

EVALUATION OF INSECTICIDAL AND REPELLENT PROPERTIES OF
LANTANA CAMARA L. AND *TEPHROSIA VOGELII* HOOK AGAINST THE
MAIZE GRAIN WEEVIL, *SITOPHILUS ZEAMAI* MOTSCHULSKY, IN
MAIZE GRAIN STORAGE IN KENYA

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DEDICATION

To my late father, Mzee Ogendo Olero, for the
immense material and moral contribution he
made in shaping my academic and social
destiny.

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ABSTRACT

A study was conducted to evaluate the insecticidal and repellent properties of two local plants, *Lantana camara* L. (Verbenaceae) and *Tephrosia vogelii* Hook (Fabaceae) against the maize grain weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), in maize grain storage in Kenya.

This was preceded by a farmer survey whose primary objectives were to confirm the pest status of *S. zeamais*, assess the potential and identity of local plant species with insecticidal or repellency against insect pests of stored grains and generate recommendations for the laboratory based in-depth investigation. The maize weevil was confirmed as the most important insect pest of maize in storage and two plants, *Lantana camara* L. and *Tephrosia vogelii* Hook identified and recommended for laboratory evaluations at Egerton University, Kenya. Laboratory bioassays and quality evaluations were conducted to determine the efficacy and repellency of the two plant materials against *S. zeamais* and their effects on the grain quality. The study was also expected to generate recommendations for adaptive research. Three (1.0, 2.5 and 5.0% w/w) and five (1.0, 2.5, 5.0, 7.5 and 10.0% w/w) rates of the ground powders of each plant material were used for the grain quality evaluation and laboratory bioassay experiments, respectively. An untreated (control) and synthetic insecticide, Actellic super 2% dust at 0.05% (w/w) were included for comparison in all experiments. The treatments were arranged in a completely randomised design (CRD) with six and four

replicates for the grain quality evaluation and laboratory bioassays, respectively. All the experiments were conducted under ambient conditions of temperature, relative humidity and 12-hr light: 12-hr dark regime.

Results showed that the ground powders were slow acting but effective in controlling adult *S. zeamais* causing 83-90 and 85-94% mortality for *L. camara* and *T. vogelii*, respectively, over a 21-day exposure period. The lethal mean exposure times (LT_{50}) to achieve 50% mortality varied from 5-6 days (7.5-10.0% w/w) to 7.5-8 days (2.5-5.0%w/w) for both plant materials. The probit regression lines showed significant relationship between weevil mortality and the powder concentration. The powders and insecticide were equally effective in reducing the number of adult F1 insects by $\geq 75\%$ compared to the untreated control. The botanical treatments and synthetic insecticide were equally effective in reducing (by 25%) insect damage on stored maize grains but the level of damage was independent of the amount of plant powder applied. The two plant materials showed significantly higher repellency against *S. zeamais* compared to Actellic super and the untreated control. However, *T. vogelii* at 7.5 and 10% w/w were the most repellent (87.5%) followed by *T. vogelii* at 2.5% w/w and *L. camara* at 10% w/w with 65 and 62.5%, respectively. The powders significantly minimised the magnitude of depression in grain moisture content over the 5 months storage period but no significant effect on the percent germination of stored maize grains was detected. The magnitude of depression of grain MC was less with higher dosages of the plant powders. The powders of both plants at 5% w/w had significantly different grain MC from that of the untreated control. Grain colour and odour were unaffected by the plant powders during the 5 months of grain storage. The two test plants have insecticidal and repellent properties, which if properly harnessed, can be effective in

the protection of grains in storage. The plant option appears viable, cost-effective and sustainable if the recommendations are tailored to meet the specific user domains, varying levels of pest attack and toxicity requirements.

CHAPTER 1

1.0 INTRODUCTION

1.1 Post-harvest Losses and Past Pest Control initiatives in Maize Storage

Cereals constitute an important component in the diets of people in the developing countries particularly in sub-Saharan Africa. It could be argued that the greatest challenge facing tropical and sub-tropical countries today is inadequate food supply for an ever growing human population (Patenius, 1988). For instance, in 1991 the developing countries experienced a net deficit of 25.4 million tonnes (FAO, 1992). FAO (1997) also reported that about 50% of the aggregate cereal grain shipments to low-income food-deficit countries are destined for sub-Saharan Africa.

Maize, among other cereals, is the first staple food grain for most people in sub-Saharan Africa and second on a world scale, after wheat (Dichter, 1978). In Kenya, small-scale farmers under seasonal agro-climatic conditions in which only one satisfactory harvest is possible every two seasons, (or following an even more irregular pattern) mainly grow maize. Under such circumstances, the food supply and food security depends not only on production, but to a very high degree also on the successful storage of the grain (Dichter, 1978 and Richter, 1988).

Although substantial yield increases have been realized in food grain production in Kenya, particularly maize, this achievement has been threatened by field and storage pests which cause losses estimated at 20% of total production (Wanjekeche, 1997) and a declining per capita cereal grain production index (93.8 to 84.0) over the past decade

(FAO, 1998). Saxena *et al.* (1990) observed that pests and diseases cause 30-80% pre- and post-harvest losses in yields or stored grains and that insect pest attack constitutes a major cause of grain losses in storage accounting for 20-60%. Richter (1988) reported that maize grain losses in storage were 2-50% depending on storage duration, pest control and pest species. In a related study, post-harvest grain losses in maize, sorghum and cowpea was reported to be 27-39% in western Kenya (Saxena *et al.*, 1990).

Patenius (1988) observed that improved post-harvest protection measures at farm level in developing countries could increase food supply by 30-40% since up to 90% of cereals produced are intended for human consumption. Insect pests are known to attack maize and other grains in storage in Kenya. The important species include the maize weevil, *Sitophilus zeamais* Motsch.; rice weevil, *Sitophilus oryzae* L; angoumois grain moth, *Sitotroga cerealella* Oliver; tropical warehouse moth, *Ephestia cautella*; red flour beetles, *Tribolium* spp. and the bostrichid beetles, *Prostephanus truncatus* Horn and *Rhyzopertha dominica* Fabricius (Bekele *et al.*, 1996 and NRI, 1999a). Of the six insect pests above, maize grain weevil, *Sitophilus zeamais*, is the most serious pest of stored maize kernels in the tropics (Victor and Ojuarega, 1993). Storage studies in Togo using traditional granaries revealed that 80-90% of overall grain losses were ascribed to this insect feeding (Patenius, 1988).

Storage of grains for future use as food or feed has been practiced by mankind for thousands of generations (Freeman, 1980). Storage serves three noble roles in society: ensuring reliability of quality food supply (food security), stabilization of market prices

and improvement of health and productivity (Dichter, 1978 and McFarlane, 1988). To realize the above, storage must observe three cardinal rules namely, keeping the stored grain *dry, secure and free from pest damage*, particularly insects and rodents. Most agricultural produce is stored on-farm for short or long duration depending on whether the produce is for sale or home consumption. Farmers in sub-Saharan Africa who face perennial food deficits often can not rely on government institutions to procure and recirculate the food grains. Hence, there is need to improve grain handling and storage at farm level to ensure food security. This improvement should consider the fact that the majority of African farmers are resource poor, small-scale producers (subsistence) and lack cash to invest in farming enterprises (Saxena *et al.*, 1990; Ogendo, 2000 unpubl.).

Insecticides have been used widely and successfully to control insects in storage (Golob, 1988; Pierce and Schmidt, 1992). However, concern over the development of insect resistance, toxic residues, workers' safety, erratic supply and prohibitive costs have given impetus to the search for alternative methods of pest control which will reduce or eliminate their use (Tembo and Murfitt, 1995). Labreque (1983) observed that cost remains the primary deterrent to the development and commercialization of new pesticides. The majority of farmers in Africa are resource poor and have neither the means nor the skills to obtain and handle pesticides (Saxena *et al.*, 1990; Ogendo, 2000 unpubl.). The hazards posed by pesticides to the environment, man and animals are well documented and makes their use unpopular and incompatible in most African countries.

Jensen (1985), in his evaluation of agro-chemical use in sub-Sahara Africa, revealed that

Kenya spent US \$12.0 million on agro-chemicals. It is a fact that pest control as practiced today in most developing countries relies on the use of imported insecticides. This dependence has to be reduced (Mengech *et al.*, 1997). The use of botanicals should not be viewed as total replacement of any particular pesticide, but rather as an important input of the integrated pest management (IPM) package to reduce dependence on synthetic chemicals (Mengech *et al.*, 1997). Increased awareness of the significance of pesticide residues in recent years has increased legislation regarding use of insecticides. Revelations in recent studies that insecticides, such as malathion and meothrin, penetrate into grains during storage and may affect internal organs of rats is worrisome to the grain consumers (El Sheamy *et al.*, 1988; Lalah and Wandiga, 1996; Tigar and Pinniger, 1996).

In Kenya, the adoption rate of recommended synthetic insecticides in the mainly traditional farming communities has remained remarkably low (Ogendo, 2000 unpubl.). This reluctance to adopt insecticides, probably influenced by lack of financial resources and toxicity concerns, offers a stimulating challenge to scientists to search for cost effective and safe alternatives compatible with the farmers' circumstances. With the development of insect and consumer resistance to insecticides, there are pest management and economic incentives to increase emphasis on alternative non-residual or non-chemical measures (Longstaff, 1994). The need for safer and more natural pesticides that are readily available, cost-effective, less detrimental to the environment and compatible with the resource poor farmers' circumstances has rekindled the entomologists' interest in botanicals that could be used for IPM. This opens the door for plant based alternatives, which have proven useful for the survival of mankind over several generations. Bioactive

products of plant origin, which are known to be biodegradable, cheaper than synthetic pesticides and less persistent in the environment, are potential alternatives to synthetic pesticides (Regnault-Roger and Hamraoui, 1993; Facknath, 1994). Botanicals are readily available in many places and their extracts are relatively easy to prepare. Labreque (1983) reported that botanicals were ideally suited for regional development and provided monetary supplement to many areas where agricultural production can not compete with the highly developed agro-industrial advances of developed countries. The use of plant materials (whole plants, leaves, roots, twigs, flowers, extracts and ashes) all over the world for protection of field crops and stored grains against insect pest attack is as old as mankind (Regnault-Roger and Hamraoui, 1993; Dales, 1996). Dales (1996) reported that wide-scale commercial use of plant extracts as insecticides began in the 1850s with the introduction of nicotine, rotenone, *Derris* dust and *Pyrethrum*. Thousands of plant species worldwide have been found to exhibit insecticidal and repellent properties with more than two dozen species locally available in Kenya (Dales, 1996). Tropical plants have been singled out as rich sources of plant-based pesticides which may be used in insect pest control (Facknath, 1994). For example, in the traditional farming communities in western Kenya such plants are widely available at almost no cost, have no major toxicity problems and are highly compatible with the existing circumstances.

1.2 Insect Pests of Stored Maize Grains

The main primary and secondary insect pests of economic importance in Kenya in maize grain storage belong to four Coleopteran families, Bostrichidae, Curculionidae, Dermestidae, Tenebrionidae and a Lepidopteran family, Gelechiidae (Haines, 1991;

Bekele, 1994). The primary insect pest species of importance to stored maize include curculionid beetles: *Sitophilus zeamais* Motschulsky (maize weevil) and *Sitophilus oryzae* L. (rice weevil); bostrichid beetles: *Rhyzopertha dominica* Fabricius and *Prostephanus truncatus* Horn; and gelechiid moth, *Sitotroga cerealella* (Angoumois grain moth). The main secondary insect pests of maize in storage in Kenya are *Trogoderma granarium* (Coleoptera: Dermestidae), *Tribolium castaneum* (Coleoptera: Tenebrionidae), *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) and *Ephestia cautella* (Lepidoptera: Pyralidae) ((Haines, 1991; Bekele, 1994).

Studies done in the tropics indicate that 80-90% of the losses in stored food grains at farm level is due to insect pest attack (Dichter, 1976; Patenius, 1988). Tigar *et al.* (1994), in their study of insect pests infesting rural stores of maize in Mexico, reported that *S. zeamais*, *S. cerealella* and *P. truncatus* were the most numerous and most damaging primary pests. This concurs with the findings of Ogendo (2000 unpubl.) in which the two former species were the most important primary pests of stored maize grains in western Kenya. *Sitophilus* spp. are the most widely distributed and most destructive primary insect pests of stored whole cereal grains such as maize, sorghum, wheat and rice (Haines, 1991). The maize weevil, *S. zeamais*, has been identified as the most serious pest of stored maize kernels in the tropics (Victor and Ojuarega, 1993).

1.2.1 Maize weevil (*Sitophilus zeamais* Motschulsky)

This insect pest species is commonly referred to as the “maize weevil” because it is predominantly found associated with maize (Haines, 1991; NRI, 1999a). It is a major

primary pest throughout the warm-temperate to tropical regions (Anon, 1980). *Sitophilus zeamais* closely resembles the rice weevil, *S. oryzae* L. and can only be distinguished from the latter by examination of the genitalia. The male aedeagus in *S. zeamais* has a central ridge between the two longitudinal depressions as opposed to a completely smooth and convex one in *S. oryzae* (Anon, 1980; Haines, 1991; NRI, 1999a). *S. granarius* L. is the other species similar to *S. zeamais* and *S. oryzae* but can be distinguished by the absence of metathoracic flight wings (has only small vestigial wings) and by the oval shape of the punctures on the prothorax. Also *S. granarius* is commonly found in temperate regions and in rare occasions, in the cool upland areas in the tropics where it is out-competed by the other two species (Haines, 1991).

S. zeamais thrives best at warm temperatures of about 28°C and in grain in which the moisture content is not much less than 13% (NRI, 1999a). The adults are 2.5-4.5 mm in size, long-lived (several months to one year) and are characterized by a narrow snout-like forward extension of the head, which carries mouthparts. The adult flies strongly and often infests cereals pre-harvest in the field. The adult female lays up to 150 eggs throughout adult life, at temperatures between 15 and 35°C (optimum: 25°C) and at grain moisture contents of over 10%, in tiny holes in the grain and then seals the cavity with a plug of waxy secretion (egg-plug). The incubation period of the egg is 4-6 days (Haines, 1991; NRI, 1999a). The larval (25-30 days) and pupal (4-5 days) stages of development take place entirely within a single grain. The larval feeding hollows out the inside of an infested grain. The newly developed adult chews its way out leaving a large characteristic emergence hole roughly circular in shape. The adults also feed on whole cereal grains

leaving large irregularly shaped cavities. Total developmental period (egg-adult) range from 33-41 days under optimal conditions to over 110 days in unfavourable conditions.

1.3 Role of Botanicals in Stored -Product Insect Pest- Control

The use of bioactive plant products as protectants in grain storage has a long history worldwide. These plant products range from whole plants, leaves, flowers, fruits, ground powders and ash to plant extracts (oils, acetone extracts). The bulk of grain production in sub-Saharan Africa comes from small farm holdings and some of these traditional farmers use different kinds of plant products for pest control (Poswal and Akpa, 1991). Farmers generally mix grains with bioactive plant based products. For example, peasant farmers in northern Nigeria indigenously use various plants to protect cereals and legumes against pest damage during storage (Fatope *et al.*, 1995). Similarly, small-scale farmers in western Kenya predominantly admix stored grains with ash obtained from local plants (Ogendo, 2000 unpubl.).

A large number of plant species contain natural anti-insect materials which offer protection through mechanisms such as repellency, insecticidal activity, anti-feeding and anti-oviposition (Bekele, 1994). Past research work on the bioactivity of plant products against insect pests of stored products covers a wide variety of products ranging from grains (cereal and legume) to non-grain products such as dried cassava. Research has attempted to establish the scientific basis for the continued use of plants with regard to their active constituents, efficacy and methods of application. However, the current literature review has focused mainly on documented use of two plant species, *Lantana*

camara L. (Verbenaceae) and *Tephrosia vogelii* Hook (Fabaceae), in the control of both field and storage pests. The choice of test plants was based on the findings and recommendations of a farmer survey conducted in the study area (Ogendo, 2000 unpubl.). Of particular interest is the use of such plants in the control of *Sitophilus zeamais* in stored cereal grains.

1.3.1 *Tephrosia vogelii* Hook

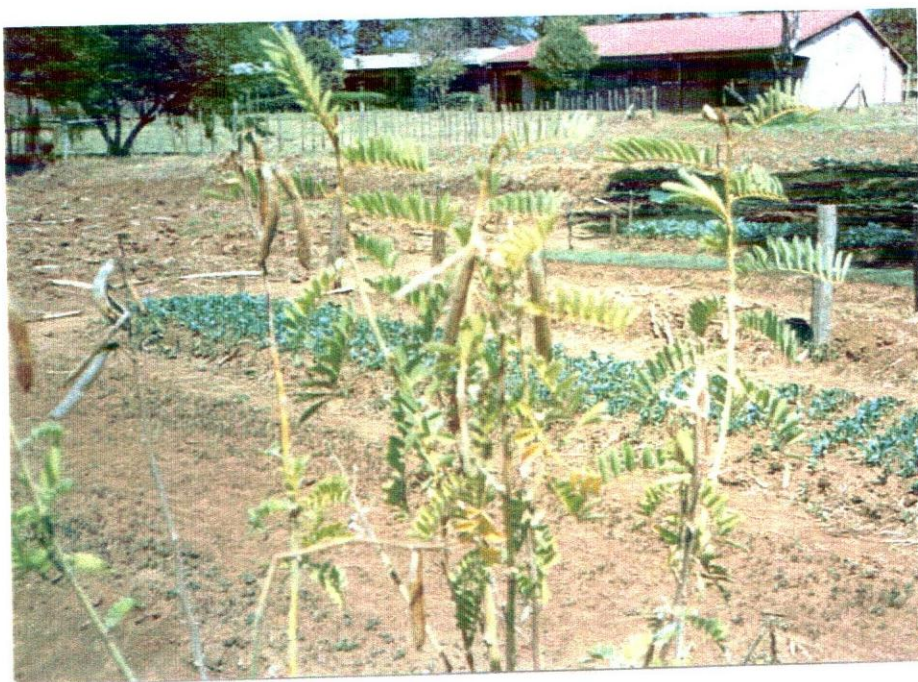


Photo by: J. O. Ogendo

Plate 1: Vegetative and reproductive structures of *Tephrosia vogelii* Hook plant

The genus *Tephrosia* [Family: Papilionidae (Fabaceae/ Leguminosae/Papilionaceae)] has a total of 400 species (Dale and Greenway, 1961; Allen and Allen, 1981; Sommerlate and Sommerlate, 1990). *Tephrosia* spp. are annual or perennial shrubs or herbs with erect or prostrate stems arising from a woody crown, woody roots and grey (long, fibrous, thick, yellowish and have characteristic odour due to tephrosal, a volatile oil), hairy twigs. The

leaves are imparipinnate with numerous petioluled leaflets (often >3) usually silky-pubescent beneath. Their inflorescence consist of several clustered flowers with 4-6 flowers in each cluster and are usually red, purple or white coloured in leaf-opposed, axillary or terminal racemes. The fruits consist of pods, which are sessile, 8 cm long, covered with silky hairs, obliquely narrowed and beaked at apex, dehiscent and many seeded (nonseptate within or thinly septate) (Allen and Allen, 1981; Sommerlate and Sommerlate, 1990). Allen and Allen (1981) reported that *Tephrosia* spp. are adapted to sandy soils and generally found in grassy fields, thickets and along roadsides throughout warm regions of both hemispheres. They observed that 70, 50 and 30 species are native to South Africa, Equatorial Africa and Southwest, United States, respectively.

Tephrosia spp have been found to be beneficial to the soil and man. Though of limited forage value (due to coarse and unpalatable foliage), these plants are beneficial for green manure, soil cover, soil improvement and reclamation. Their leaf litter is abundant and high in nitrogen (N) content, nodulation is copious and plant growth is prolific. For example, among 16 varietal introductions of *T. vogelii* in Puerto Rico, the leaf N content ranged between 4.18 to 4.83% (Irwine and Fryere, 1959 cited by Allen and Allen, 1981). Similarly, *Tephrosia* spp are classified as promiscuous and effective plants (Cowpea group) nodulating effectively with a wide range of strains from different genera and species within the group; their symbiotic effectiveness (host dry wt) is usually $\geq 80\%$ that of the N control (Date and Halliday, 1980) and three times greater than that of *Leucaena leucocephala* (Niyungeko *et al.*, 1990). Kumwenda *et al.* (1998) reported that *T. vogelii* could produce significant quantities of biomass (1.5-4.7 t/ha) in areas where maize yields

had greatly decreased due to declining soil fertility. The perennial, shrubby growth, dense foliage and good anchorage have enhanced the use of *Tephrosia* spp in Africa and Asia for hedges, windbreaks, as shade on tea and coffee plantations and to prevent soil erosion (Allen and Allen, 1981). *T. vogelii* is probably not indigenous to East Africa. It is differentiated from the other species as an erect shrub (≤ 3 m tall) and clothed with a dense rusty or yellow tomentum. The leaves have oblanceolate leaflets (7-12 pairs) which are 5 cm in length and 1.25 cm wide. Flowers are purple, red or white in terminal racemes (Dale and Greenway, 1961). *T. interrupta* Hochst & Steud. Ex Engl is the other species reported present in Kenya (Sommerlate and Sommerlate, 1990).

Interest in *Tephrosia* spp as a source of contact poisons dates back to the 1900s when their native use as fish and arrow poisons and the similarity in their physiological action to that of *Derris* and *Lonchocarpus* species were reported (Little, 1931; cited by Allen and Allen, 1981). *T. vogelii* is traditionally used in west Africa for its ichthyotoxic, insecticidal, and food parasitocidal properties (Lambert *et al.*, 1993) and medicine for treating wounds in Uganda (Worumann, 1998). Three major toxic constituents have been identified from the leaves of *Tephrosia vogelii*: tephrosin ($C_{31}H_{26}O_{10}$, which killed fish in 1 hr at concentration of 1.25 ppm), toxicarol ($C_{23}H_{22}O_7$; deguelin, an isomer of rotenone) and rotenone ($C_{23}H_{22}O_6$) (Hanriot (1907; Clark, 1931, 1933; all cited by Allen and Allen, 1981; Lambert *et al.*, 1993). Early attempts to commercially produce *T.* spp for insecticidal purposes proved unimportant because of low and variable yield of insecticidal compounds (Roark, 1937; Irvine and Freyre, 1959; all cited by Allen and Allen, 1981). Kapur *et al.* (1972) reported that *T. vogelii* leaves contain 2% rotenoids. Allen and Allen (1981) highlighted the following species as basic sources of rotenoid

compounds: *Tephrosia vogelii*, *T. virginiana* (L.), *T. toxicaria* (SW.) Pers., *T. macropoda* Harv., and *T. grandiflora* Pers.

It has been reported that pounded leaves of *Tephrosia vogelii* Hook were used in West Africa and western Kenya as fish intoxicant in local rivers (Oliver-Bever, 1986; Ogendo, 2000 unpubl.). This practice has been prohibited in southern Nigeria due to toxicity concerns. *T. vogelii* leaves, roots, fruit capsules and seeds have been found to have two main constituents, deguelin and tephrosin, which are responsible for their pungent odour (Oliver-Bever, 1986). The leaves also contain chemical compounds such as hydrocyanic acid (oral human LD₅₀ 570 µg), rotenone (oral rat LD₅₀ 132 mg) and rutin (intravenous mouse LD₅₀ 950 mg). The *Tephrosia* rotenoids are also toxic to insects, worms and snails. Dried leaves of *Tephrosia* were found to have killed goldfish and larvae of worms after 22 years of storage (Watt and Breyer-Brandwijk, 1962 cited by Oliver-Bever, 1986). Recent findings suggest that live *T. vogelii* plants can effectively repel mole rats thereby reducing damage caused to field crops (Ayoub, 1999) and that powdered (sun-dried) leaves can effectively control maize weevils and the larger grain borer and cowpea and bean bruchids in grain storage (Ayoub, 1999; Soko, 1999; Worumman, 1998;). However, the amount of literature available on the safe and effective use of *Tephrosia vogelii* in grain storage proves insufficient as far as efficacy, dosage and effects on grain quality are concerned.

1.3.2 *Lantana camara* L. (Verbenaceae, Verbena family)



Photo by: J. O. Ogendo

Plate 2: Vegetative and reproductive structures of *Lantana camara* L. plant

This plant has a host of common names in many places around the world namely, wild sage, red-flowered sage, lantana, tick berry, white sage or prickly lantana (Holm *et al.*, 1977). In East Africa where this study was conducted, it is commonly referred to as lantana or tick berry (Holm *et al.*, 1977). This species belongs to the genus *Lantana* in the family Verbenaceae. *Lantana camara* L is a perennial, erect or spreading, thicket-forming, woody shrub with multicoloured flowers (Plate 1) and is strongly odorous. It can be 2 to 5 m tall with four-angled stems bearing recurved prickles on the angles. Dales and Greenway (1961) identified three other species within the genus *Lantana* as *L. rhodesiensis* (crimson or purple flowers), *L. trifolia* (leaves in threes and pink or mauve flowers) and *L. viburnoides* (leaves always opposite and flowers usually white). *L. camara* is distinguished from the other indigenous species by the strong unpleasant odour of its leaves when crushed, its four-angled stems with recurved prickles, and its flat-top

with pink, yellow, orange or white showy flowers (Holm *et al.*, 1977; Dale and Greenway, 1961). The fruits are usually greenish-blue or black and are mono seeded.

Lantana camara L., referred to as the "Curse of India", is classified as one of the most successful noxious weeds in Kenya and of the world (Holm *et al.*, 1977; Dale and Greenway, 1961). It is among the top six most prominent plant species in the roadside habitats (Henderson, 1989). This plant is a weed of plantation crops and pastures from lat. 45° N to 45° S and is most troublesome in the Caribbean area, eastern Africa, South Africa, southern Asia, Australia, and the Pacific Islands (Holm *et al.*, 1977). Kumar and Rohatgi (1999) described *L. camara* as an example of a weed species that dominates fields, forests and wastelands occupying almost the whole area with little or no growth of other species thus resulting in a total loss of biodiversity. The species is commonly associated with sandy soils along roadsides, wastelands, yards and gardens. It is occasionally cultivated as an ornamental in northern areas of the United States.

This ornamental shrub contains lantanin, a triterpenoid, hepatoxins lantadene A and B, as well as other structurally and toxicologically related pentacyclic triterpene acids including reduced lantadene A, dihydralantadene A and icterogenin known to cause irritation of the mucosa of the gastrointestinal tract (lantanin) and cholestasis, hepatic necrosis and ruminal paralysis secondary to liver injury (Kellerman *et al.*, 1988). Morton (1994) reported that ingestion of 340-453 g of leaves causes liver and kidney damage, photosensitization, intestinal haemorrhage, paralysis of gall bladder and death within 1-4 days of ingestion in horses and cattle. All parts of the plant are said to contain toxins and that poisoning often occurs when clippings are thrown into the pasture. Such poisoning occurs almost exclusively in cattle (Kellerman *et al.*, 1988). The poisonous effects may

be treated through detoxification with activated charcoal, removal of victims from direct sunlight, and the use of antibiotic injections and topical applications of protective antibiotic creams.

Although classified among poisonous plants, *L. camara* has been used as tonic and stimulant and in traditional folk medicine to treat cuts, ulcers, rheumatism, eczema and gonorrhea (Getahun, 1976; Siddiqui *et al.*, 1995; Masinde, 1996). Past scientific studies have shown that *L. camara* has natural compounds with insecticidal, repellent, antifeedant, anti-oviposition, ovicidal or larvicidal activity against certain important field and storage insect pests. Laboratory and field evaluations have been conducted on the efficacy of aqueous and organic (alcoholic and petroleum ether) extracts and topical application of ground powders from roots, leaves, stems and flowers on several insect pests. Sharma *et al.* (1992) in their study using aqueous extracts of *L. camara* reported significant antifeedant activity against the jute stem weevil, *Apion corchori* (Coleoptera: Apionidae), the jute semilooper, *Anomis sabulifera* (Lepidoptera: Noctuidae) and the Bihar hairy caterpillar, *Spilosoma obliqua* (Spodoptera: Tiliaceae). Other studies with aqueous extracts of *L. camara* reported significant inhibition of egg hatching and juvenile mortality of *Meloidogyne incognita* and that flower extracts produced stronger nematocidal activity than leaf or stem extracts (Joymati *et al.*, 1998). Significant inhibition of adult longevity and fecundity, reduced egg hatchability, percent adult emergence and ovicidal action of/on tea mosquito bug, *Helopeltis theivora* (Hemiptera: Miridae), have been observed (Deka *et al.*, 1998a, b). Aqueous extracts were also found to prevent further spread of tukra disease in mulberry (Babu *et al.*, 1994). Similarly, aqueous extracts of *L. camara* exhibited strong antifeedant effect on *Plutella xylostella*

(Lepidoptera: Plutellinae) but were not repellent. In the same study, the volatile oil from flowers and leaves of *L. camara* was found, based on LD₅₀ values, to be moderately toxic to *Sitophilus zeamais* (Rejesus and Tantengco, 1985).

Extracts (petroleum ether, methanol or ethanol) of *L. camara* effectively reduced larval population (90% larval mortality) of *Henosepilachna vigintioctopunctata* F. and the 2nd instar larvae of *Amsacta moorei* (Lepidoptera: Arctiidae) (Reddy *et al.*, 1990; Satpathi and Ghatak, 1990; Chitra and Rao, 1993; Patel *et al.*, 1993), caused 80% mortality against *Myzus persicae* (Hemiptera: Aphididae) in the laboratory (Stein and Klingauf, 1990) and were as effective as synthetic insecticides, Polytrin and Profenofos, against safflower aphid, *Dactyonotus carthamii* (Hemiptera: Aphididae) (Patil *et al.*, 1997). Kulkarni *et al.* (1997) and Kulkarni and Joshi (1998), using leaf (methanol and petroleum ether) extracts of *L. camara* and other plants in a laboratory bioassay, reported significant antifeedant activity against sissoo defoliator, *Plecoptera reflexa* and maharukha webworm, *Atteva fabriciella*. Alcoholic extract of *L. camara* produced 96% short-term repellency against the German cockroach, *Blattella germanica* (Dictyoptera: Blattellinae) (Scheffler and Dombrowski, 1993). Pandey *et al.* (1987) and Kulkarni and Joshi (1997) reported that leaf extracts of *L. camara* at 1.0-1.5 and $\geq 3\%$ caused 67 and 84.5% mortality and reduced fecundity of *Lipaphis erysimi* (Hemiptera: Aphididae) and *Rhesala imparata*, respectively, in the laboratory. Leaf extracts from *L. camara* have been reported to have insecticidal and growth regulating effects on leaf miners, *Liriomyza* spp. (Diptera: Agromyzidae) (Facknath, 1994).

A survey of documented scientific work indicates that attempts have been made to use *L.*

camara in the protection of stored agricultural (grain and horticultural) products against insect pests with positive results. Studies using shredded and powdered foliage of *L. camara* reported effective control against gelechiid, *Phthorimaea operculella* (Lepidoptera) on stored potato tubers for 4 months (Raman *et al.*, 1987; Wahundeniya, 1990). Pandey *et al.* (1986) observed that leaf and flower extracts of *L. camara* and other plant species, at 0.5-1.5% (w/w), were highly repellent, inhibited oviposition and protected seeds of *Vigna radiata* against *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) and had no adverse effects on seed germination. However, extracts of *L. camara* were less effective in protecting *V. unguiculata* seeds against *C. maculatus* (Soedomo and Sunaryono, 1988). Osman *et al.* (1997) reported that extracts of cut flowers of *L. camara* at 5 and 10% (w/w) were deleterious on rice moth, *Corcyra cephalonica* St. (Lepidoptera: Pyralidae). Bioassays using alcoholic extracts from leaves of *L. camara*, tested alongside five other botanical plants, found moderate toxicity against the fully grown larvae of khapra beetle, *Trogoderma granarium* (Coleoptera: Dermestidae) but was inferior to organophosphate insecticides, malathion 50 EC and chlorpyrifos-methyl 50 EC (Tripathi *et al.*, 1996).

Extracts of *L. camara* resulted in lower fecundity per female, reduced adult emergence, reduced grain damage and significant adult mortality of the lesser grain borer, *Rhyzopertha dominica* F. in wheat grains (Mrig and Mahla, 1996 and Singh *et al.*, 1996).

On the contrary, Ambadkar and Khan (1994), using a choice chamber, reported that fresh and dried leaves of *L. camara* showed significant attractant qualities against adult cigarette beetles, *Lasioderma serricorne* F. (Coleoptera: Anobiidae).

However, it should be noted from the literature survey that there is insufficient

information for an effective appraisal of the effectiveness and appropriateness of such plants against stored-product insect-pests. Most of the articles have focused on the use of plant extracts, which may not be compatible with the farm level storage practices in sub-Saharan Africa. Interestingly, little research has been conducted in Kenya to investigate the potential and understand the scientific basis for the use of local plant materials as grain protectants against major insect pests of stored product such as the maize weevil, *Sitophilus zeamais*. In particular, information on the bioactivity of ground powders from local plants against such insects and quality of stored cereal grains is lacking. Accordingly, this study was initiated and commenced with a survey in Kasipul division of Rachuonyo district, Kenya, aimed at determining the pest status of *Sitophilus zeamais*, assessment of pest control methods in the area, and the potential of local plant materials as suitable and sustainable alternatives to synthetic insecticides. As a result of the preliminary survey, two plant species, *Lantana camara* L. and *Tephrosia vogelii* Hook were identified and selected based on availability and their potential as botanical pesticides. The plants were later recommended for detailed laboratory investigations to address the following objectives:

1. To determine the effect of the two plant materials and rate of application on:
 - (a) The mortality, F1 progeny production and repellency against maize grain weevil, *Sitophilus zeamais*
 - (b) The grain quality parameters (moisture content, odour, colour, and insect damage)
 - (c) Seed viability and
2. To recommend plant and dosage(s) that can be used for adaptive research (testing) at farm level as possible alternatives to Actellic Super (Pirimiphos-methyl+ Permethrin) 2% dust in the control of *Sitophilus zeamais* Motschulsky in maize-grain storage.

CHAPTER 2

MATERIALS AND METHODS

2.1 Diagnostic Field Survey

A preliminary diagnostic field survey was conducted in the resource-poor small-holder farming communities of Kasipul Division of Rachuonyo District, Kenya, from 12th to 22nd September 1999. The objectives of the survey were to: (a) confirm the pest status of *Sitophilus zeamais* Motsch; (b) identify the insect pest control methods used by local farmers; (c) assess the potential and identity of local plant species with insecticidal and repellent properties against stored-product insect-pests; (d) establish the role of gender in grain storage; (e) study the value of grain quality in maize grain storage, and (f) finally, generate recommendations for the current and future laboratory investigations.

Two administrative locations, Koderia and Kojwach, were identified as predominantly maize growing areas. The farmers in these locations store grains and mainly use bioactive plant products for insect pest control. The Ministry of Agriculture crop production statistics and other related literature (CMRT, 1995; Ogendo, 1999) guided the choices made. A total of 25 (44% women) representative farmers were randomly sampled, without bias towards sex and age, from two study locations and interviewed guided by survey guidelines pre-developed by the Principal Investigator. The sampling procedure used in the selection of interviewees was unbiased and conformed to that that described by Poate and Daplyn (1993). The constraints to safe maize grain storage were diagnosed, ranked and solutions suggested according to methodologies used by CIMMYT (1988) and Tripp and Woolley (1989). Examination of grain storage structures and samples of

stored maize for damage and insects supplemented the information from interviews. Compton and Sherington's (1999) rapid assessment methods were used to examine grain samples for damage and presence of insect pests. On spot recognition and identification of insect and plant species mentioned by the farmers was done through Investigator's expertise, pictorial (NRI) aids and available literature (Agnew and Agnew, 1994; Dales, 1996; Capon, 1998; Kokwaro and Johns, 1998; Ogendo, 1999). Insect and plant specimens were also collected, preserved and forwarded to the Department of Botany at Egerton University, Kenya, for further identification.

2.2 Test Plant Materials

Plant materials used for insect mortality and repellency bioassays and grain quality evaluations were obtained from fresh (mixture of leaves, inflorescence and succulent stems) samples of two local plants, *Lantana camara* L. (Common name: Wild sage; Dholuo: Onyalobiro) and *Tephrosia vogelii* Hook (Common name: Tephrosia; Dholuo: Jinga/ Yadh rech) (Plates 1 & 2). Specially preserved plant samples were prepared and the scientific identity of the two plants was confirmed at the Department of Botany, Egerton University, Kenya.

The plant samples were collected from Kasipul Division of Rachuonyo District, Kenya, during September 1999. All fresh plant samples were dried under shade at room temperature of 27-30°C for three days before being transported to Egerton University, Kenya. The samples were further oven-dried in the laboratory at 35°C (Niber, 1994; Venskutonis, 1997; Wisniewski, 1999) for 48 hours. The dry plant samples were ground to fine powder using an electric laboratory hammer mill.

2.3 Mass rearing of test insects

Heavily infested maize grains were collected from the survey area and screened in the laboratory for confirmation of the insect species. Two hundred unsexed adult *Sitophilus zeamais* insects were reared in 1-litre jars containing 500g of disinfested whole maize grains as described by Haines (1991) and Wright *et al.* (1987). The top of each rearing jar was covered with nylon mesh fastened tightly with rubber bands, and the insects were allowed a 7-day period for egg laying (oviposition). At the end of 7 days, all adults were removed. Each jar was allowed to stand for 25 days during which emerging adult insects were monitored and kept in separate jars according to age. The rearing of test insects was done in the laboratory at ambient temperature of 17-28°C, 38-69 % RH and at L12: D12 regime. A local maize (*Zea mays* L.) variety, Nyar Maragoli, purchased from Kasipul Division of Rachuonyo District, was used for rearing.

2.4 Disinfesting of test maize grains

Test maize grains in 90kg jute bags were disinfested under a gas tight sheet using phostoxin tablets at 5 g PH₃ per tonne for 10 days to kill any latent insect pest infestation. The choice of phosphine as fumigant was based on convenience for use in small-scale sealed bags, virtually having no effect on seed germination, ease of removal by subsequent ventilation, easily handled solid and not requiring specialist equipment (Meuser *et al.*, 1977; AFHB-ACIAR, 1989; Annis and Graver, 1994; NRI, 1999a). Meuser *et al.* (1977) also reported that phosphides are easily hydrolyzed by air moisture and that the absolute amount of water in gaseous space remains constant thus keeping the RH unchanged. The disinfested grains were thereafter kept in the laboratory at room

temperature, RH and a L L12: D12 regime. Sub-samples used for the bioassays and grain quality evaluations were further disinfested at 40°C in an oven for 4 hr (Bekele *et al.*, 1996) just before use and allowed to cool for at least 2hr.

2.5 Grain Quality Evaluations

Ground dry-powders of the two plant materials, *Lantana camara* L. and *Tephrosia vogelii* Hook each at three rates: 1.0, 2.5 and 5.0% (w/w) were admixed with 5kg-disinfested test maize grains in separate jute bags (Plate 3). The botanical treatments were tested against an untreated control and an insecticide, Actellic Super (16g/kg Pirimiphos-methyl + 3g/kg Permethrin) 2% dust at the recommended rate of 0.05% (w/w). A total of eight treatments (Table 1) each replicated six times were arranged in a completely randomized design (CRD) on 1m-high wooden benches in the laboratory (Plate 3). This experiment was set up on November 4, 1999 and kept at ambient temperature of 17-28°C, 38-69 % RH and a 12hr L: 12hr D regime.

Twenty representative random samples of the reference test grains were obtained from various parts of two holding jute bags (NRI, 1999c) at the onset of the experiment. The samples were used to determine the baseline grain quality parameters: moisture content (%), insect damage (%), seed viability index (germination %), grain colour and odour. Approximately 250g grain sub-samples were obtained from each the 48 experimental units (jute bags) after 0, 30, 60, 90, 120 and 150 days of grain storage. A simple cylindrical grain sampler (25 mm in diameter) was used to take sub-samples from five points (i.e. the middle and approximately 5-cm from the sides of the jute bag) as shown in the sketch diagram below. Each sub-sample, after dusting off the treatment, was evaluated for change in the five-grain quality parameters mentioned above. The procedures used to measure each parameter are as described below.



Photo by J. O. Ogendo

Plate 3: Grain quality evaluation experiment: 5-kg grain samples in jute bags on 1-m high wooden platform

Sampling points within an experimental jute bag

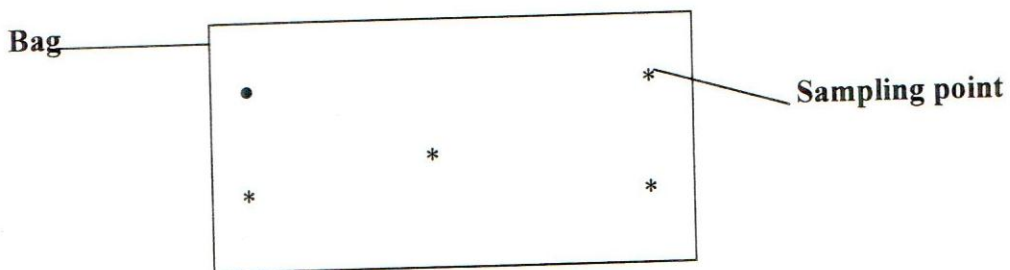


Table 1: Description of treatments used for the grain quality (colour, odour, and moisture content, germination and insect damage) evaluations

Treatment	Description	Dosage (% w/w)
T1	Untreated control	-
T2	Actellic super 2% dust*	0.05
T3	Ground powder of <i>L. camara</i>	1.0
T4	Ground powder of <i>L. camara</i>	2.5
T5	Ground powder of <i>L. camara</i>	5.0
T6	Ground powder of <i>T. vogelii</i>	1.0
T7	Ground powder of <i>T. vogelii</i>	2.5
T8	Ground powder of <i>T. vogelii</i>	5.0

*Actellic super 2% dust used was a cocktail of an organophosphate, Pirimiphos-methyl, and a synthetic pyrethroid, Permethrin (16g/kg Pirimiphos-methyl + 3g/kg Permethrin).

2.5.1 Grain Moisture Content

Moisture contents of the test grains were evaluated for all the treatments after 0, 30, 60, 90, 120 and 150 days of storage. Grain sub-samples were obtained from each experimental unit as described in 2.5 above. Direct temperature-corrected moisture content (%) readings were recorded using a capacitance grain moisture meter (Eaton®).

2.5.2 Insect Damage

Grain samples (≈100g) obtained from untreated and treated grains described in 2.5 & 2.5.1 above were assessed for damage arising from natural insect infestation after 0, 30, 60, 90, 120 and 150 days of storage. Each sample was separated into undamaged and

insect-damaged grains. The number of grains in each category was counted, weighed and the percentage weight loss (percent insect damage) of maize grains in storage computed using NRI (1999a) formula:

$$\% \text{ Weight loss} = \frac{UN_d - DN_u}{U(N_d + N_u)} \times 100$$

Where U = weight of undamaged grains, D = weight of insect-damaged grains, N_u = number of undamaged grains and N_d = number of insect-damaged grains.

2.5.3 Evaluating effect of Botanicals on Seed Viability

The effect of the eight treatments (see 2.5.1), storage duration and their interactions on seed viability, expressed as viability index (VI), were investigated over a 150-day grain storage period. Sub-samples of 100 undamaged grains were obtained from each experimental jute bag in section 2.5.2 above according to NRI (1999c) to ensure unbiased sampling. The 100-grain samples were then germinated on moistened filter paper (Whatman No. 1) in petri-dishes arranged in a completely randomized design (CRD) with six replicates. The set up was maintained at ambient temperature, RH and a L12: D12 regime. The number of emerged seedlings from each petri dish was counted and recorded after 7 days. The percent germination was computed according to Zibokere (1994) as follows: **Viability Index (%) = (NG*100)/TG**

Where NG = number of seeds that germinated and TG = total number (=100) of test seeds in placed in each petri dish.

2.5.4 Grain Colour and Odour Evaluations

The change in colour and odour of untreated and treated maize grains (see 3.5) during storage was assessed on a monthly basis for 5 months (150 days). Grain sub-samples ($\approx 50\text{g}$) obtained from the samples in section 2.5 were assessed for change in colour and odour. Kramer's (1956) quick assessment method was modified into a scoring scale of 1-5 defined separately for each of the two parameters.

Scoring for change in grain colour was done according to the following scale:

<u>Score</u>	<u>Description</u>
1	No visually detectable change i.e. natural white with a few yellow grains
2	Slight change ($\leq 5\%$) from natural white/yellow to light brown
3	Moderate change (>5 to $\leq 30\%$) from natural white/yellow to brown
4	Great change (>30 to $\leq 50\%$) from natural white/yellow to dark brown
5	Highly significant change ($>50\%$) making grain unacceptable for human consumption

Similarly, scoring for the change in grain odour was done as follows:

<u>Score</u>	<u>Description</u>
1	Grain is odourless
2	Grain has less offensive odour
3	Grain has moderately offensive odour
4	Grain has offensive odour
5	Grain has very offensive odour making grain unacceptable for human consumption

To obtain unbiased scores, each grain sample was coded and presented in a well-lit and ventilated room for assessment. Grain samples from the baseline sample lot were made available during assessment for reference in as far as the original grain colour and odour were concerned. A sensory panel consisting of six independent judges (Kramer, 1956)

was asked to score, using the pre-set scoring scales above, for change in grain colour and odour. The judges were allowed into the assessment room, one at a time, in rotation to ensure that their scores were independent from each other. This procedure was repeated on a monthly basis for 5 months (150 days) and the same six panelists retained over the entire assessment period (Kramer, 1956). Blank scoring (data) sheets were used for the different assessment dates to ensure that the previous ones do not bias subsequent scores.

2.6 Laboratory Bioassay Experiments

2.6.1 Insect Mortality and F1 progeny production

The contact toxicity of five rates of dry ground powders of *L. camara* and *T. vogelii* and Actellic Super 2% dust on the maize grain weevil, *Sitophilus zeamais*, were evaluated in the laboratory under ambient temperature, relative humidity and a 12hr: 12hr light: dark regime. Two hundred and fifty grams (250g) of disinfested maize grains were put into 1-litre jars and admixed with the powders from the two plants each at five rates and Actellic Super 2% dust (Table 2). Untreated grains were also included as treatment for comparison. The jars were arranged in a CRD with four replicates. Forty unsexed (5-8 day old) adult *S. zeamais* insects were then introduced into each jar. The top of each jar was covered with nylon mesh held tightly with rubber bands. A 2 cm width band of fluon (Polytetrafluoroethylene) was smeared, at three-quarter height of the jar, round the circumference to deter the test insects from climbing to the top of the jar.

The number of dead insects in each jar was counted, on a cumulative basis, daily for the first ten days. Additional counts were taken on the 14th and 21st day. The percent adult mortality was calculated using the formula:

$$\text{Insect mortality (\%)} = \frac{\text{No. dead insects}}{\text{Total No. Insects (=40)}} \times 100$$

Total No. Insects (=40)

All adult insects, dead and alive, were removed from each jar after 21 days of exposure to the treated and untreated grains. Cumulative counts of emerging adult F1 (first filial generation) insects commenced on the 35th day to avoid overlap of insect generations (Bekele *et al.*, 1996) and continued weekly for 3 weeks. The counts stopped on the 56th day.

Table 2: Description of treatments used for laboratory bioassays (mortality, repellency and F1 progeny)

Treatment	Description	Dosage (% w/w)
T1	Untreated control	-
T2	Actellic super 2% dust*	0.05
T3	Ground powder of <i>L. camara</i>	1.0
T4	Ground powder of <i>L. camara</i>	2.5
T5	Ground powder of <i>L. camara</i>	5.0
T6	Ground powder of <i>L. camara</i>	7.5
T7	Ground powder of <i>L. camara</i>	10.0
T8	Ground powder of <i>T. vogelii</i>	1.0
T9	Ground powder of <i>T. vogelii</i>	2.5
T10	Ground powder of <i>T. vogelii</i>	5.0
T11	Ground powder of <i>T. vogelii</i>	7.5
T12	Ground powder of <i>T. vogelii</i>	10.0

*Actellic super 2% dust used was a cocktail of organophosphate, Pirimiphos-methyl , and synthetic pyrethroid, Permethrin (16g/kg Pirimiphos-methyl + 3g/kg Permethrin)

2.6.2 Repellency

The repellency of untreated grains and those treated with Actellic Super 2% dust and five rates each of dry ground powders of *L. camara* and *T. vogelii* against the maize grain weevil, *S. zeamais*, were evaluated in a choice bioassay system similar to Talukder and Howse (1993) and Bekele *et al.* (1996) with modifications. Instead of two jars connected via nylon mesh band, each choice bioassay system consisted of a circular plastic basin measuring 45 cm (diameter) by 30 cm (height). The circular base of the basin was divided into four clearly marked equal portions. Alternate treated (one treatment) and untreated control 100g-grain samples were placed equidistant from centre of the circular base (Plate 4b).

A similar arrangement, as in the diagram below (Plate 4), was repeated for each of the other ten treatments and a no choice control. Each basin was smeared with fluon, 15cm from the bottom, all round the circumference and the top covered with tightly fitting meshed nylon for relatively free airflow. Four replicates of the circular basin were used per treatment for all treatments (Table 2) including a no choice control (grain entirely untreated). A complete set of twelve choice bioassay basins was set up daily for each exposure period for four consecutive days.

Using a funnel, 40 unsexed (5-8 day old) adult *S. zeamais* were introduced at the centre of the circular basin. The top of basin was then immediately covered using the nylon mesh (Plate 4a). The bioassay system remained undisturbed during the first three hours to give the insects time to settle. The number of insects that settled on the control (N_C) and treated (N_T) grains were recorded after 3, 4 and 24 hours of exposure. The insect counts in control and treated grains were summed up separately and recorded as total counts. The total counts were used to compute the percent repulsion (PR) according to Talukder and Howse (1993) as follows:

$$PR (\%) = 2 \times (C-50)$$

Where C = the percentage of insects on the untreated control grains. Positive values (+) represented repellency whereas negative (-) values were interpreted as attractancy or arrestment on treated grain.

The mean PR values were assigned into classes using the following scale (Talukder and Howse, 1993):

<u>Class</u>	<u>Percent Repulsion</u>
0	>0.01 to <0.1
1	0.1 - 20
2	20.1-40
3	40.1-60
4	60.1-80
5	80.1-100

Test weevils introduced in the middle of the circular base divided into four equal quarters containing alternate treated –untreated maize grains placed equidistant from the centre of the circular base.

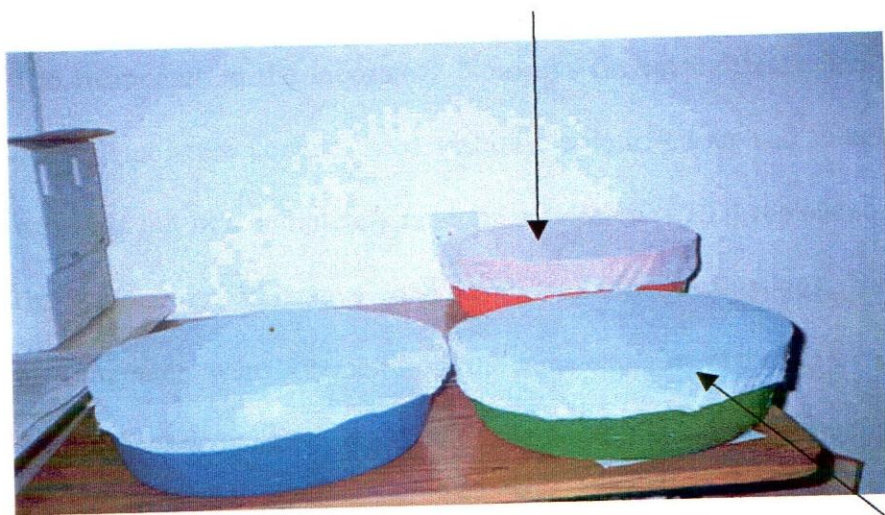


Photo by J.O. Ogendero

Tightly fitting meshed nylon
for relatively free air flow

Plate 4a: Repellency test: an alternate treated-control choice bioassay system

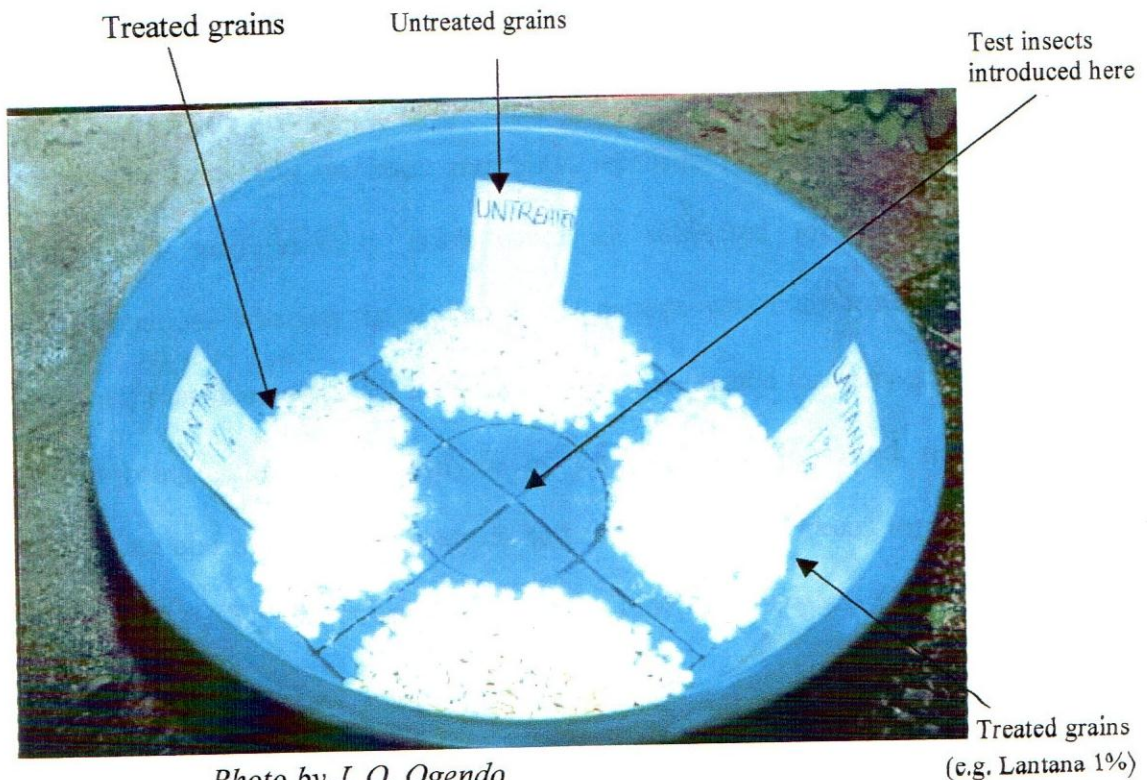


Photo by J. O. Ogendero

Fig. 4b: Repellency test: an example showing the four portions of the circular base with alternate treated-control grains

2.7 Experimental Design and Analysis

The treatments in the laboratory bioassays (insect mortality and repellency) and grain quality (moisture content, seed viability, colour, odour and insect damage) evaluations were laid out in a completely randomized design (CRD) replicated four and six times for the laboratory bioassays and grain quality evaluations, respectively. Repeated measurements were obtained from the same experimental units over time.

As usually encountered, the data of the current study on counts, percentages and visual scores violated some of the assumptions of the analysis of variance. The chi-square test for homogeneity of variances (Gomez and Gomez, 1984) and graphing of the means against the variances were applied and revealed heterogeneity. Accordingly, logarithmic [$\log_{10}X$ or $\log_{10} (X+1)$] and square root [$\sqrt{(X+1)}$] transformations were applied on the

original data (Gomez and Gomez, 1984). As is always the case with assays based upon quantitative responses (Finney, 1964), logarithmic transformations often linearizes the response curve. Data on insect mortality, repellency, grain moisture content, colour, odour and seed viability index were all subjected to appropriate logarithmic transformations whereas the percent insect damage was transformed using square root [$\sqrt{(X+1)}$] transformation. All transformed data were tested for homogeneity of variances before being subjected to analysis of variance (ANOVA) and repeated measures (univariate and multivariate) analysis and the transformed means separated by Tukey's studentized (HSD) test (Gomez and Gomez, 1984; Scheiner and Guvevitch, 1993; SAS Institute Inc., 1995).

Non-parametric exact tests [Wilcoxon rank sum test (Mann-Whitney U test)] were used to test for any significant changes in grain colour and odour during storage. The choice of this statistical test was based on the manner in which the scoring was done and its power to test the hypotheses under study (Siegel and Castellan, 1988). For purposes of the final presentation of results, the treatment means were converted to the original scale with the transformed means in parentheses.

Differences between specific treatment means were tested using orthogonal contrasts. The treatments comparisons made were (a) untreated (control) versus all the treated grains, (b) Actellic Super 2% dust treated versus powder-treated (*L. camara* and *T. vogelii*) and (c) *L. camara* powder versus *T. vogelii* powder. Linear and quadratic contrasts were used to test intra-plant linear and quadratic relationships with increased dosage.

The relationship between concentration of powder and the adult insect mortality was investigated using probit regression analysis of $\log_{10}$ transformation of concentration of *L. camara* and *T. vogelii*. Regression lines for $\log_{10}$ concentration and insect mortality were computed (Finney, 1964; Talukder and Howse, 1994). Any two probit lines were considered significantly different (Talukder and Howse, 1994) if their standard errors did not overlap.

CHAPTER 3

RESULTS

3.1 Diagnostic Survey

The key findings of the survey were as follows: (i) maize was the priority crop (ranked 1st by 64% of the respondents) followed by sorghum, beans, sweet potato, groundnuts and bananas; (ii) bag storage (ranked 1st by ≥80% farmers) is preferred over granaries because of grain security, ease of admixing grains with ash or insecticides and ease of moving grain in and out of store; (iii) *Sitophilus zeamais* Motsch. (≥96% of grain samples) and *Sitotroga cerealella* (5% of grain samples) were the major insect pests of maize in storage; (iv) farmers do experience recurring pest problems due to poor timing of grain harvesting and application of grain protectants, insufficient knowledge and skills about detection of insect pest damage and poor input (pesticide) distribution; (v) farmers use bioactive plant ash, drying or insecticides for insect pest control with 57, 27 and 12% of the farmers ranking 1st the use of ash, drying and insecticides, respectively; (vi) insecticides are viewed as potential poisons, are expensive, change the taste, texture, colour and odour of the grain, not readily available and occasionally not effective over the entire storage period; (vii) a dozen local plant species were reported as potentially insecticidal and repellent. Out of these, *Lantana camara* L. and *Tephrosia vogelii* Hook were recommended by the survey respondents and resource persons as test materials in this study. This was based on their wide distribution, potential insecticidal and repellent potency and likelihood of being used as sustainable alternatives to synthetic insecticides; (viii) 92% of the respondents were either aware and using or aware but not using plant materials in and out of storage against insect pests (Table 3a). (ix) Gender emerged as a strong factor in grain storage with women being responsible (72% of respondents) for storing grains and making decisions on the release of grains for domestic use and sale. This makes the involvement of women in adaptive post-harvest initiatives an important consideration. (x) And finally, farmers highlighted insect damage, moisture content, colour and odour of the grain as the most important quality parameters in maize grain storage. The parameters were reported as having very strong effect on market price and acceptability of grain for human consumption.

The effects of bioactive plant-based ash and synthetic insecticides on grain quality parameters, as reported by the farmers, were as shown in Table 3b below:

Table 3a: Evaluation of farmers' knowledge and use of plants as protectants against insect pests in maize grain storage

Category	% of Respondents
1. That farmers are aware and use specific plants to obtain "Ash" for control of insect pests of stored grains	28
2. That farmers are aware and using a general (mixture of plants) source of "Ash" for control of insect pests of stored grains	4
3 That farmers are aware but not using plant materials against storage insect pests	24
4. That the farmers are aware and using specific plants as repellents against non-storage insect species	4
5. That farmers are aware and using specific plants against insect pests in storage and as repellents against non-storage insects	32
6. That farmers are not aware of plants being used for control of insect pests both in and outside grain stores	8
TOTAL (%)	100

Table 3b: Relationship between grain quality and insect control method

Effect of Insect Pest Control Method	% Respondents
1. That "ash" changes grain colour from white to brown	30.5
2. That neither "ash" nor "insecticide" influences grain colour	9.0
3. That insecticide does not affect colour, weight and moisture content of grain	3.0
4. That insecticide imparts offensive odour on grain/flour	12.0
5. That ash imparts offensive odour on grain	6.0
6. That ash-treated grains give better tasting "Ugali or Porridge" than insecticide-treated grains	3.0
7. That ash changes taste of flour	3.0
8. That ash treatment increases grain density	30.5
9. That ash does not offer protection over entire storage period	3.0
TOTAL	100

In conclusion, the survey noted that agriculture in the study area was mainly subsistence ($\geq 90\%$) which made investment in pest control in grain storage uneconomical. The best way forward is for research to focus on the potential of specific local plant materials as grain protectants against the most important insect pest, *S. zeamais*, in maize storage.

3.2 Insect Mortality

Table 4a shows the percent insect mortality of *S. zeamais* in all treatments after 21 days. No mortality was recorded in the untreated grains during the first 21 days. There was significant ($p < 0.01$) treatment, exposure time and treatment by exposure time interaction effects on the adult insect mortality (Fig 1a, b).

Highest insect mortality was recorded in the grains treated with Actellic Super in which 100% kill of *S. zeamais* was achieved within 48 hours. The botanical treatments, on the other hand, were slower acting and induced mortality of the test insect species over much longer exposure duration compared to the synthetic insecticide. The two plant materials induced comparable percent insect mortality. Both plants induced $< 50\%$ mortality during the first five days of exposure but registered $> 60\%$ by the end of ten days. The botanicals achieved almost 100% mortality by the end of 21 days exposure. The lethal mean exposure times (LT_{50}) to achieve 50% adult insect mortality varied from 5-6 days for the higher rates (7.5 and 10.0% w/w) of the two plants to 7.5-8 days for the lower rates (2.5 and 5.0% w/w). This showed that the test insects differed in their susceptibility to different dosages of the plant materials. The contact toxicity of the ground powders on the adult *S. zeamais* insects was remarkably increased with the higher dosages. These results indicated that insect mortality was dependent upon the exposure period and the dosage of the plant materials tested. The results of the probit regression analysis and regression lines for adult *S. zeamais* mortality for 3-21 days of exposure to powders of the two test plant materials are presented in Table 4b. The regression lines for the effects of botanicals showed a significant relationship between weevil mortality (%) and the powder concentration applied. The slopes of the probit lines, for both plant materials, were steeper for the period 3 to 5 days of exposure. Thereafter, the slopes became increasingly less steep with exposure time. The calculated regression lines (Table 4b)

for the two plants have shown the existence of a linear relationship between concentration and the mortality (%) of adult maize weevils treated

Table 4a: Mean cumulative percent adult mortality of *S. zeamais* in grains treated with *L. camara* and *T. vogelii* powders after 21 days exposure

		Mean percent	
Treatment Code	Dose (% w/w)	mortality ±SE; n=4	
T1 Untreated control	-	0.00±0.00	(-----) ^a
T2 Actellic super 2% dust	0.05	100.00±0.00	(2.303) ^{bc}
T3 Lantana powder	1.0	88.75±6.30	(2.242) ^{ab}
T4 Lantana powder	2.5	82.75±9.60	(2.204) ^b
T5 Lantana powder	5.0	95.00±4.10	(2.277) ^{ab}
T6 Lantana powder	7.5	87.50±8.70	(2.234) ^{ab}
T7 Lantana powder	10.0	90.00±7.10	(2.249) ^{ab}
T8 Tephrosia powder	1.0	92.50±8.70	(2.262) ^{ab}
T9 Tephrosia powder	2.5	85.00±7.10	(2.220) ^{ab}
T10 Tephrosia powder	5.0	93.75±4.80	(2.270) ^{ab}
T11 Tephrosia powder	7.5	90.00±9.10	(2.248) ^{ab}
T12 Tephrosia powder	10.0	93.75±9.50	(2.269) ^{ab}

Means of four replicates of cumulative percent adult mortality of 40 unsexed adult *S. zeamais* recorded for 21 days in the untreated and treated grains. Means in a column followed by different letters are significantly different at α= 0.05 by Tukey’s studentized range (HSD) test. Figures in the parentheses are the log-transformed values.

3.3 F1 Progeny Production

The number of adult F1 progeny produced by the test insect species, *S. zeamais*, in untreated grains and those treated with Actellic super 2% dust and five dosages of each of the two plant materials are presented in Table 5. Statistical analysis showed significant (p<0.01) treatment and exposure period effects on the number of adult F1 progeny produced. The number of adult F1 insects emerging increased with exposure period (from 28 to 42 days) and the dosage (5.0 - 7.5 and 2.5-10% w/w for *Lantana* and *Tephrosia*, respectively) of the two plant materials (Table 4 & Fig.1c). Untreated grains (control) and those treated with Actellic super 2% dust, *Lantana* at 1-2.5% w/w and *Tephrosia* at 1% showed no significant changes in F1 counts after the 28th day. It emerged clearly from the results that all the botanical treatment and the synthetic insecticide effectively reduced the adult F1 progeny by ≥75%.

Fig.1a: Cumulative mean (n=4) percent adult mortality of *S. zeamais* over a 21-day exposure period (effect of *L. camara* powder)

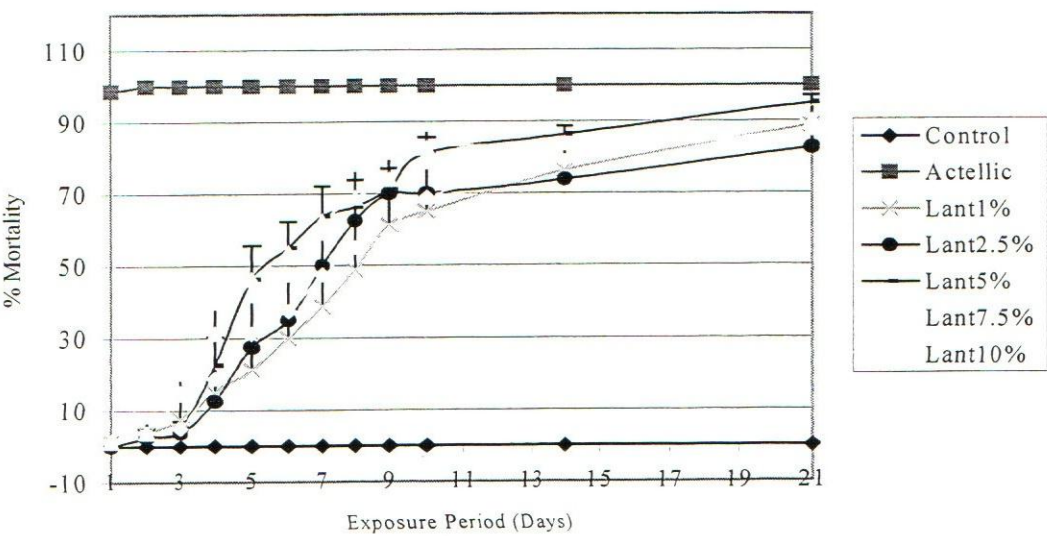


Fig.1b: Cumulative mean (n=4) percent adult mortality of *S. zeamais* over a 21-day exposure period (effect of *T. vogelii* powder)

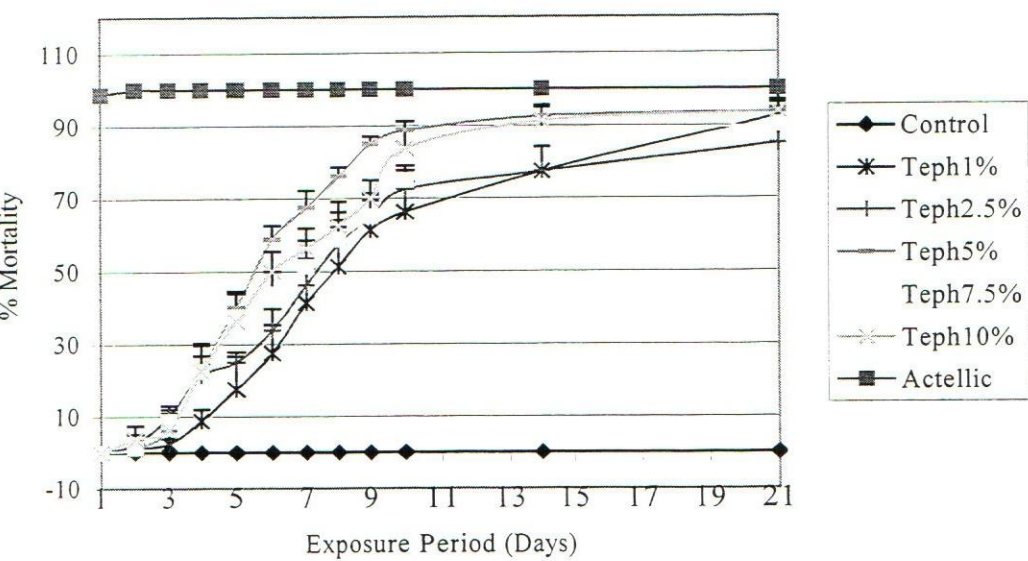


Table 4b: Probit Regression Analysis: Concentration of *L. camara* and *T. vogelii* versus mortality (%) of adult *S. zeamais* over 3-21 days period

Day	Pearson		
Day	χ^2 value	Prob.	Regression line ($\pm$ SE)
<i>Lantana camara</i>			
3	26.780*	0.083	Y= 0.299($\pm$ 0.270-1.049) X
4	26.155 ^{ns}	0.096	Y=0.399($\pm$ 0.203- 0.277) X
5	32.868*	0.017	Y=0.443+0.612($\pm$ 0.187) X
6	30.111*	0.036	Y=0.527+0.523($\pm$ 0.179) X
7	40.06**	0.002	Y=0.733+0.484($\pm$ 0.177) X
8	32.586*	0.019	Y=0.767+0.365($\pm$ 0.178) X
9	29.261*	0.045	Y=0.661+0.159($\pm$ 0.181) X
10	29.68*	0.041	Y=1.184+0.397($\pm$ 0.186) X
14	27.313 ^{ns}	0.073	Y=1.198+0.261($\pm$ 0.197) X
21	22.749 ^{ns}	0.200	Y=1.385+0.123($\pm$ 0.230) X
<i>Tephrosia vogelii</i>			
3	23.873 ^{ns}	0.159	Y=0.309($\pm$ 0.287-1.074) X
4	24.442 ^{ns}	0.141	Y=0.519($\pm$ 0.214-0.153) X
5	29.959*	0.038	Y=0.401+0.649($\pm$ 0.269) X
6	26.828 ^{ns}	0.082	Y=0.689+0.631($\pm$ 0.180) X
7	31.879*	0.023	Y=0.619+0.401($\pm$ 0.177) X
8	35.502**	0.008	Y=0.719+0.312($\pm$ 0.255) X
9	30.812*	0.030	Y=0.862+0.258($\pm$ 0.182) X
10	26.536 ^{ns}	0.088	Y=1.470+0.514($\pm$ 0.191) X
14	30.704*	0.031	Y=1.923+0.615($\pm$ 0.210) X
21	28.799 ^{ns}	0.051	Y=1.465+0.089($\pm$ 0.248) X

Values based on five concentrations, four replicates of 20 weevils each giving a total of 400 weevils.
 *, ** and ^{ns} refer to significant (p<0.05), highly significant (p<0.01) and non-significant (p>0.05), respectively.
 X and Y refer to the powder concentration and percent insect mortality, respectively.

Fig 1c: Number of F1 progeny in grains treated with botanicals

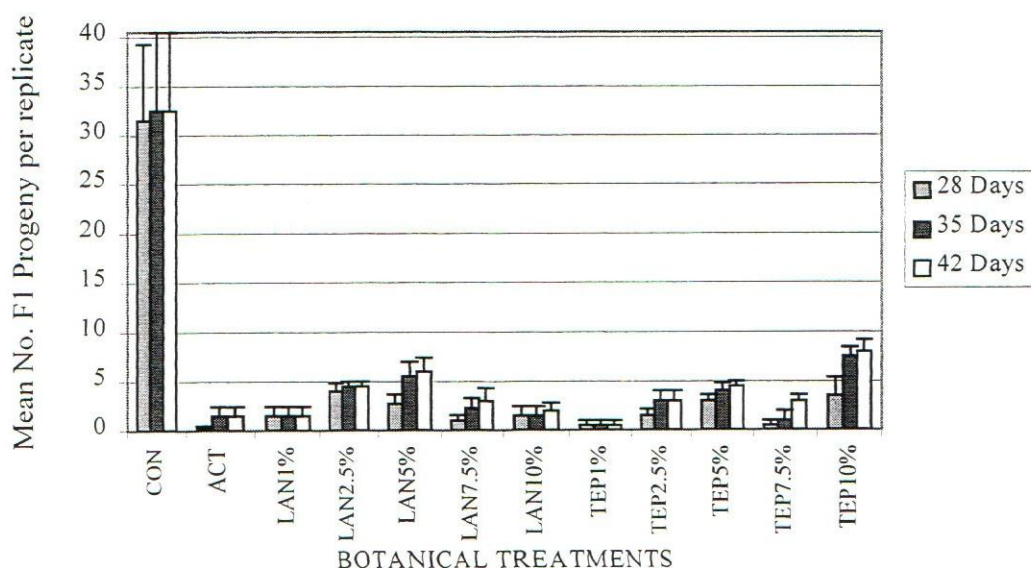


Table 5: Mean adult F1 counts (*S. zeamais*) in grains treated with ground powders of *L. camara* and *T. vogelii* after 42 days

Treatment Code	Dose (% w/w)	Mean number of adult F1 produced $\pm$ SE ;n=4
T1 Untreated control	-	32.20 $\pm$ 7.93 (1.462) ^a
T2 Actellic super 2% dust	0.05	1.10 $\pm$ 0.72 (0.2211) ^f
T3 Lantana powder	1.0	1.50 $\pm$ 0.96 (0.2940) ^{ef}
T4 Lantana powder	2.5	4.30 $\pm$ 0.61 (0.7170) ^{bc}
T5 Lantana powder	5.0	4.70 $\pm$ 1.29 (0.6929) ^{bcd}
T6 Lantana powder	7.5	2.08 $\pm$ 0.97 (0.3923) ^{cdef}
T7 Lantana powder	10.0	1.70 $\pm$ 0.91 (0.3338) ^{def}
T8 Tephrosia powder	1.0	0.50 $\pm$ 0.50 (0.1193) ^f
T9 Tephrosia powder	2.5	2.50 $\pm$ 0.88 (0.4645) ^{bcdef}
T10 Tephrosia powder	5.0	3.80 $\pm$ 0.80 (0.6679) ^{bcde}
T11 Tephrosia powder	7.5	1.50 $\pm$ 0.69 (0.2946) ^{ef}
T12 Tephrosia powder	10.0	6.30 $\pm$ 1.32 (0.7910) ^b

Means of four replicates of F1 progeny produced by 40 unsexed adult *S. zeamais*. Means in a column followed by different letters are significantly different at $\alpha=0.05$ by Tukey's studentized range (HSD) test. Figures in the parentheses are the log-transformed values.

3.4 Repellency

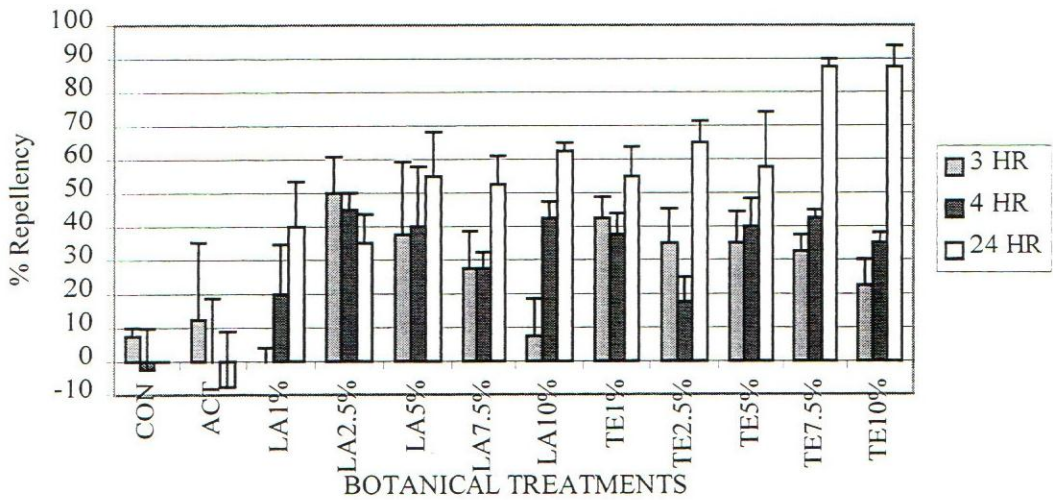
The mean percent repellency (PR) values for a 3, 4 and 24-hour exposure obtained from a choice bioassay system for all treatments are presented in Table 6 and Figure 2. Data analysis of the log-transformed PR values showed significant ($p<0.01$) differences in repellency between the untreated control and all the treated grains. There were significant differences in repellency between Actellic super 2% dust and the two plant materials at all rates except for *L. camara* at 1.0% (w/w). However, grains treated with *T. vogelii* powder had significantly ($p<0.01$) higher PR values than those treated with *L. camara* powder. *L. camara* powder showed a linear relationship in PR values as the dosage increased. The PR values for all treatments, except for the untreated and Actellic super treatment, increased significantly with increase in exposure time in the choice bioassay system.

Table 6: Mean percent repellency of untreated grains and grains treated with botanical treatments against *S. zeamais*

Treatment Code	Dose (% w/w)	Mean percent Repellency (PR) $\pm$ SE after 24 hrs		Repellency Class; n=4
T1 Untreated control	-	0.00 $\pm$ 0.00	(0.7433) ^d	0
T2 Actellic Super 2% dust	0.05	-7.50 $\pm$ 16.52	(2.1659) ^c	I
T3 Lantana powder	1.0	40.00 $\pm$ 13.54	(2.3365) ^c	II
T4 Lantana powder	2.5	35.00 $\pm$ 8.66	(3.3244) ^{ab}	II
T5 Lantana powder	5.0	55.00 $\pm$ 13.23	(3.4623) ^{ab}	III
T6 Lantana powder	7.5	52.50 $\pm$ 8.54	(3.0783) ^b	III
T7 Lantana powder	10.0	62.50 $\pm$ 2.5	(3.3239) ^{ab}	IV
T8 Tephrosia powder	1.0	55.00 $\pm$ 8.66	(3.5742) ^{ab}	III
T9 Tephrosia powder	2.5	65.00 $\pm$ 6.46	(3.3573) ^{ab}	IV
T10 Tephrosia powder	5.0	57.50 $\pm$ 16.52	(3.5187) ^{ab}	III
T11 Tephrosia powder	7.5	87.50 $\pm$ 2.50	(3.8168) ^a	V
T12 Tephrosia powder	10.0	87.50 $\pm$ 6.29	(3.4356) ^{ab}	V

Means of four replicates of 40 unsexed adult *S. zeamais* tested in a choice bioassay of alternate untreated-treated grains recorded after 24 hours exposure. Means in a column followed by different letters are significantly different at $\alpha= 0.05$ by Tukey’s studentized range (HSD) test. Figures in the parentheses are the log-transformed values.

Fig. 2: Repellency of botanicals against *S. zeamais* as affected by exposure time



3.5 Grain Quality Assessments

3.5.1 Percent grain moisture content (%MC)

The results of the baseline grain quality evaluations at the start of investigation (day 0) are presented in Table 7. Tables 8a-8c shows the percent grain moisture content for all treatments. The tables highlight the effect storage duration (8a & 8c) and the botanical treatments (8b & 8c). Analysis of variance results indicated significant ($p < 0.05$) storage duration, treatment and storage duration by treatment interaction effects on the percent grain moisture content. The treatment effects were significant for 60, 90 and 150 days of grain storage. Storage duration, on the other hand, influenced the moisture content of both untreated and treated grains.

Their grain moisture content increased significantly between 0 and 30 days for all treatments including the untreated control. Thereafter, there was significant reduction ($p<0.05$) in percent grain moisture content for all the treatments for the next 3 months (up to 120 days) of grain storage (Fig.3a & 3b). Grains treated with the synthetic insecticide had consistently lower ($p<0.05$) %MC than grains treated with botanicals or untreated during the entire 5 months of storage. The magnitude of depression of grain MC was generally less with higher dosages of the powdered botanical treatments from the two plant materials. The lowest grain MC was recorded after 120 days of storage. The magnitude of change in MC of grains treated with *L. camara* and *T. vogelii* powders at 5.0% (w/w) were significantly different from that of the untreated control.

Table 7: Mean (n= 20) quality characteristics of the test grains (baseline grain samples) at the start (Day 0) of the grain quality evaluation activity

<u>Quality Parameter</u>	<u>Value ($\pm$SE where applicable)</u>
1. Grain moisture content (%)	13.6 $\pm$ 0.178
2. Insect damage (%)	0.00 $\pm$ 0.00
3. Seed viability index (%)	89.4 $\pm$ 4.630
4. Grain Odour	Odourless
5. Grain Colour	Natural white with few yellow grains

Table 8a: Mean percent grain moisture, across all treatments, as affected by the storage duration

Storage duration	Mean percent grain	
(Days)	moisture content (n=48)	
30	14.38	(2.666) ^a
60	13.99	(2.638) ^b
90	14.46	(2.600) ^c
120	12.89	(2.556) ^d
150	12.96	(2.562) ^d
MSD (HSD)*	0.089	(0.0066)

Means of six replicates each of untreated and treated grains over 150 days of storage. Means in a column followed by different letters are significantly different at $\alpha = 0.05$ by Tukey's studentized range (HSD) test. Figures in the parentheses are the log-transformed values. MSD (HSD)* is the minimum significant difference at $\alpha = 0.05$ by Tukey's studentized range (HSD) test.

Table 8b: Mean (n=30) percent grain moisture content as affected by botanical treatments during 5 months of storage

Treatment	Dose(% w/w)	Mean % MC (n=30)
T1 Untreated	-	13.88 ^b
T2 Actellic Super	0.05	13.85 ^b
T3 <i>Lantana</i>	1.0	13.97 ^{ab}
T4 <i>Lantana</i>	2.5	14.07 ^{ab}
T5 <i>Lantana</i>	5.0	14.10 ^{ab}
T6 <i>Tephrosia</i>	1.0	14.02 ^{ab}
T7 <i>Tephrosia</i>	2.5	13.85 ^b
T8 <i>Tephrosia</i>	5.0	14.18 ^a
MSD (HSD)*		0.31

Means of six replicates each of untreated and treated grains over 150 days of storage. Means in a column followed by different letters are significantly different at $\alpha = 0.05$ by Tukey's studentized range (HSD) test. Figures in the parentheses are the log-transformed values. MSD (HSD)* is the minimum significant difference at $\alpha = 0.05$ by Tukey's studentized range (HSD) test.

Fig. 3a: Change in moisture content of grains treated with *L. camara* powder during storage

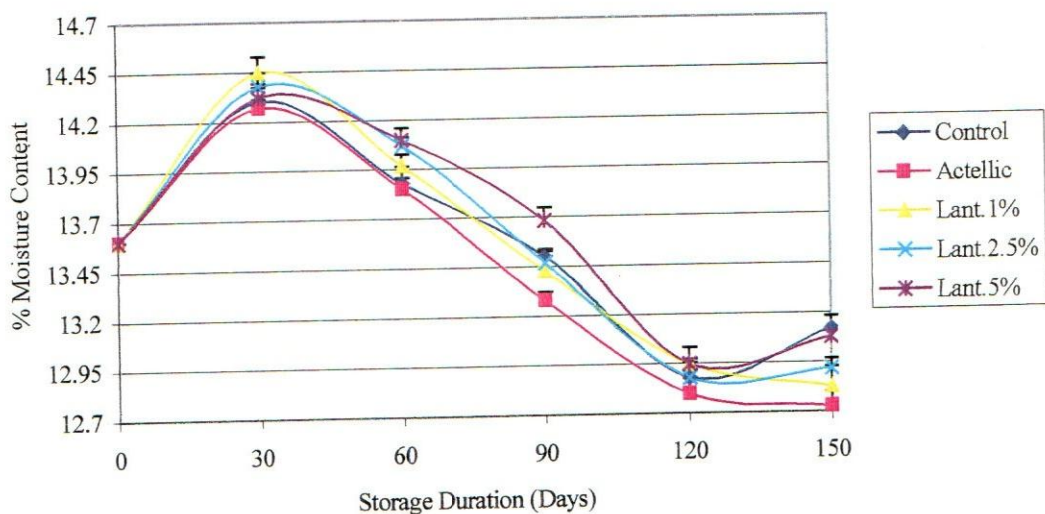


Fig. 3b: Change in moisture content of grains treated with *T. vogelii* powder during storage

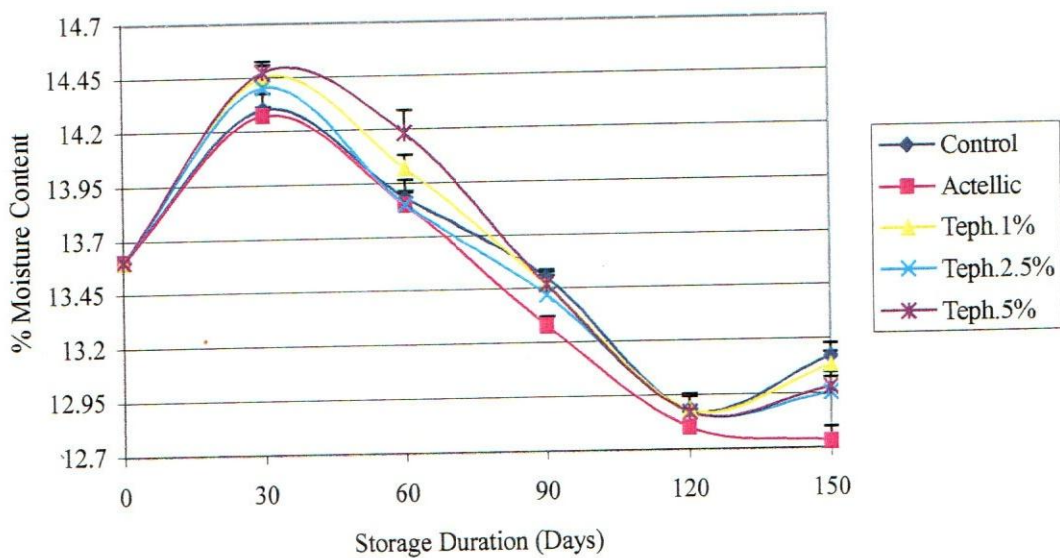


Table 8c: Mean percent maize grain moisture content as a function of storage duration and botanical treatments.

Storage Duration	T R E A T M E N T S.....							
(Days)	T1	T2	T3	T4	T5	T6	T7	T8
0	13.6	13.6	13.6	13.6	13.6	13.6	13.6	13.6
30	14.30 ^a	14.27 ^a	14.45 ^a	14.38 ^a	14.32 ^a	14.45 ^a	14.40 ^a	14.47 ^a
60	13.88 ^b	13.85 ^b	13.97 ^b	14.07 ^b	14.10 ^b	14.02 ^b	13.85 ^b	14.18 ^a
90	13.50 ^c	13.28 ^c	13.43 ^c	13.47 ^c	13.68 ^b	13.47 ^c	13.42 ^c	13.47 ^c
120	12.88 ^d	12.80 ^d	12.95 ^d	12.88 ^d	12.95 ^d	12.88 ^d	12.87 ^d	12.87 ^c
150	13.12 ^d	12.73 ^d	12.83 ^d	12.92 ^d	13.08 ^c	13.08 ^d	12.95 ^d	12.98 ^c
MSD(HSD)*	0.280	0.237	0.286	0.212	0.260	0.240	0.300	0.310

Means of six replicates each of untreated and treated grains over 150 days of storage. Means in a column followed by different letters are significantly different at $\alpha=0.05$ by Tukey's studentized range (HSD) test. MSD(HSD)* is the minimum significant difference at $\alpha=0.05$ by Tukey's studentized range (HSD) test.

3.5.2 Seed Viability Index

The effect of storage duration and botanical treatments on the seed viability index, expressed as percent germination, is presented in Table 9 and Fig. 4a & 4b. The analysis of variance (ANOVA) and multivariate analysis results indicated that the percent germination significantly ($p<0.05$) increased, except for 120 days, with storage duration for both untreated and treated maize grains. Germination was lowest after 120 days of grain storage. However, there was no significant treatment and storage duration by treatment interaction effects on the percent germination. There were no linear and quadratic relationships for both plant materials. Correlation analysis results show that the seed viability index was weakly correlated with the grain MC, botanical treatments and the storage duration (Appendix 4).

With the exception of synthetic insecticide, all the botanical treatments and the control recorded decrease in percent germination for the first 30 days of grain storage.

Untreated (control) grains had the lowest viability index which was judged significantly (no overlap of the SE bars) different from the botanical treatments. Grains treated with Actellic super 2% dust registered increased percent germination during the first 30 days. *Lantana* at 5% w/w and the control treatment had the lowest viability index at 120 days compared to its lower dosages (1-2.5% w/w) and Actellic super. A similar response trend was observed with *Tephrosia* (all dosages) except only grains treated with Actellic Super were significantly different from the powders.

Table 9: Mean percent germination of untreated and treated grains during 150 days of storage

Storage Duration _____	T R E A T M E N T S.....							
(Days)	T1	T2	T3	T4	T5	T6	T7	T8
0	89.4 ^{ab}	89.4 ^{ab}	89.4 ^{ab}	89.4 ^{ab}	89.4 ^{ab}	89.4 ^{ab}	89.4 ^{ab}	89.4 ^{ab}
30	81.50 ^{bc}	91.67 ^{ab}	83.00 ^b	86.5 ^b	85.33 ^{ab}	85.83 ^{bc}	85.17 ^{ab}	85.83 ^{bc}
60	93.33 ^a	92.33 ^a	90.00 ^{ab}	93.17 ^{ab}	92.17 ^a	91.83 ^{ab}	93.33 ^a	94.83 ^a
90	95.17 ^a	93.50 ^a	97.33 ^a	96.67 ^a	94.83 ^a	96.5 ^a	92.67 ^{ab}	94.00 ^a
120	77.83 ^c	83.50 ^b	81.00 ^b	86.83 ^{ab}	77.17 ^b	82.17 ^c	82.33 ^b	83.50 ^c
150	90.33 ^{ab}	93.33 ^a	90.18 ^{ab}	88.33 ^{ab}	89.83 ^{ab}	96.17 ^a	90.67 ^{ab}	92.33 ^{ab}
MSD(HSD)*	9.32	8.38	12.78	9.94	10.14	9.50	10.93	

Means of six replicates each of untreated and treated grains over 150 days of storage. Means in a column followed by different letters are significantly different at $\alpha=0.05$ by Tukey’s studentized range (HSD) test. MSD (HSD)* is the minimum significant difference at $\alpha=0.05$ by Tukey’s studentized range (HSD) test. T1-T8 is as defined Table 1 above.

Fig. 4a: Change in viability of grains treated with *L. camara* powder during storage

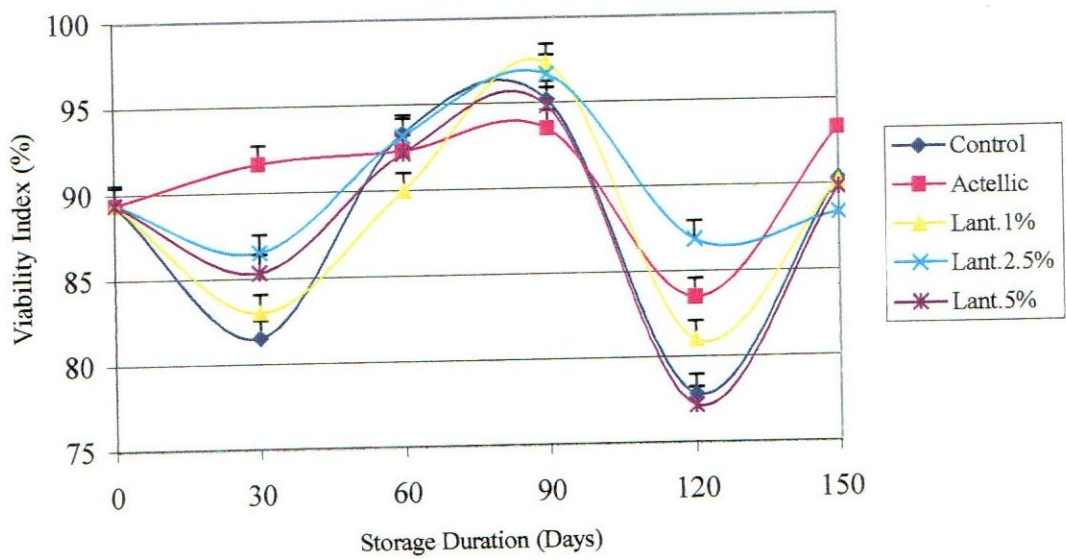
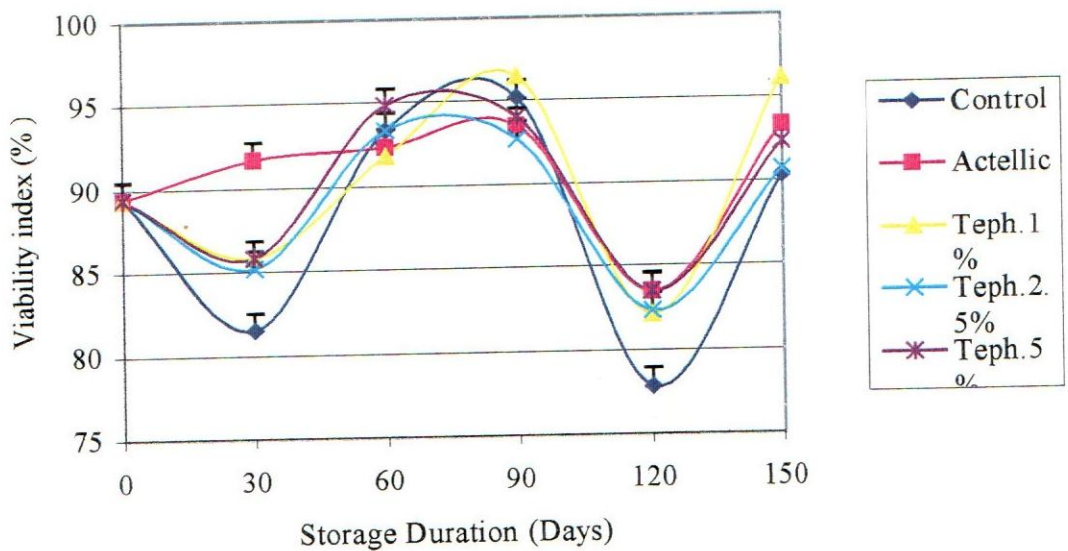
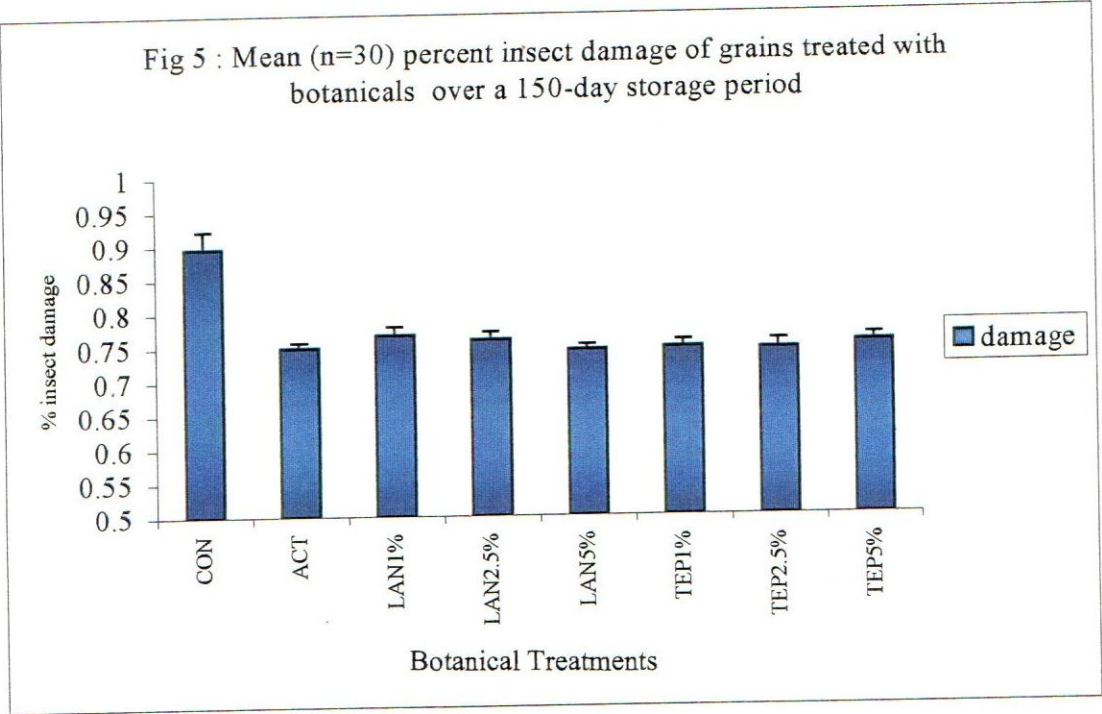


Fig. 4b: Change in viability of grains treated with *T. vogelii* powder during storage



3.5.3 Insect Damage

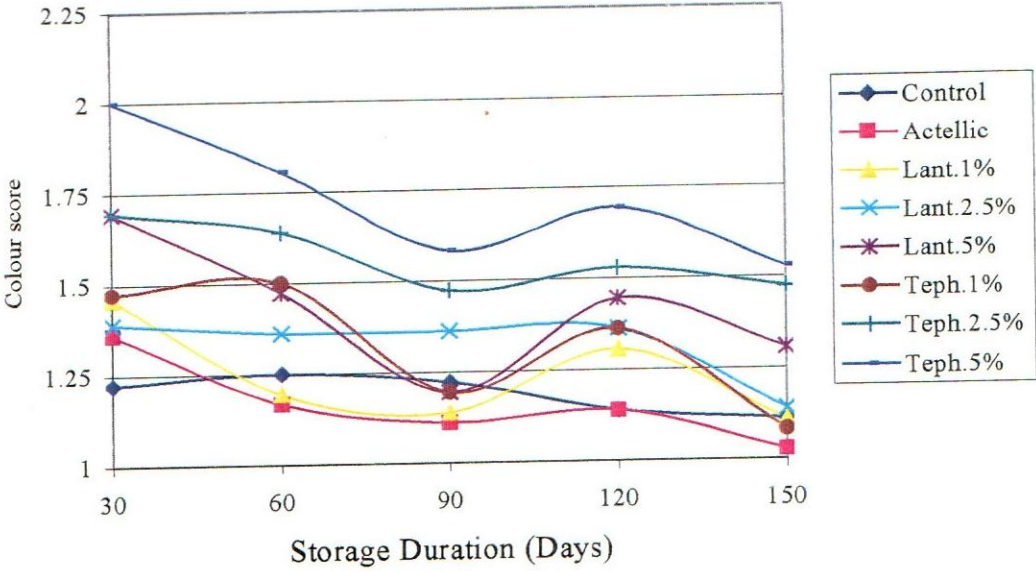
The results of the percent insect damage during 150 days of storage of untreated grains and those treated with different rates of the two test plant materials, *L. camara* and *T. vogelii*, are presented in Figure 5. The MANOVA analysis results of the log-transformed data showed significant treatment ($p<0.01$), storage duration ($p<0.01$) and treatment by storage duration ($p<0.05$) interaction effects on the level of insect damage on stored maize grains. The untreated grains suffered significant ($p<0.01$) percent insect damage compared to grains treated with Actellic Super 2% dust and the different rates of the two test plant materials. However, there were no significant differences between grains treated with Actellic super 2% dust and the different rates of the two plant materials tested. Similarly, increasing the rates of application of the two plant materials had no linear and quadratic relationships with the level of insect damage recorded.



3.5.4 Grain Colour

Figure 6 below shows the mean evaluation scores for change in the colour of untreated maize grains and those treated with Actellic super 2% dust and three rates each of the ground powders of *L. camara* and *T. vogelii*. Statistical analysis results indicated that there were significant ($p<0.01$) storage duration, quality assessors, botanical treatments and storage duration by botanical treatments interaction effects on the colour of stored maize grains. Highly significant ($p<0.01$) differences were recorded amongst the treatments. The Wilcoxon scores (rank sums) decreased with storage duration but increased with the dosage of the two plant powders applied (Appendices 2a & 2b).

Fig.6: Mean (n=36) evaluation scores for grain colour (+SE bars) as affected by botanical treatments during storage



3.5.5 Grain Odour

The results of changes in odour of untreated maize grains and those treated with Actellic super 2% dust and three rates each of ground powders of *L. camara* and *T. vogelii* are presented in Fig.7a and 7b. Statistical analyses showed highly significant ($p<0.01$) storage duration, treatment, storage duration by treatment, treatment by judges and storage duration by judges interaction effects on the odour of stored maize grains. The Wilcoxon Scores (Rank Sums) increased with the dosage of ground powder of the two plant materials but decreased with duration of grain storage (Appendices 3a & 3b). The grains treated with Actellic super 2% dust had the lowest rank scores followed by the untreated grains. Highest rank scores were recorded with *T. vogelii* at 5% w/w compared to *L. camara* at the same dosage.

The change in grain odour, compared to the reference grain sample (untreated control), was strongest early in storage and this effect decreased with increase in storage period. Throughout the grain storage period, *T. vogelii* powder at 2.5 and 5% w/w imparted the strongest odour followed by *L. camara* powder at the same rates. However, the changes in grain odour, in all the cases, were mainly between odourless and less offensive. Except for *T. vogelii* powder at 5% w/w, all the other treatments recorded assessment scores less than 2 and the untreated (control) grains remaining odourless throughout the storage period.

Fig. 7a: Mean (n=180) evaluation scores for grain odour as affected by botanical treatments during 5 months of storage

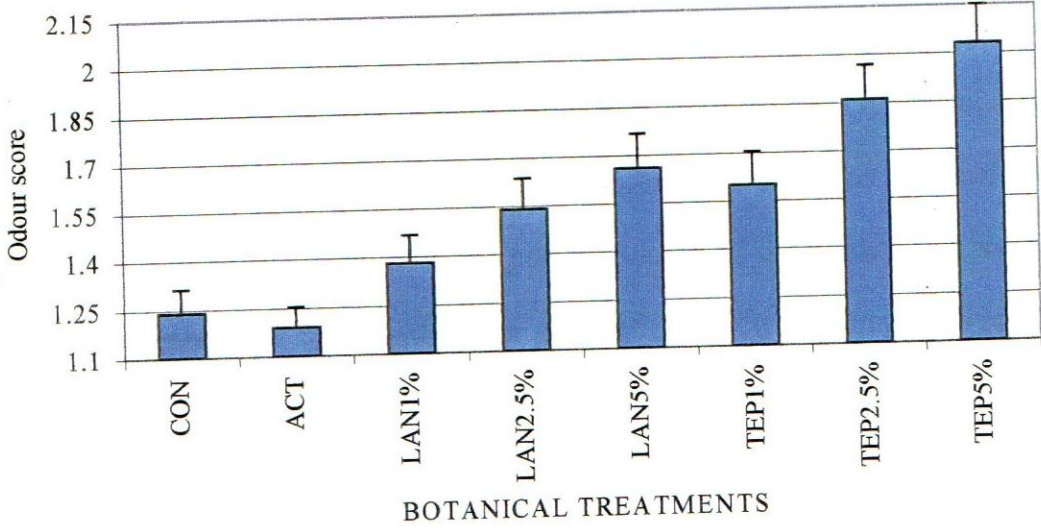
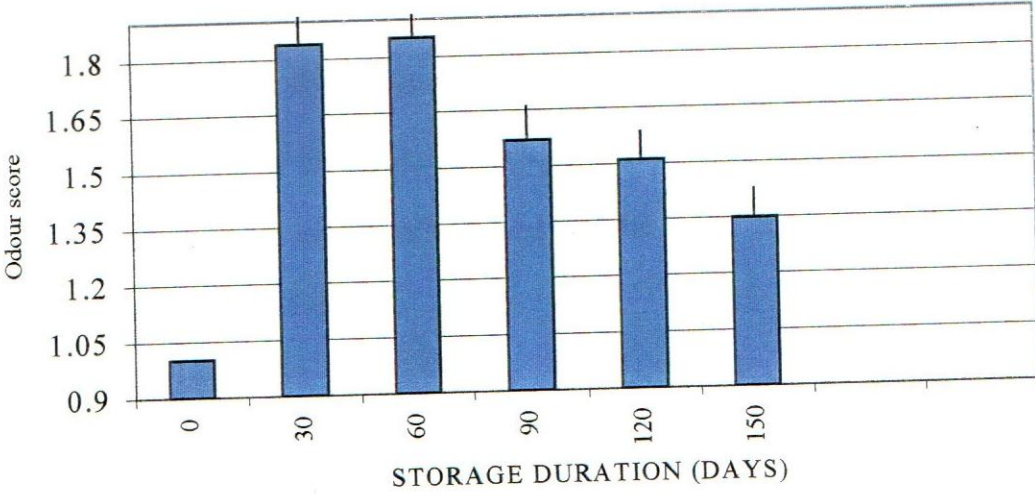


Fig. 7b: Mean (n=288) evaluation scores for grain odour as affected by storage duration



NB: The odour score at day 0 represents the value just before the treatments were applied.

CHAPTER 4

DISCUSSION

The responses observed are consistent with Regnault-Roger and Hamraoui (1993) findings in which plant materials produced a slow insecticidal effect on the bean beetle, *Acanthoscelides obtectus*, during a 12-day period. Results of the adult insect mortality and F1 progeny emergence reveal two distinct types of effects by the botanical treatments on *S. zeamais*. There was direct contact toxicity as manifested in the cumulative adult insect mortality over the test period. This direct insecticidal effect was an important mechanism through which the impact of the ground powders of *L. camara* and *T. vogelii* on existing insect infestation was evaluated. Compared to the Actellic super 2% dust, which achieved 100% kill within 2 days, the powders were slow acting achieving 65-85% kill after 10 days of exposure. The mortality results achieved by the botanical treatments are comparable to the findings of Kasa and Tadese (1996) in which crude powders from eight plant species inflicted 58-88% adult insect mortality to *S. zeamais* after 14 days of exposure. It is evident from the results that the two plant materials, *L. camara* and *T. vogelii*, induced comparable insect mortality and that the insect mortality increased with increase in amount of powder applied.

The botanical treatments, on the other hand, showed an indirect effect on the test adult insects by interfering with their reproduction. This effect on reproduction was assessed after a complete cycle of development of *S. zeamais* from oviposition to adult (F1 progeny) emergence. Victor and Ojaruega (1993) reported that humidity is an important factor affecting the oviposition and reproductive potential of insects in storage and that *S.*

zeamais appeared to prefer humidities within the range of 30-50% RH. The ambient conditions (Appendix 1) in the laboratory during the developmental cycle were within those described by (Haines, 1991) and were, therefore, conducive for egg laying and subsequent development to adult stage. The mean number of F1 adult insects produced was highest (32.2 insects) in the untreated grains and least in the grains treated with Actellic super 2% dust (1.1 insects). The variability in the number of F1 adult insects with increase in concentration of ground powders of the two plant materials (1% to 2.5% w/w) appeared erratic (up and down), inconsistent and numerically insignificant. The general reduction may be due to suppressed oviposition and/ or ovicidal and/ or larvicidal effects of the ground powders. The insecticidal (killing adults) effects of *T. vogelii* may be due to the presence of such constituents as rotenone, rotenolone, tephrosin and deguelin reported to have both insecticidal and larvicidal effects even after 22 years of storage (Oliver-Bever, 1986; Lambert *et al.*, 1993). Similarly, leaf extracts of *Lantana camara* have been reported to have insecticidal and growth regulating effects against *Liriomyza trifolii* and *L. huidobrensis* (Facknath, 1994). Saxena *et al.* (1992) also reported that a 5% solution extracted from *L. camara* caused 40% mortality in adult *Callosobruchus chinensis* within 7 days, and an application of 40 mg/100 g grain prevented oviposition. The exact chemical constituents of the two plant materials and the modes of action against storage pests remain for further investigation.

Repellency of the ground powders from *L. camara* and *T. vogelii* against the maize weevil, *S. zeamais*, increased significantly with exposure time and amount of powder applied. *T. vogelii* at 7.5 and 10% w/w were the most repellent (87.5%, class V) followed

by *T. vogelii* at 2.5% w/w and *L. camara* at 10% w/w with 65.0 (class IV) and 62.5% (class IV), respectively. On average, *T. vogelii* was more repellent (70.5%) than *L. camara* (49%). The significant PR values observed against *S. zeamais* give clue at the presence of natural insect repellent compounds in the tissues of plants such as *L. camara* and *T. vogelii*. *Tephrosia* leaves, roots, fruit capsules and seeds contain two main constituents, deguelin and tephrosin, responsible for their pungent odour (Oliver-Bever, 1986). *L. camara* has been reported to have toxic compounds triterpenes, lantadenes A and B (Morton, 1994) and two novel compounds, camarinic and camaric acids, which made the plant important in traditional folk medicine (Siddiqui *et al.*, 1995). The presence of such compounds, some of which are volatile and aromatic, form a strong basis upon which the observed insect mortality and repellency may be explained. It is worth noting that although a lot of insect mortality and repellence studies have been conducted in the past (Bowry *et al.*, 1984; Regnault-Roger and Hamraoui, 1993; Talukder and Howse, 1993, 1994; Niber, 1994; Bekele *et al.*, 1996), no chemical composition and powder bioassay data are available on the bioactivity of *L. camara* and *T. vogelii* as grain protectants against major insect pests of maize such as *S. zeamais* in storage. This means, therefore, that further studies are needed to isolate the chemical constituents of the two plant species contributing to the contact toxicity, repellence and the suppressed emergence of F1 progeny.

Results showed that the grain moisture content (MC) and seed viability index (expressed as percent germination) of untreated grains and those treated with Actellic super 2% dust and different rates of ground powders of *L. camara* and *T. vogelii* were all significantly

influenced by the duration of storage. Except for the periods 0-30 and 90-120 days, the results showed increased seed viability index with duration of storage for all treatments. On the other hand, the effect of amount of powder applied was insignificant. However, storage duration, botanical treatments and their interaction effects influenced the grain MC. An increase in MC during the first 30 days was observed which was negatively correlated to the seed viability index. Thereafter, the grain MC decreased during storage for 3 months (30-120 days) and the magnitude of depression of MC decreased with concentration of plant powder. The grain MC and viability index were lowest after 120 days of storage. This extremely low MC and viability index observed after 120 days (February 2000) coincides with the time when the ambient relative humidity was only 38%. The changes in grain MC can be attributed to variations in ambient temperature and relative humidity during storage.

All durable stored products are hygroscopic and can therefore absorb or desorb (release) moisture from and to the surroundings. The moisture holding capacity of the air increases three times the amount of change in temperature (NRI, 1999b). It follows from this argument that stored products release moisture to the surrounding air with a low relative humidity resulting in decrease in grain MC. Where the air temperature and relative humidity are variable, the grain MC will also vary. This explanation seems plausible in this study where the ambient relative humidity and temperature were variable throughout the duration of storage (Appendix 1). There was an inverse relationship between grain MC and the seed viability index. The seed viability index increased significantly (82 to 97%) for all treatments as the grain MC decreased (14.5 to 13.3%) during the period 30-

90 days of grain storage. There was a sharp drop in seed viability index (97 to 78-85%) for all treatments when the grain MC fell below 13.25% between 90 and 120 days. This indicates that 13.3% could be the acceptable critical minimum moisture content of stored maize grains below which a marked decrease in seed viability index is realised. NRI (1999a) reported that *S. zeamais* thrives best in grains with moisture contents not much less than 13%.

Statistical analysis of the results showed significant treatment, storage duration and their interaction effects on the percent insect damage on stored maize grains. The level of damage was significantly higher in the untreated (control) grains than in those treated with the synthetic insecticide and different dosages of the ground powders of the two plant materials. However, the differences between the insecticide and plant materials were insignificant. No linear relationships were detected between the concentration of powder applied and the level of insect damage observed. The low moisture content of the grains, extremely dry weather conditions and lack of other grains stored in the neighbourhood could explain the exceptionally low damage levels (between 0.7 and 0.9% for all treatments). These results agree well with Schulten (1996), working with *Prostephanus truncatus*, who observed that the progress of infestation in traditional maize varieties in large parts of eastern Africa was very slow during the dry season and that measurable losses only develop after the onset of rains. The level of insect damage recorded (0.7 to 0.9%) fits well into their findings of 0.5-4% during the same months (November-April) of the year. Further research may be needed to establish the seasonality of *S. zeamais* infestation in relation to periods of bumper harvests and crop

failures. Also it has been suggested that altitudes above 1500 m are likely to slow down insect breeding and hence cause less damage (Schulten, 1996).

The botanical treatments, storage duration, quality assessors and treatment-storage duration interaction significantly influenced the grain colour and odour evaluation scores. For both quality parameters, the Wilcoxon scores (rank sums) increased with dosage of plant powder but decreased as storage progressed. This implies concentration influences the amount of odour emitted by a plant material and that the concentration decreases with time. The decrease could possibly be due to the presence of volatile compounds in the plant tissues, which volatilize easily under ambient conditions. Grains admixed with synthetic insecticide had the lowest rank scores followed by the untreated control but the differences were insignificant. This observation disapproved the farmers hypothesis that insecticides impart offensive odour to maize grain and flour (Ogendo, 2000). It appears that insecticide treatment is likely to improve the grain colour and odour. However, conclusion should be subject to further investigation. The evaluation results indicated that admixing maize grains with plant powders changed, though at very low level, grain colour and odour over 5 months of storage. Except for *T. vogelii* powder at 5.0% w/w which had significantly stronger odour (mean score >2) than *L. camara* at same rate, all the other treatments had mean assessment scores less than 2. This means, therefore, that the colour and odour changes detected were expected to affect the market value marginally. It is important to note that the literature survey conducted revealed no reported data on the evaluation of effect of botanicals on the grain colour and odour.

Conclusions and Recommendations

The farm survey has confirmed that insect pest problems in storage are real and that the maize weevil, *S. zeamais*, remains the number one insect pest of stored maize grains in the small holder farming communities. Despite decades of campaigns, the adoption rate of synthetic insecticides has remained remarkably low in these environments. Research has lately focused on exploring the possibilities of using bioactive plant based products with potential insecticidal and repellent properties as cost-effective and compatible alternatives to the synthetic insecticides.

Based on the results of laboratory bioassays and grain quality evaluations, the following conclusions can be made:

- (1) Although slow acting, the powders of *L. camara* and *T. vogelii* effectively reduced adult F1 progeny emergence (by $\geq 75\%$) and caused 83-90 and 85-94% mortality after 21 days, respectively. They are, therefore, potential botanical pesticides for use against insect pests of stored maize.
- (2) Both plant materials were repellent against the maize weevil, *S. zeamais*. However, *T. vogelii* was more repellent and has better prospects as protectant of stored maize grains than *L. camara*.
- (3) The botanical treatments significantly minimized the magnitude of depression in the % MC and virtually had no effect on the % germination of stored maize grains. The magnitude of grain MC depression was dependent upon the concentration of powder applied. Minimisation of fluctuations in grain MC is an important positive attribute for successful storage of all types of grains.

- (4) The botanical treatments effectively reduced the insect damage on stored maize grains arising from natural infestations. The damage levels recorded were, however, not dependent upon the dosage of plant powder. But this needs to be verified further under conditions of high insect pest infestations.
- (5) Finally, admixing maize grains with ground powders of *L. camara* and *T. vogelli* results in slight change in grain odour but no effect on grain colour during storage.

The scope of this study was obviously limited to just observing the response of adult insects, exclusive of reproduction, to ground powders of the two plant materials tested. The plant materials used consisted of succulent stems, leaves, flowers and fruits obtained from the two plant species. It was not possible to investigate the sources of and modes of action involved in the observed insect mortality, repellency and odours imparted on stored grains. There is, therefore, need for further investigations to address the following:

- (a) The isolation, characterization and quantification of the exact chemical compounds and their modes of action in relation to the variables that were the subject of this investigation;
- (b) Determinative investigation on the most effective plant part (roots, stems, leaves, flowers or fruits/seeds) and form in which applied (powder; aqueous, petroleum ether, or alcoholic extracts). Attempts be made to relate form of application to compatibility and cost-effectiveness with the target user groups;
- (c) Effect of drying method at graded temperature regimes on the chemical composition and insecticidal and repellent potency against insect pests in grain storage. These should be partitioned, possibly in a split-plot arrangement, according to plant part used.
- (d) This is food for thought for future research: How does altitude (location), ambient (or controlled) relative humidity and seasons (dry Vs wet) affect the insecticidal and repellent potency of these botanicals and the levels of natural insect pest infestations?

CHAPTER 5

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CHAPTER 8

APPENDICES

Appendix 1: Summary of the rainfall, temperature and relative humidity of the air at Egerton University, Njoro Division, Kenya, October 1999 to April 2000.

	<u>1999</u>			<u>2000</u>			
	<u>Oct.</u>	<u>Nov.</u>	<u>Dec.</u>	<u>Jan</u>	<u>Feb</u>	<u>Mar</u>	<u>April</u>
Mean ambient Temp ($^{\circ}\text{C}$)	18.7	19.7	19.1	21.3	22.2	22.3	21.0
Mean rel. humidity (%)	65	69	64	45	38	54	66
Rainfall (mm)	65.2	118.9	56.8	1.2	0.8	2.0	56.8
No. rainy days	22	15	9	2	1	3	13

Appendix 2a: Wilcoxon rank sum test (Mann-Whitney U test) for Grain Colour (NPAR1WAY PROCEDURE FOR STORAGE DURATION)

Storage duration	Sum of	Expected	Std Dev	Mean
(Days)	Scores	Under H0	Under H0	Score
30	232050.0	207504.0	5167.61443	805.729167
60	218457.5	207504.0	5167.61443	758.532986
90	194820.0	207504.0	5167.61443	676.458333
120	208495.0	207504.0	5167.61443	723.940972
150	183697.5	207504.0	5167.61443	637.838542

Kruskal-Wallis Test (Chi-Square Approximation)

Chisq = 43.472 DF = 4 Prob > Chisq = 0.0001

Appendix 2b: Wilcoxon rank sum test (Mann-Whitney U test) for Grain Colour
(NPAR1WAY PROCEDURE FOR TREATMENT)

Treatment	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
Control	110467.5	129690.0	4272.56958	613.708333
Actellic super	106992.5	129690.0	4272.56958	594.402778
Lantana 1%	115589.0	129690.0	4272.56958	642.161111
Lantana 2.5%	124835.0	129690.0	4272.56958	693.527778
Lantana 5.0%	136211.5	129690.0	4272.56958	756.730556
Tephrosia 1%	125760.0	129690.0	4272.56958	698.666667
Tephrosia 2.5%	151044.5	129690.0	4272.56958	839.136111
Tephrosia 5.0%	166620.0	129690.0	4272.56958	925.666667

Kruskal-Wallis Test (Chi-Square Approximation)

Chi-square = 143.07

DF = 7

Prob > Chisq = 0.0001

Appendix 3a: Wilcoxon rank sum test (Mann-Whitney U test) for Grain Odour
(NPAR1WAY PROCEDURE FOR STORAGE DURATION)

Storage duration (Days)	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
30	242026.5	207504.0	5618.16868	840.369792
60	205999.0	207504.0	5618.16868	715.274306
90	214936.5	207504.0	5618.16868	746.307292
120	199049.0	207504.0	5618.16868	691.142361
150	175509.0	207504.0	5618.16868	609.406250

Kruskal-Wallis Test (Chi-Square Approximation)

Chi-square = 59.422

DF = 4

Prob > Chisq = 0.0001

Appendix 3b: Wilcoxon rank sum test (Mann-Whitney U test) for Grain Odour
(NPAR1WAY PROCEDURE FOR TREATMENT)

Treatment	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
Control	95655.0	129690.0	4645.08661	531.416667
Actellic super	91347.5	129690.0	4645.08661	507.486111
<i>Lantana</i> 1%	112222.5	129690.0	4645.08661	623.458333
<i>Lantana</i> 2.5	130445.0	129690.0	4645.0866	724.694444
<i>Lantana</i> 5.0%	138305.0	129690.0	4645.0866	768.361111
<i>Tephrosia</i> 1%	134870.0	129690.0	4645.0866	749.277778
<i>Tephrosia</i> 2.5%	161497.5	129690.0	4645.0866	897.208333
<i>Tephrosia</i> 5.0%	173177.5	129690.0	4645.0866	962.097222

Kruskal-Wallis Test (Chi-Square Approximation)

Chi-square = 240.81 DF = 7 Prob > Chisq = 0.0001

Appendix 4: Correlation Coefficients for Grain Quality Parameters

	%MC	%GERM	TREATMENT	STOR.DURATION
MC	-	0.069	0.054	-0.923
%GERM	0.069	-	0.040	-
COLOUR	-	-	0.290	-0.167
ODOUR	-	-	0.394	-0.213

Appendix 5: Summary of farm survey guidelines

1. **General information:** farmer's name, age, farming experience, village/location; farm size, land cropped (acreage) and crops grown in order of socio-economic importance.
2. **Grain storage:** Does farmer store grains? If so, storage methods/structures used; storage period.
3. **Insect Pest Problems & Control in Storage:** Does farmer experience insect pest problems? Any current infestation? Observe grains and store for insect species present, recognize and identify; what control measures does farmer use? Does he/she use insecticides? If yes, which type(s) (Name) and rate applied? If using insecticides, are these insecticides effective against all insect pests in storage? What problems / dangers does farmer associate with use of insecticides? If not using insecticides, why?
4. **Use of plant materials in control of stored products insect pests:** Is farmer aware of plants being used against storage insect pests? If yes, does he/she use plant materials? What forms or plant parts are used? What problems does farmer associate with use of plant materials? Farmer is then asked to name by common or local names, the plants with pesticidal activity. Obtain samples of such plants, preserve and use for confirmation of scientific identity.
5. **Role of Gender in grain storage:** Who is in-charge of grain store? Decision-making process regarding domestic use and release of sale; impact of gender in community based research and extension interventions.
6. **Importance of Grain quality in Storage:** Is grain quality an important consideration in storage? If yes, what are the most important quality parameters in storage and price determination? Reasons why such parameters are important; Are changes in grain

colour, moisture content, insect damage and odour of importance in grain quality? If yes, is there any relationship between insect control methods used and these quality parameters? If a relationship exists, why (possible explanation)?

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