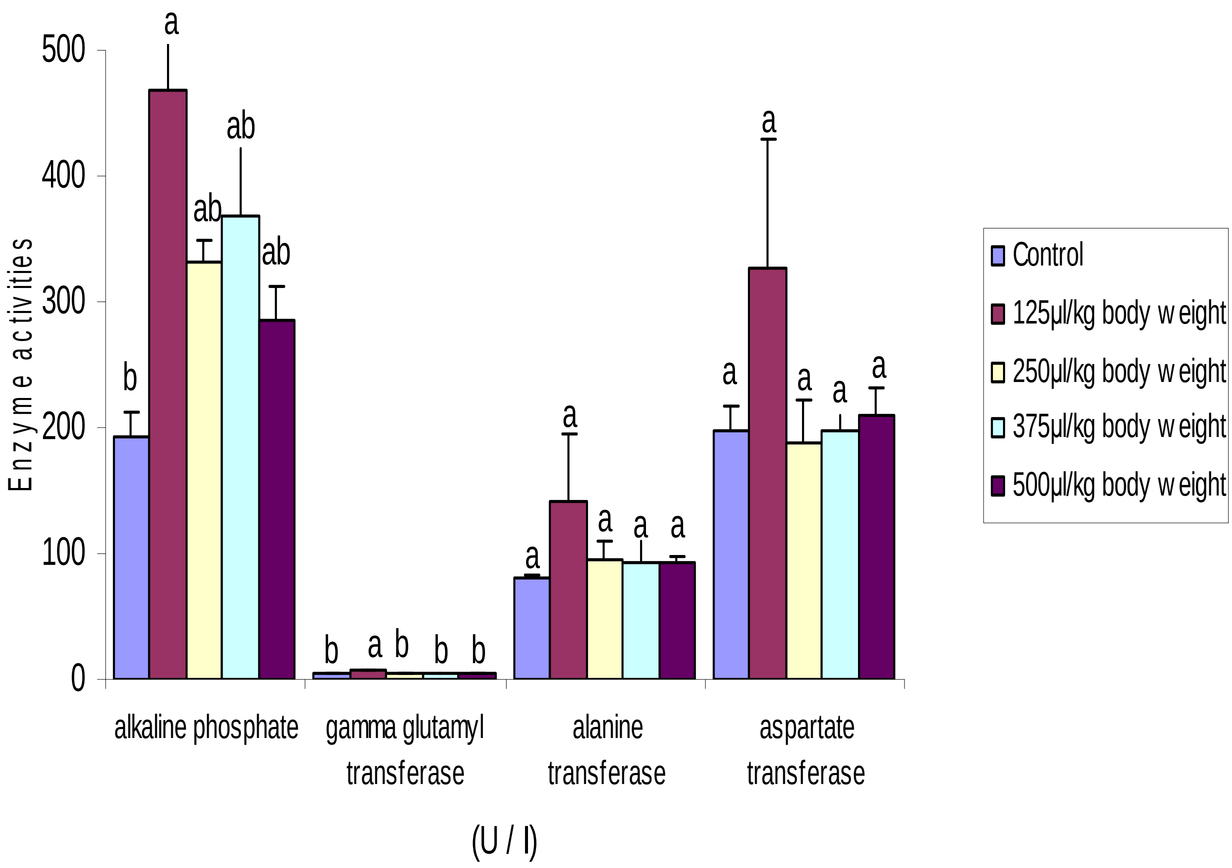

Figure 2: Effect of the essential oil from *Mentha longifolia* L. on some enzyme activities of rat serum. Histograms (in a group) with the same letter are not significantly different (P< 0.05).

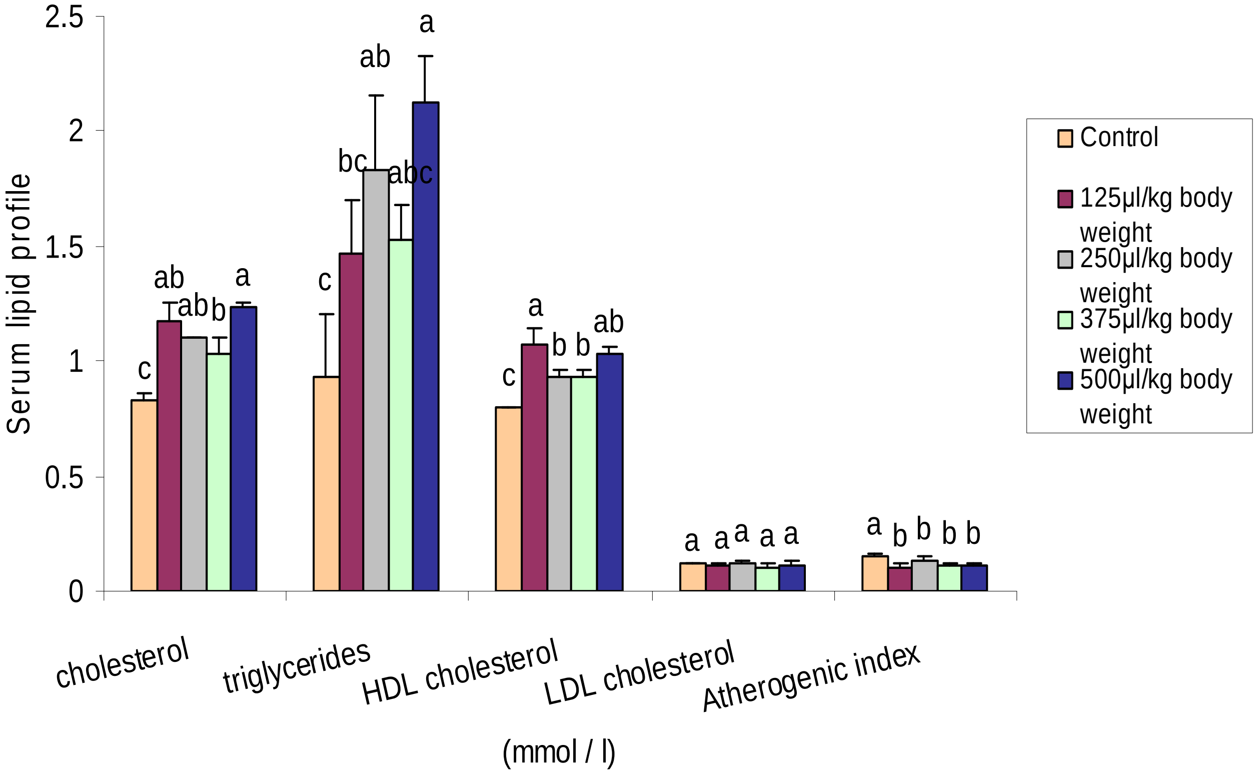

Figure 3: Effect of the essential oil of *Mentha longifolia* L. on serum lipid profile of rats. Histograms (in a group) with the same letter are not significantly different (P< 0.05).

CHAPTER 9

GENERAL DISCUSSION AND CONCLUSIONS

Chapter 9

DISCUSSION AND CONCLUSIONS

Postharvest losses due to insect damage is a major challenge facing farmers in tropical and sub-tropical Africa. In the absence of insect control, the threats of malnutrition and hunger loom over millions of people in the developing world especially in africa where most of the grains are grown for consumption and the ravages of stored-product insects are the greatest (Donahaye, 2000). Thus, the protection of farm produce against the deleterious effect of insect pests is very important in order to attain food security in the phase of the ever increasing African annual population (Schult 1994, in Eicher and Byerlee, 1997). To ensure food security among the people, there is a need for adequate control of insect pest damage on stored food.

The search for safe and environmentally-friendly pest control agents has led to the exploration of natural products from plants for potential alternatives. In this study, information gathered from the literature shows that the protection of stored agricultural products (including maize) using plant materials has been an age-long practice in South Africa. Also, plants from several families are known to exhibit various modes of actions against a wide range of insects, some of which have been scientifically validated (Chapter 2)

The present study has highlighted the importance of farmers' knowledge and experience of indigenous insect pest control. A survey conducted as part of the study revealed that 63% of the farmers were aware of indigenous knowledge of insect pest control, despite the fact that such methods are currently being neglected and the knowledge of its application fast eroding. Insects and other pests were reported to be a major threat to most of the cultivated crops. The maize storage weevil (*Sitophilus zeamais*) was identified as the cause of huge losses of harvested maize. Poor storage condition were observed in most of the visited farms, where maize grains were either spread on the ground inside an open room, stored in plastic containers and synthetic bags or spread on top of the roofs. Some of these storage conditions may encourage rapid insect development (Chapter 3). Interestingly, from this study, majority of farmers were eager to adopt new insect control methods if available.

Recently, there has been a growing interest in research regarding the possible use of plant extracts as alternatives to synthetic insecticides. Essential oils are among the best known plant products tested against insects (Papachristos and Stamopoulos, 2002, Formisano et al, 2008). Plant essential oil is a group of botanical insecticide that has recently been commercialized in the United States (Isman, 2008). However, thousands of plants with potential insecticidal properties are still there in the forest /vegetation unexplored. In the course of the present study, the essential oil of *M. longifolia* exhibited different levels of toxicity against *S. zeamais* at the various concentrations investigated. The dose of the oil at 0.50µl /g of maize grains in the contact bioassay caused 100% mortality of S. *zeamais.* The level of fumigation toxicity exhibited by the oil was moderate at 24 and 32 µl of oil per litre of air. However, the oil evoked a very high repellency against the insect. Also, *T. minuta* oil acted similarly in the contact bioassay causing 100% mortality at 0.375µl /g of maize grains. The repellent effect of the oil against *S. zeamais* was well pronounced. Contrarily, the essential oils of *R. officinalis, H. oddoratissimum* and *P. graveolens* had weak contact and fumigation effects against *S. zeamais*. The results from this study shows that the essential oils of *M. longifolia* and *T. minuta* have the potential to be employed in the control of *S. zeamais.*

This study further revealed that the essential oil of *M. longifolia* and *T. minuta* had no major adverse effect on the proximate composition of maize grains stored with the oils over a period of three months. *M. longifolia* had no significant change in the colour of the treated grain throughout the storage period. However, the oil affected on the grain odour at the earlier storage period, which was found to decrease as the storage period progressed. A similar trend was also observed for the oil of *T. minuta.*

Several cases of pesticide poisoning have been reported in human food across the globe. The problems of chemical residues in treated crops and grains have been of a great concern to the public. This has led to the increasing demand for quality food uncontaminated by insecticidal residues (Donahaye, 2000). The results obtained from this study indicated that the essential oil from *M. longifolia* leaves may not lead to atherosclerosis but might disrupt the cell membrane, and cause dysfunction in the rat liver. *T. minuta* oil had mild effect on the parameters investigated at certain doses. It is, therefore, advised that the essential oils from these plants not be used as insecticides for protection against *S. zeamais,* in maize grains that are intended for human consumption, at the doses investigated.

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APPENDICES

Appendix 1. Questionnaire administered for the survey on the farmer's knowledge and experience of indigenous insect pest control in the Eastern Cape, South Africa. (Chapter 3).

The aim of this questionnaire was to obtain baseline information on the plants used for insect pest control by the farmers.

Questionnaire

Village……………………

Location…………………..

Questionnaire…………………..

Date…………………………..

Sex…………………………….

Age……………………………

PERSONAL INFORMATION

1. Educational background………………….
2. Major source of income…………………..

FARMING EXPERIENCE

3. Do you grow crops? If yes
4. What crops do you grow?

5. Why do you grow these crops?

i) For home consumption ( )

ii) Selling ( )

iii) For livestock ( )

iv) Others ( )

6. How long have you been growing these crops?

Crop	<1 year	2-5 years	6-10 years	>10 years
Maize Beans Cabbage Others				

7. Where do you plant these crops?

	Size
Home gardens	
Arable fields	

8. Do you have any insect problem in growing/ storing these crops? If yes

9. What type of insect (s)?

10. What is the nature of damage caused by these insects?

11. At what stage are your crops affected?

i) Seedling ( )

ii) Vegetative ( )

iii) Flowering ( )

iv) Fruiting ( )

v) Post-harvest ( )

12. In what way does this damage affect your yield, quantity of produce or quality of produce?

13. At what time of the year do you experience these problems? (Summer, Spring……..

14. What other type of pest problem do you encounter…………..?

15. What do you do with the harvested produce?

Keep in;

Barn

Tanks

Others…………….

16. What materials do you use to make the storage units?

17. What are the various ways you control the insect pest problems?

i) At growing stage

ii) In storage

18. Do you buy any of the materials you use to control the insect pest?

If yes, what do you buy?

- Where do you buy them from?
- How do you pay for them?
- How do you use them?

19. Do you know of any other methods that these insect problems were controlled in the olden days?

20. Are there plants that are used to control insect pest? If yes?

i) What is the name of the plant?

ii) Which part of the plant do you use?

a) Leaves ( )

b) Stem ( )

c) Bark ( )

d) Root ( )

iii) How is it prepared?

iv) How is it used?

21. What are your suggestions for the control of these insects?

Appendix 2a. Assessment sheet for grain quality parameters (Chapter 6)

ASSESSMENT SHEET FOR MAIZE GRAIN

Sheet no…….					
Date of assessment…………. **Assessed by………………….**	**Odour**				
Sample	**1**	**2**	**3**	**4**	**5**
A1					
A2					
A3					
B1					
B2					
B3					
C1					
C2					
C3					
D1					
D2					
D3					
E1					
E2					
E3					
F1					
F2					
F3					

Scale: 1-5

1- No difference in grain odour.
2- Grain has little offensive odour.
3- Grain has moderately offensive odour.
4- Grain has offensive odour.
5- Grain has very offensive odour.

Appendix 2b. Assessment sheet for grain quality parameters (Chapter 6)

ASSESSMENT SHEET FOR MAIZE GRAIN

Sheet no…….					
Date of assessment………….	**Colour**				
Assessed by………………….					
Sample	**1**	**2**	**3**	**4**	**5**
A1					
A2					
A3					
B1					
B2					
B3					
C1					
C2					
C3					
D1					
D2					
D3					
E1					
E2					
E3					
F1					
F2					
F3					

Scale: 1-5

1- No detectable change i.e natural white with a few yellow/ red grains.
2- Slight change (≤ 5%) from natural white/ yellow/ red to light brown.
3- Moderate change (> 5 to 30%) from natural white/ yellow/ red to brown.
4- Great change (> 30- 50%) from natural white/ yellow/ red to dark brown.
5-Highly significant change (> 50 %) making grain unacceptable for human consumption.

Appendix 4. Typical output on Analysis of Variance and DMRT for the effect of administration of the essential oil from *Tagetes minuta* L. leaves in Wistar Rats (Chapter 7).

Dependent Variable: WBC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	28.30518000	7.07629500	2.98	0.0539
Error	15	35.63407500	2.37560500		
Corrected Total	19	63.93925500			

Duncan's Multiple Range Test for WBC

Alpha	0.05
Error Degrees of Freedom	15
Error Mean Square	2.375605

Number of Means	2	3	4	5
Critical Range	2.323	2.435	2.505	2.552

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	trt
A	7.820	4	C
B	5.353	4	D
B			
B	5.235	4	B
B			
B	5.235	4	E

B			
B	4.250	4	A

Dependent Variable: RBC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	2.35432000	0.58858000	3.21	0.0433
Error	15	2.75377500	0.18358500		
Corrected Total	19	5.10809500			

Duncan's Multiple Range Test for RBC

Alpha	0.05
Error Degrees of Freedom	15
Error Mean Square	0.183585

Number of Means	2	3	4	5
Critical Range	.6458	.6769	.6963	.7095

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	trt
A	9.1375	4	E
A			
B A	9.0375	4	D
B A			
B A C	8.9775	4	C
B C			
B C	8.3975	4	B
C			
C	8.3225	4	A

Dependent Variable: Neutrophils

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	89.1126117	22.2781529	5.39	0.0068
Error	15	61.9953935	4.1330262		
Corrected Total	19	151.1080052			

Duncan's Multiple Range Test for Neutrophils

Alpha	0.05
Error Degrees of Freedom	15
Error Mean Square	4.133026

Number of Means	2	3	4	5
Critical Range	3.064	3.212	3.304	3.366

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	trt
	A	16.394	4	A
	A			
B	A	14.598	4	B
B	A			
B	A	14.281	4	D
B				
B	C	12.306	4	E
	C			
	C	10.232	4	C

Dependent Variable: Hb

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	3.20300000	0.80075000	2.63	0.0764
Error	15	4.57500000	0.30500000		
Corrected Total	19	7.77800000			

Duncan's Multiple Range Test for Hb

Alpha	0.05
Error Degrees of Freedom	15
Error Mean Square	0.305

Number of Means	2	3	4	5
Critical Range	.8324	.8725	.8975	.9145

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	trt
	A	16.1250	4	E
	A			
B	A	16.0250	4	C
B	A			
B	A	15.3500	4	A
B	A			
B	A	15.2500	4	D
B				
B		15.2000	4	B

Dependent Variable: PCV

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.00238000	0.00059500	1.04	0.4202
Error	15	0.00860000	0.00057333		
Corrected Total	19	0.01098000			

Duncan's Multiple Range Test for PCV

Alpha	0.05
Error Degrees of Freedom	15
Error Mean Square	0.000573

Number of Means	2	3	4	5
Critical Range	.03609	.03783	.03891	.03965

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	trt
A	0.53500	4	E
A			
A	0.53250	4	C
A			
A	0.51750	4	D
A			
A	0.51250	4	A
A			
A	0.50750	4	B

Dependent Variable: MCV

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F

Model	4	41.38700000	10.34675000	4.62	0.0125
Error	15	33.62250000	2.24150000		
Corrected Total	19	75.00950000			

Duncan's Multiple Range Test for MCV

Alpha	0.05
Error Degrees of Freedom	15
Error Mean Square	2.2415

Number of Means	2	3	4	5
Critical Range	2.256	2.365	2.433	2.479

Means with the same letter are not significantly different.

Duncan Grouping			Mean	N	trt
	A		61.400	4	A
	A				
B	A		60.225	4	B
B	A				
B	A	C	59.200	4	C
B		C			
B		C	58.425	4	E
		C			
		C	57.225	4	D

Dependent Variable: MCH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	5.46700000	1.36675000	6.48	0.0031
Error	15	3.16250000	0.21083333		
Corrected Total	19	8.62950000			

Duncan's Multiple Range Test for MCH

Alpha	0.05
Error Degrees of Freedom	15
Error Mean Square	0.210833

Number of Means	2	3	4	5
Critical Range	.6920	.7254	.7462	.7603

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	trt
	A	18.4750	4	A
	A			
B	A	18.0750	4	B
B	A			
B	A	17.9000	4	C
B				
B		17.6750	4	E
	C	16.9000	4	D

The SAS System