EVALUATION OF TERMITE RESISTANT PLANT ATTRIBUTES FOR THEIR BIOACTIVITIES AGAINST *MACROTERMES* TERMITE

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Abstract

Choice, toxicity, antifeedant tests and repellency effects of indigenous trees (*Croton macrostachyus, Juniperus procera* and *Hagenia abyssinica*) of Ethiopia on *Macrotermes* termite workers were studied under field and laboratory conditions. Fresh leaves of *J. procera* and *C. macrostachyus* fresh leaves attracted more worker termites than *H. abyssinica*. Volatiles collected from the leaves and wood stakes of the above mentioned plants, showed no significant differencee (P> 0.05) in attraction. Acetone, ethanol and water extracts of *J. procera* and *H. abyssinica* showed no toxic effect on the insects after 24 hours of treatment application. Aqueous extract of *Azadirachta indica* (neem) seed powder also showed no toxicity. However, aqueous extract of *H. abyssinica* female flower powder killed all the insects at three levels of extraction (10%, 15% & 20% w/v) and at 3ml level of application unlike 1ml & 2ml levels. Moreover, aqueous extracts of *Milletia ferruginea* seed powder extract showed 100% mortality at all the three extraction levels and 1ml, 2ml and 3ml rates of application. *C. macrostachyus* stakes immersed into acetone, chloroform and ethanol extracts of *H. abyssinca* and *J. procera* saw dust for three minutes and buried around termite mound were damaged at different levels after a month. *C. macrostaychyus* stakes were highly damaged (82.9%) compared to *J. procera* stakes which were not even scratched. This indicated that worker *Macrotermes* termites feed less on *H. abyssinica* and do not feed on *J. procera.*

1. Introduction

Termites are social insects found mainly in the tropics between 45^{o} north and 45^{o} south latitudes. These distribution areas cover over two-thirds of the landmass, involving some 100 countries (Abdurahman, 1991). Termites hold two positions from the economic point of view. They damage buildings, forestry and wide range of crops including cash crops such as maize, wheat, groundnuts, and rice; and pastures but on the other hand they are beneficial in that they assist in the conversion of dead trees and other plant products to substances that can be utilized by plants (Boror *et al.*, 1989; Dawes-Gromadzki, 2005).

Any material that incorporates cellulose can be devoured by termites, from paper to palaces (building) and fungi to fir trees. Termites also damage dam linings, fires and electrical faults in large cables. In many of their distribution areas, the termite pest species pose a serious threat to agricultural crops, forest seedlings, rangelands and wooden structures (Wood, 1986). Natural forest is also damaged by termites.

Termite damage is a major problem in tropical forestry especially where exotic tree species are used. Geer (2005) estimated the over all cost of damage to agricultural and forestry resource by termites is over $ 30 billion dollars per year worldwide (Geer, 2005). Stressed trees are generally the most susceptible to attack. Dry wood termites (Kalotermitida*e*) live and feed in dead wood, but sometimes attack living parts of mature trees. They are pests only in humid tropics, causing local, but sometimes serious damage. *Coptotermes (*Rhinotermitidae*)* causes more wide spread and serious damage to mature trees. The most serious losses (up to 100%), due predominantly to various Macrotermitinae (Termitidae) such as *Macrotermes*, *Odontotermes* and *Mictrotermes,* occur in young, exotic trees such as Eucalyptus in dry regions in Africa and India.

So far successful termite control measures in agriculture have depended largely on the use of persistent organochlorine insecticides. They are usually applied at higher rates and are toxic to the environment. Moreover, these insecticides are less readily available and severe restrictions are being placed on their use (Pearce, 1997). Chemical control of dry wood termites is not feasible; use of resistant trees is probably the only satisfactory strategy. Control of *Coptotermes* by various methods have been suggested; but only insecticide injection into nests within affected trunks, destruction of nests with explosives prior to planting followed by destruction of queens in subsequently located nests are effective and economically viable (Pearce, 1997).

Attack on seedlings, especially by Macrotermitinae in Africa and India, can be prevented by the increasingly unacceptable persistent cyclodienes used as mound poisons or as a barrier around the roots preventing attack by subterranean species. Controlled-release formulations of otherwise non-persistent insecticides are being developed, but are expensive and not widely available. Many non chemical measures have been suggested, but none has been rigorously evaluated. The need for alternative strategies is becoming acute. Biological control shows little promise.

Use of resistant tree species and development of resistant varieties offers the only long term solution, but until these are available there will be a need to continue using cyclodienes or rapidly to develop alternative control methods. Therefore, products which are environmentally friendly and potentially useful in integrated pest management such as the use of resistant varieties or their extracts and locally available plant extracts have frequently been claimed to be effective in control of termites (Cowie *et al.*, 1989).

The present research was initiated with an over all objective of studying some resistant plants (*Hagenia abyssinica* and *Juniperus procera*) attributes if they have repellent, antifeedant or toxic principles which contributed to their resistance against termite damage. These plants are locally available and reported to be resistant (Getachew Desalegn *et al.*, 2003).

Specific objectives

1. To investigate attributes of termite resistant plants that contribute to the resistance of the plants against termites damage
2. To evaluate toxicity and repellence of some plant materials of the resistant plants against worker termites
3. To undertake solvent extraction of resistant plants and evaluate for their toxicity
4. To collect volatiles from the termite resistant plants and test for their repellency

2. Literature review

2.1 Termites as insects

Termites belong to the insect order Isoptera, and are characterized by their colonial behaviour. They are often referred to as ‘white ants’, however, morphologically they are very different from the ants and other social Hymenopterans (bees and wasps). The word isoptera originated from the Greek words, in which ‘ isos’ means equal and ‘ pteron’ means wing, and refers to the two pairs of identical wings in the adults (Harris, 1957). They are polymorphic, eusocial insects, living in large communities of several hundred to several million individuals, composed of reproductives (winged) forms together with numerous apterous sterile soldiers and workers. Their numerous colonies have great influence in ecosystems. The dominance of termites in tropical ecosystem is mainly related to their ability of utilizing dead plant material rich in cellulose (the most abundant organic matter on earth) (Peakins and Josens, 1978; Wood, 1986).

These social insects are found mainly in the tropics between 45^o north and 45^o south latitude. These distribution areas cover two-thirds of the landmass, involving some 100 countries (Abdurahman, 1991). Termites hold two positions from the economic point of view. They can be very destructive, since they feed upon and often destroy various structures or materials that man utilizes i.e. wooden portions of buildings, furniture, books, utility poles, fence posts, many fabrics, and the like. Termites damage buildings, forestry and a wide range of crops including cash crops such as rubber, cocoa, coconut, oil plum, sugar cane, and cotton; and food crops such as maize, wheat, groundnuts, and rice; and pastures. Any material that incorporates cellulose can be devoured by termites, from paper to palaces and fungi to fir trees. Termites, in their quest for food, also destroy other material that stands in the way; this has led to breaks in dam linings, fires and electrical faults in large cables (SU and Scheffrahn, 1998).

However, on the other hand they are beneficial in that they assist in the conversion of dead trees and other plant products to substances that can be utilized by plants. Moreover, termites are important part of the food chain for many animals including man. They supply materials for many food chains, soil engineering (translocating and altering soils physically and chemically and maintaining soil fertility) (Lee and wood, 1971; Wood, 1988), providing a possible input of nitrogen through symbiont fixation, methane gas release and carbon flux (Collins, 1984).

2.2 Termite classification

There are seven extant families of termites (Pearce, 1997) in the order Isoptera: Mastotermitidae, Kalotermitidae, Hodotermitidae, Termopsidae, Serritermitidae, Rhinotermitidae and Termitidae. These families are divided in to 14 sub families, 270 genera and over 2000 species (Kambhampati et al., 1996). Based on the composition of the symbiont microbiota in the gut, termites are divided into two groups , ‘ lower termites’ and ‘ higher termites’, where lower termites house flagellate protozoans and bacteria. Higher termites house a variety of prokaryotic microbes, but no flagellates. Some Termitinae also house cellulolytic amoebae. The different termite families are described as follows.

1. Mastotermitidae

They are represented by *Mastotermes darwiniensis* Forggatt, a single living species. It is confined to Australia and New Guinea. These species usually nest in the trees and stumps, but can be very destructive to buildings as well as trees and crops including sugar cane (Lamb, 1974).

2. Kalotermitidae

Species in this family are often referred to as the dry wood termites (from their nesting habit, in sound wood) and believed to be a sister group to Rhinotermitidae and Termitidae (Kambhampati *et al*., 1996). This is the largest family of lower termites, with 25 genera and 350 species (Krishna, 1970; Wood, 1986). These termites occur in small numbers in rain forests, mainly confined to dead limbs and trunks in the forest canopy (Collins, 1988). Many species in this family are serious pests of forest products. Soldiers normally have robust, phragmotic heads, which are of particular value in blocking and defending nest galleries (Collins, 1988).

3. Hodotermitidae

The Hodotermitidae are called harvester termites (Harris, 1971). They are small groups of subterranean species. Found in drier parts of Africa and the Middle East. They forage the above ground part of grasses which are cut and stored in underground nests (Lamb, 1974).

4. Rhinotermitidae

Rhinotermids are the most important family of lower termites and are often referred to as damp wood termites. They are found in standing or fallen trunks and limbs, and can cause severe damage to timber and living trees (Collins, 1988).

5. Termopsidae

These are damp-wood termites and usually found in standing trees or fallen logs. Very few of them are pests (Lamb, 1974). The Termopsidae, a small family of termites living in damp, rotten wood, partially or wholly buried underground, have a scattered, essentially temperate distribution and are rarely pests (Logan *et al*., 1990).

6. Termitidae

The family Termitidae contains three-quarters of all known species, comprising four subfamilies: Macrotermitinae, Apicotermitinae, Termitinae, and Nasutitermitinae (Wood, 1986; Collins, 1988). Members of this family are often termed as the higher termites as they possess more advanced features (Pearce, 1997). They are mainly wood or grass eaters with subterranean habits and many are mound builders. One of the most important sub- families is the Macrotermitinae. Genera in this sub- family are known to cultivate species of the symbiotic basidiomycete fungus *Termitomyes* on faecal combs within their nests. These termites have high weight-specific consumption rates and a correspondingly greater impact on decomposition processes than other termites. Macrotermitinae are known to originate from Africa.

7. Serritermitidae

These are subterranean termites which were previously categorised under Rhinotermitidae

The rare monotypic subfamily Serritermitinae from Brazil has recently been elevated to full family status, serritermitidae (Emerson and Krishna, 1975)

2.3 Termite biology and behavioural ecology

2.3.1 Termite colony structure and life cycle

Termites live in large communities and the colony members are of four castes: the reproductive (king and queen), soldiers and workers. In addition, colonies have a large number of young immature forms in all stages and of all castes (Collins, 1984). Each caste varies morphologically and behaviourally but they have to live cooperatively or else the colony will die (Collins, 1984). The number of individuals and ratios of each caste in a colony is very difficult to determine and it varies between species and also depends on the age and size of the colony (Bignell and Eggleton, 1998). Large colonies may include a number of supplementary reproductives, producing eggs to augment or replace the founding queen (Edwards and Mill, 1986; Bignell and Eggleton, 1998).

The parent termites, the king and the queen are the functional reproductives. The queen's major role is to lay eggs. She develops an enlarged abdomen containing ovarioles and associated tissues, a condition known as being physogastric (Collins, 1984). The queen is also involved in phermonal regulation of the production of each caste in a colony (Noirot and Noirot-Timothee, 1969).

Soldiers and workers are wingless and can be either sterile male or female. Soldiers usually represent one-tenth of the population at most (Harris, 1957). There are also termite genera that lack of this caste, such as *Anoplotermes* and *Protohamitermes*. Termites are the only social insects with a true soldier caste whose major role is only to defend the colony (Bignell and Eggleton, 1998). For this reason, morphologically they are bigger in size and have defensive adaptations such as enlarged mandibles or stopper-like heads. In the subfamily Nasutitermitinae, the mandibles are reduced and non-functional. Instead the soldiers have a nasus, an elongated projection of the fontanelle and their way of defence is by squirting irritating chemical substances through it (Collins, 1984). Besides having mandibles and a sclerotized head, soldiers of some genera such as *Coptotermes* have a frontal gland that discharges a defensive secretion through a frontal pore (Richards and Davies, 1978). This secretion can be toxic or repellent to intruders, such as ants, or tacky and entangle their legs and antennae.

The worker caste is the most numerous and plays the major role in the survival of the colony. They collect food, process the digesta, feed other castes and construct the mound or nest (Harris, 1957). All living termites, except the Kalotermitidae, are known to have a true worker caste. In Kalotermitidae, there is no distinct worker caste and the work of the colony is done by immature adults, whose development is stopped temporarily according to the needs of the colony (Harris, 1957).

Winged reproductives or alates of both sexes are produced in large number in a mature colony. These alates swarm out from mature nests at particular times of the year (often during or just before rains) (Bignell and Eggleton, 1998). They make short, often rather feeble, dispersal flights, and then pair-up the ground after the wings have been shed (dealation) (Bignell and Eggleton, 1998). The paired termites will then select a new nesting site and once they are established, mating takes place. The first batch of eggs is produced by the female within a few days.

Termites are hemimetabolus in their life cycle. The hatched young are translucent white and feeble at first, but very active from the moment they hatch (Edwards and Mill, 1986). These larvae are fed from nutrient-rich salivary secretions produced by the parents. They normally undergo a number of moults until they achieve the mature form as sterile workers or soldiers, depending to the needs of the colony (Harris, 1957). These developments are determined by extrinsic factors such as pheromones and hormones (Krishna, 1970). Usually, at the beginning of a colony foundation the entire larva become workers and after sometime, an occasional larvae is found with large head and jaws of quite a different shape, and this grows into a soldier (Harris, 1957). The colony grows slowly for many years, accompanied by a continuous increase in the number of individuals, enlargement of the nest and much building activity (Bignell and Eggleton, 1998). Once the colony is well organised, larvae appear with wing buds, which later will become winged termites and the full cycle of development is complete (Harris, 1957).

2.3.2 Communication in termite colony

To maintain the social structure of the colony, termite communication predominantly relies on the use of sophisticated chemical (pheromone) communication system (Higashi ***et al.,*** 2000). There is wide range of categories of pheromones such as trail, alarm, aggregation, recruitment, mating, etc. The signals induce and modulate a wide range of individual's and collective behavioural responses such as territorial behaviour, attendance of the reproductives and brood and foraging for food or searching for nesting sites. Each colony develops its own characteristics odour. An intruder is instantly recognized and an alarm pheromone is secreted that triggers the soldiers to attack. If a worker finds a new source of food, it lays a chemical trail for others to follow. The proportion of termites in each caste within the colony is also regulated chemically. Nymphs or immatures can develop into workers, soldiers or reproductive adults depending on colony needs (Michael, 2000).

Sound is another means of communication. Soldiers and workers may bang their heads against the tunnels creating vibrations perceived by others in the colony and serving to mobilize the colony to defend it self. Mutual exchange of foods enhances recognition of colony members (Kamble, 2002).

2.3.3 Feeding and feeding groups of termites

Termite colony success depends on the adaptability to eat different foods. Although termites are soft-bodied insects, their hard, saw-toothed jaws work like shears and are able to bite off extremely small fragments of wood, a piece at a time. The characteristic food of termites, considered as a whole, is wood. Almost certainly termites are attracted to wood by its odor, which they are able to sense at some distance even through the soil. Many species, however, seldom if ever eat wood but concentrate upon grass and general plant debris. Almost all species of termites are detritivorous (Harris, 1957). They consume wide range of freshly dead or decaying plant material including dry grass, leaf litter, decaying wood, dung and humus. Living plant tissues, including lichen and mosses are taken by a few species. Although cellulose is the major part of food in wood eating forms termites can not digest it. This is because the glands in their digestive tract do not secret cellulose digesting enzymes. But digestion is assisted by symbiont organisms in their tract.

Another feeding group that may be common and important in many tropical forests is the soil-feeding termites. Nonetheless, termite species can be categorized into six broad trophic categories according to their food, foraging galleries or columns, and biology (Martius, 1994; De Souza and Brown, 1994; Eggleton *et al*., 1995; 1996; 1997). The feeding categories are described below.

1. Wood- feeders (Xylophagous): are termites that feed on live wood and/or sound to partially decayed dead wood excluding extremely decayed and friable wood including branches still attached to trees. They may live in their feeding galleries which in some cases become colony centres (Eggleton *et al*., 1996, Wardell, 1987). The condition of wood taken is very important. This may include living trees (*Coptotermes*, *Schedorhinotermes* and *Microcerotermes dubius*), sound dead wood (Kalotermitidae), or fungus-attacked wood (Nasutitermitinae, some Termitinae and Macrotermitinae) (Wood, 1976; Collins, 1984). Most of these termites are arboreal (attached to trees), subterranean or epigeal nesters (Eggleton *et al*., 1997).

2. Wood/soil interface-feeders: termites that feed on extremely decayed wood that has lost its structure and has become friable and soil-like wood, the soil under logs or soil plastered to logs, or soil mixed with leaf litter in slit-root complexes (Eggleton *et al*., 1996; 1997). They are found in the Termitinae, Apicotermitinae and Nasutitermitinae sub families. Most of them nest within dead logs, build epigeal nest or form colony centres in the soil (Eggleton *et al*., 1996; 1997).

3. Soil-feeders (Geophagous): termites that feed on humus and upper mineral soil, with some degree of selection of silt and clay fractions. The vast majority of species in this group ingest topsoil rich in organic matter. They normally are distributed in the soil profile, in the organic litter layer (leaves and twigs) and in epigeal mounds (Eggleton *et al*., 1995; 1997). This form is found in many Termitinae (the *Capritermes*-group and *Labritermes*), several Nasutitermitinae (*Subullitermes*- group), and most Apicotermitinae (the *Anoplotermes*-group) (Wood, 1976; Eggleton *et al*., 1997). Soil feeders are very common and abundant in many tropical rain forests (Wood, 1976). In Southeast Asian regions, soil feeders are dominated by the Termitinae with small number of Nsutitermitinae and Apicotermitinae (Abe, 1987).

4. Grass-harvesters: termites that feed on living and dead grass. They will also take dung and may sometimes scavenge vertebrate corpses. They are mainly of the family Hodotermitidae, found only in savannah and deserts (Krishna, 1970).

5. Litter-feeders (Humivorous): termites that feed predominantly on leaf-litter and small wood items. Food sources are often taken back and stored temporarily in the nest. This group includes some subterranean and other mound-building Macrotermitinae (with fungal association), as well as certain Nasutitermitinae that forage on the surface of the ground or litter layers (Eggleton *et al.*, 1997; Collins, 1984). Genera such as *Laccessititermes* and *Longiditermes* are also known as arboreal foragers.

6. Micro-epiphyte- fedeeders: Termites of this group forage on mosses, algae, lichens and fungi on tree barks (Collins, 1988).

2.4 Termites as agricultural and structural pests

Termites can have significant impact on plantation and urban forestry as well as on agricultural tree crops and buildings (Cowie *et al.,* 1989). Many other insect pest species cause damage to various parts of trees, but often do not cause mortality. Some termite species, however, are able to kill apparently healthy trees and therefore, have the potential to cause much greater losses. Even where termites do not cause damage to the bole, they attack the wood by consuming the heartwood and, thereby, hollowing the trunk and reducing the value of the tree as a source of timber. The extent to which termites are problems to trees and the nature of loss they cause are very much related to the geographic region concerned (Logan *et al*., 1990).

Termites feed on wood and serve an important function in nature by converting dead trees into organic matter. Unfortunately, the wood in building is equally appetizing to termites and they cause serious damage to residential and commercial buildings (Wood and Sands, 1977; Wood *et al*., 1980). In many of their distribution areas, termite pests pose serious threat to agricultural crops, forest seedlings, range lands and wooden structures (Wood, 1986). They often infest buildings and damage lumber, wood panel, flooring, sheetrock, wall paper, plastics, paper products, and fabric made of plant fibres. The most serious damage is the loss of structural strength. Other costly losses include attacks on flooring, carpeting, art work, books, clothing, furniture and valuable papers. The annual economic cost of structural damage to buildings from termites in urban areas is about $ 15-20 billion dollars world wide. When this is combined with the cost of damage to agricultural and forestry resources, the over all cost to the society is over $ 30 billion dollars per year (Geer, 2005).

Within the wide limits of their geographical distribution, termites will destroy any unprotected timber used in construction work or as fittings, unless it has been rendered unpalatable or is naturally resistant termites (Harris, 1971). Termites can tunnel through mud and known to tunnel through mortar between bricks and thatching in African houses are expected to last for only 5-6 years (Pearce, 1997).

Termites are very serious pests in several parts of Ethiopia, particularly in the western parts of the country. They cause considerable damage on agricultural crops, range lands, forestry seedlings and wooden structures such as rural houses, stores, fences and bridges crossing streams (Abdurahman, 2000). Gauchan *et al.* (1998) in their study of termite situation in west Wollega reported that the houses made of wood can only last for about 2-3 years. About 61 species of termites belonging to 25 genera and four families have been recorded in the country. Only few of these are important pests of agricultural crops, forestry seedlings, rangelands and wooden structures. The rest are harmless, feeding either on dead plant materials, dung or soil organic matter. The major termite species that cause damage on wooden structures belong to the fungus-growing subfamilies and to the genera *Macrotermes*, *Odontotermes, Pseudacanthotermes*, *Microtermes* and *Ancisrotermes*. The fungus growing forms depend on the fungus cultivated within the nest for digestion of their food (Abdurahman, 2000).

Abdurahman (1990) reported that in western Ethiopia thatched roof huts are destroyed in less than five years and corrugated iron roof houses in less than eight years. Many wooden structures in the same area require maintenance every year. This undesirable consequence of repeated rebuilding of wood straw thatch houses leads to excessive clearing of native woodland and forest. The vicious cycle of cause and effect of the termite problem negatively affects the socio-economic situation of the population of the area. As a result trees are cut frequently to replace the structures destroyed by termites which would in turn lead to deforestation, erosion and environmental degradation.

Temperature and humidity are the main factors affecting termites in buildings. The equilibrium moisture content of wood is affected by temperature and water vapour in the air. Pearce (1997) produced predictions for the dry-wood termites' hazard to tropical and sub-tropical buildings in coastal regions of Africa. He found that sea fogs and early morning dews in semi-desert coastal regions provide water for building soil runways. As the temperature is often low at this time, termites can even forage on the outside of the runways.

The general picture of termite damage to buildings is straight forward; the worker termites remove all palatable wood excepting only the outer layers which are left to provide the shelter and freedom from disturbance that are necessary to termites. Dry-wood termites eat out galleries in the timber and these provide accommodation for the king and the queen, the soldiers, and the various young stages of the community. The community is found in the vicinity of maximum feeding activity at any time. In the course of time, their galleries coalesce to form large cavities (Harris, 1971). Subterranean termites fix nests from which the workers move out in search of food, and to which they return with their spoil. Distances of one hundred yards (30 meters) may be travelled by the small workers with their loads of wood. Communities of these termites number many thousands of individuals, and those which make the distinctive large mounds in the tropics are estimated to run into millions. For this reason, the rapidity and scale of their attack on new buildings is much more spectacular than that of dry wood termites whose colonies consist of only a few hundred individuals (Harris, 1971).

Termites can cause direct physical damage often affecting the structural support of crop plants. For example, fungus growing termites, *Microtermes, Ancistrotermes, Macrotermes, Allodontermes* and *Pseudoacanthotermes* species are the predominant pests of maize in Southern Africa (Sands, 1977; Uys, 2002). They can also cause indirect damages by interfering with the food crops and water supply, causing the eventual death of part, of all, of the plant (Pearce, 1997). Hickin (1971) stated that termites by no means confine their attentions to dead plant tissues such as wood. In many parts of the world termite species are serious pests of growing crops including living trees. Guachan *et al.* (1998) described that termites cause widespread damages to a great variety of crops in tropical Africa. The damages can occur from the seedlings to harvest and usually occur every year; as termites form almost stable population and foraging by various combinations of several species occur throughout the year. The author reported that termites lowered the yield of maize, sorghum, teff, millet and beans in Manasibu district (west Wollega, Ethiopia).

Termites primarily feed on dead grasses' litter and much of the small amounts of dry grass biomass to such low level that grass consumption by termites result in the denudation of the area. Massive termite damage was observed in grazing land in Manasibu and Jarso districts that led to degradation of the pasture land (Wood, 1986). Degradation of pasture land is the consequence of lack of fodder and erosion. Lack of fodder leads to, weak animals that are: susceptible to diseases, give low milk and meat yield, and are weak in traction. Lack of fodder also leads to overgrazing. Animals trampling on overgrazed land again results in surface soil compaction. Since infiltration in compact soil is very low it would lead to high runoff, and subsequent erosion.

Wood (1986) observed *Macrotermes subhyalinus, Pseudacanthotermes mititaris* and several species of *Odontotermes* feeding on dry grass in west Wollega. He concluded that overgrazing was an important and possibly the major factor for termites' damage to the range land in west Wollega, Ethiopia. Termites damage to the growing trees fall into two distinct divisions- damage to the seedlings in the nursery and young trees in the field, and damage to the mature trees in the natural habitat, in plantations and as specimens in towns or botanical gardens. Resistance to termites attack is apparent as a character of the wood in some species of trees, but while still young, the trees have not had time to develop this trait and are just as liable to damage as any other tree (Harris, 1971).

Plantations in wet places are not usually subject to much termite damage, but in dry countries it is common to find that termites are the main obstacles to afforestation (Harris, 1971). Throughout tropical Africa (south of the Sahara), damage is caused mainly by ground-dwelling termites whose nests contain fungus gardens, mound-builders and subterranean-nesting species together. Wood (1986) reported that: 1) the problem of damage to forestry trees in Ethiopia is confined to exotic trees, particularly Eucalyptus planted in the low areas (i.e. altitudes less than 2000m), 2) the damage is mainly caused by *Macrotermes*, *Odontotermes*, *Pseudoacanthotermes, Ancistrotermes* and *Microtermes* which attack either nursery seedlings or seedling trees of up to 3 years after transplanting, 3) loses are sever and in some areas total.

Similarly, termites often burrow through non-cellulose materials that lie in their path. Pearce (1997) pointed that plastics are often eaten by termites and this causes leakage in plastic pipes and power cuts in cables. Hickin (1971) explained the reasons to remove obstacles from the path leading towards cellulose otherwise it is certain that plastic and allied materials do not serve in any way as a source of nutrition for termites.

In general, the following general principles can be derived from published works (Wood, 1996). The likelihood of damage is greater in exotic rather than indigenous crops, in rain-fed rather than irrigated crops and in crops under stress rather than healthy and vigorous crops. Damage occurs from seedling to maturity in annual and perennial crops and forestry trees. Losses occur throughout the tropics, but particularly in Africa and Indo-Malasia where the major pest species belong to the *Macrotermitinae* (fungus growing termites), particularly *Microtermes, Macrotermes* and *Odontotermes.* These species have semi-permanent nest-systems throughout farmers' field and are a potential threat every year. Damage varies from superficial damage to dead parts of the plant (e.g. outer bark) to penetration of root systems, stems and death of the plant. Yield losses of up to 20% are common and localised losses can exceed 50%. Crops suffering greatest losses are wheat, maize, sugar cane, groundnuts, cotton some vegetables and locally in west Africa and South America, Cassava and Yam. Some perennial crops such as cocoa, coconut and rubber suffer low-level, localised damage but throughout the tropics tea are infested by a wide range of Kalotermitidae, particularly *Kalotermes* in South America and Asia. It should be emphasised that the majority of termite species in farmers' fields do not damage crops (Cowie *et al.*, 1989).

Exotic forestry trees have been increasingly incorporated into agricultural enterprises and there are wide spread losses of Eucalyptus in South America, Africa and India and Pinus in South America and northern Australia. The termites causing greatest losses are Macrotermitinae in Africa and Indo-Malaysia, *Mastotermes* in Australia and *Comitermes* and *Procomitermes* in South America.

2.5 Termite control and management

In the past organochlorine insecticides were effective for the control of termites and other insects. These were often applied at higher rates than required to control and their break down products were not environmentally friendly. One application around a building could prevent termite attack over 30 years (Cowie *et al*., 1989). However, this persistence created potential environmental problems, the toxic chemicals entering food chains and finally humans. Current control options include placement of chemical and physical barriers, wood treatments and population control using baits.

2.5.1 Chemical control

2.5.1.1 Treatments of soil and seedlings with new generation insecticides

Soil treatment and the treatment of seedlings before transplanting have been used as classical methods of control and prevention of subterranean, arboreal and dry wood termites for many regions of the world for many years. These methods of control have a high environmental impact because of the large amounts of insecticides that have to be applied in open areas that are exposed to leaching. In recent years, new generation insecticides that are active at very low doses have become available. These chemicals generally also have a low toxicity to other forms (Su and Schefrahn, 1998).

When chemical treatment is deemed necessary, such chemicals provide an environmentally preferable alternative to traditional chemicals. Examples of these new generation insecticides are imidacloprid and fipronil. However, before adopting a chemical means of control, alternative, traditional methods of control should be considered.

2.5.1.2 Treatment of timber products by insecticides

Termite-susceptible wood can be turned into a termite resistant material by treating it with chemical toxicant (wood preservatives) that inhibits feeding by termites. Wood treatment is most successful in preventing termite infestation when used in conjunction with other termite management strategies, especially proper site preparation (removing cellulose debris and earth-to-wood contacts) and termite resistant building design. Chemicals such as chromated copper arsenate (CCA), ammoniacal copper quat compound (ACQ), and Disoddium octoborate tetrahydrate (DOT) are used as wood preservatives. Out of, these borates are gaining popularity because of their low mammalian toxicity, water solubility and ease of application. Wood preservatives are most toxic to termites when ingested. In the case of dry wood termites, treated timber may also discourage new kings and queens (alates) from establishing colonies (Su and Schefrahn, 1998).

2.5.1.3 Space fumigation

Space fumigation involves the introduction of a toxic gas inside a structure sealed inside a tarpaulin or into or around and isolated area or object infested with subterranean termite, arboreal and dry wood termites. These gases must be used in extreme care because they are extremely toxic to humans, other animals and even for plants. The most common fumigant is methyl bromide.1 (Pearce, 1997).

2.5.1.4 Baiting systems

Baiting has the advantage of not contaminating the soil with chemicals. Baits can be used where insecticide treatments are avoided for fear of contamination or where owners can not afford extensive treatment. In this method, non-toxic baits can be placed near colonies for termites to locate and forage and then to replace the baits with toxic ones (Pearce, 1997). Baits must be more attractive than the surrounding food source. For some species of termites, sugar, molasses and honey can be used to increase bait consumption. Other baits may be made attractive by treating with moulds, allowing partial decay, adding amino acids, nitrogen sources and even attractive pheromones held in vegetable oil. Water can be added to the soil since the termites are attracted to wet soil (Logan *et al*., 1990).

Baiting systems using active ingredients such as moult inhibitors have become widely used in the control of termites affecting housing (French, 1991). They are effective in eliminating colonies when used consistently over an extended period of time. However, their application against termite pests in agriculture and plantation or urban forestry has not yet been widely developed or specifically tailored for the purpose. They would almost certainly prove to be effective against subterranean pest species from among the lower termites, such as *Retuculitermes* and *Coptotermes (*Ripa and Smith, 2000). Systems specifically designed for the treatment of termites on trees will probably become available in the future, and will be a low-environmental-risk than chemical method of control. Bait systems have already been tested and have shown promising results against *Reticulitermes santonensis* in Paris, where city officials are also cooperating with the Centre National de la Recherche Scientifique (CNRS) in a pilot study to investigate colony and population structure using molecular and chemical markers (French, 1991). Hopefully this knowledge will lead to a better understanding of the pest’s invasion strategies and allow development of effective targeted control measures for Paris and other big cities (Su and Schefrahn, 1998).

2.5.2 Physical and cultural control methods

Physical or mechanical method involves physical barriers such as sand or gravel aggregates, metal mesh or sheeting. Physical and cultural control involves mound removal, queen removal, flooding, suffocating, and using repellent or toxic materials and others.

2.5.2.1 Mound (nest) destruction and queen removal

Guachan *et al*. (1998) described that digging and removal of queen exposes the other castes to direct sunshine, birds and other predators and prevent their access to crops. Nests of pest termite species that are easily visible be removed manually, thus reducing their population. This has been used in South America to control arboreal nesting species, particularly *Nasutitermes*, which may occur in high densities on fruit trees. It is also applicable to *Microtermes dubius* in Southeast Asia, which may occur very occasionally in forest plantations. Likewise, dry wood termites, such as *Neotermes tectonae* on teak, can also be controlled by removal of affected branches (Zhang *et al.*, 2000).

The queen must be dug out of nests to ensure that the colony cannot recover. However, one must be certain that there is not more than one pair of reproductives present. So killing the queen may not be sufficient to destroy the colony (Pearce, 1997). But the digging out of queen is often ineffective, laborious and sometimes useless as termites are capable of rearing supplementary reproductives. Mechanical removal of termite mounds by means of bulldozers does not always lead to complete success unless the remnants of the colonies are subsequently sprayed or dusted with chlorofyrifos, carbosulfan (marshcl), cymbush and gaucho (EECMY-WS, 1997).

2.5.2.2 Flooding and suffocating

It is also possible to reduce termite population by facilitating flooding to occur on the mounds of termites. This may lead to death of members of the colony by suffocation (Pearce, 1997). Excavating the top parts of the mounds and burning straw can suffocate and kill the colony (Guachan *et al*., 1998).

2.5.2.3 Using repellent or toxic materials

In some parts of the world termites can traditionally be controlled by farmers by burying dead animals in the middle of infested fields or planting different species of grasses at several spots in the farm to repel termites. The smell of the dead animals in the field or in the mound will cause termites to avoid their habitat or the dead animals may attract some predatory insects like ants and other termite eating insects (Pearce, 1997).

Some plant parts and plant extracts having either toxic or repellent or anti-feedant properties to termites can provide a simple means of control that can be implemented by farmers and foresters (Pearce, 1997). Many of the termite controlling parts can be extracted and used as natural insecticides against termites by grinding and placing the appropriate part of the plant in boiling water, stirring and leaving to soak. The mixture is then sprayed or painted onto the required area (Gauchan *et al.*, 1998). Various trees and leaves have been shown to be effective against termites. For example, Deoder tree bark and leaves, chiv trees, castor plants (oil) and neem tree leaves have been used in India (Pearce, 1997).

2.5.2.4 Selecting low risk sites and use of suitable species

Termite pest species have their own native habitats in which they are most abundant. For example, in Southeast Asia, the tree-killing termite species, *Coptotermes curvignathus,* is abundant in peat sites is often reflective of the history of the site. Plantations established in areas once occupied by peat swamps in Southeast Asia often have a relatively high incidence of attack by *Coptotermes curvignathus* (Su and Scheffran, 1998). One way of avoiding the problem of termites in forest plantations is not to plant susceptible species of trees on sites known to be high risk.

Tree species-site matching is an important aspect of termite management. Trees grown in regions to which they are not suited may be more stressed and, hence, more prone to attack by termites. *Acacia mangium* for example, which is widely planted in parts of Southeast Asia, does better in a climate with a marked season, such as a wet and dry season. In other areas, it tends to develop a bushy growth form, which requires more pruning and predisposes the trees to attack as a result mechanical injury to the trees. Terrain, climate tree species chosen, is bearing in mind its native habitat and its degree of tolerance outside the conditions in which it naturally grows (Cowie *et al.,* 1989).

Some tree species are naturally resistant to certain termites. However, a tree species that is resistant to one termite species may not be resistant to another. Thus, it is important to identify the termite pest species that is a problem in the geographic area concerned. In Southeast Asia, *Coptotermes curviganthus* is the primary species that kills trees in plantations, and it has a wide host range (Mitchell, 1989).

2.5.2.5 Reduction of mechanical damage

Reducing mechanical damage to trees can minimize damage by termites. Such mechanical damage can occur from pruning wounds, or during weed control or thinning operations, especially where heavy machinery is used. Wounds and scars on tree trunks or branches serve as entry points for termites into the heartwood of the tree, sometimes after fungal infections of the wounds. Termite species that kill trees, such as *Coptotermes curvignathus* in Southeast Asia, may also preferentially attack trees with wounds, and may locate trees by the exudates that flow from such wounds or dissolve in rainwater. All wounds should be treated with a wound dressing, but minimising any form of mechanical damage to trees is advisable, as wound dressings do not adequately prevent fungal and termite infestations. Besides mechanical injury, it is thought that stress also predisposes trees to attack, therefore, maintaining plant vigour is an important means of minimising damage by termites (Logan ***et al.***, 1990).

2.5.2.6 Increasing biodiversity

Taking measures to increase biodiversity may increase competition from non-pest termite species and, thereby, reduce populations of pest termite species. For example, in Southeast Asia established colonies of the pest termite species, *Coptotermes curvignathus*, have a competitive edge over other termite species in plantations of susceptible tree species because of its ability to kill trees (Lenz, 2000). However, encouraging a diversity of termite species in forest floor wood litter may reduce nesting opportunities for new founding colonies and, in the long term, reduce the population of the pest termite species. In addition to providing increased competition against termite pest species, increasing biodiversity can also increase natural enemies of termites, such as ants. Measures that can be taken to encourage biodiversity in forest plantations include inter-planting, retaining a litter layer, retaining ground cover, encouraging rapid canopy closure and reducing pesticide usage.

2.5.2.7 Improving soil quality

Since termite damage to crops is frequently associated with low soil fertility (Wardell, 1987), improving soil by increasing the quality and quantity of organic matter can reduce the effect of pests on crops (Sileshi and Mafongoya, 2003). For example, Sileshi *et al.* (2005) reported that termite damage in unfertilised maize was not significantly different from fully fertilized maize. The lack of a significant difference between fully fertilized and unfertilised maize, and the absence of significant correlation between termite damage and preseason soil inorganic nitrogen indicated that termite damage to maize is not influenced by soil nitrogen. But, the decrease in termite damage to the maize grown in legume fallows compared with monoculture maize could be due to an improvement in soil organic matter and soil water retention. Fungus growing termites feed on crop residues, mulches and soil organic matter. However, when this type of food is not available, they will eat live plants, including maize and their damage is known to be greater in soils with low organic matter content. This is because such soils do not contain enough food to support termites, and they resort to feeding on living plant material.

2.5.3 Biological control

In this method of termite control, natural enemies such as predators, parasites and pathogens are used. Termites can be preyed by a wide range of predators like birds, lizards, frogs, spiders, bats, mammals (e.g. bats) and ants. These natural enemies can destroy many swarming males and females when they leave the nests or during the flight. Humans, in some parts of the world may also feed on the winged forms. Ants are also the natural enemies of termites, which have the grate potential as biological control agents (Su and Scheffran, 1998). Biological control of termites has largely focused on the use of fungi (e.g. *Metarhizium*) and nematodes though some viruses and bacteria are also used for their control (Abdurahman, 1991). Nematodes are effective bio-control agents for termites living inside mounds or branches. They can enter their insect hosts both via natural openings (mouth, anus, and spiracles) and by active penetration of the cuticle and inter-segmental membrane. They have pathogenic action, which is at least partly due to infection of the host by bacteria with nematode (Gauchan *et al*., 1998).

It is not easily achieved in the field because of the tendency of termite colonies to cut off and avoid infected areas as soon as disease sets in. Nevertheless, such biological control agents can be a substitute for chemicals when they are used to control local infestations. The use of these agents against termites is an area of active research and, in the future, methods may be developed for colony elimination using biological control agents. Trails using the entomopathogenic nematode, *Heterorhabditis* species, have given encouraging results for the control of *Mastotermes darwinensis* in Australia (Forschler, 2000). Also, initial studies indicate that *Neotermes* colonies attacking mahogany in the south Pacific Islands can be eliminated by both the fungus *Metarhizium anisopleae* and the nematode *Heterorhabditis* species. However, because mahogany forest, re-colonisation of trees over a plantation life time of about 30 years is possible. Entomopathogenic fungi can invade their host through the integument and cause death by depletion of host metabolites, production of toxic products or by-products, destruction of vital tissues or a combination of all the three (Rath, 2000). The causal agent of green muscardine diseases, *Metarhizium anisopliae* is a naturally occurring pathogen which infects over 200 insect species, including termites (Hanel, 1982). It has been widely investigated for the control of a number of economically important insects, such as sugar cane beetles, carpenter ants and termites (Hanel and Watson, 1983; Zoberi, 1995). The infective propagules are the fungal spores, or conidia. Upon landing on the insect cuticle, they penetrate and grow into the termite, killing it within 12-48 hours, depending on the dose applied. It is a promising agent for the biological control of termites because the conidia are viable in the soil they naturally adhere to insect cuticle, and are easily transferred to other termites through ordinary, interactive colony behavior (Hanel and Watson, 1983). This involves exploiting termites' social behavior. That is, as termites are highly social insects and engage in a variety of activities that necessitate frequent, direct physical contact with other colony members. Trophallaxis (the exchange of regurgitated food), proctodeal trophallaxis (the consumption of anal excretions) and grooming are regular, necessary colony functions. It is through grooming behavior that the infective propagules of *M. anisopliae* can be transferred from one individual to another (Maniania, *et al*., 2002; Haimanot Abebe, 2002).

To create a high level of infection (an epizootic), a percentage of the colony would have to be removed from the site of infestation, dusted with conidia, and released back into the colony. As these conidia-carrying termites, or vectors (since they are the mobile carriers of the fungus), encounter other colony members, they will be groomed and the conidia is transferred. However, some early results indicated that termites act aggressively towards vectors carrying high loads of conidia. When a vector is detected within the colony, the termites begin to move rapidly back and forth in characteristic alarm behavior. This behavior is often exhibited when termites are in a state of distress. While alarm behavior is being displayed, the vectors may be attacked, dismembered, or buried alive. This aggressive response by the colony may effectively prevent the dissemination and transmission of the conidia (Zoberi, 1995).

The grooming and other social interactions between termites are seen to have the potential to have spread the fungus through colony, allowing for colony control by treatment or remote feeding sites. However, factors such as avoidance of the fungus conidia by the termites, the removal and burial of fungus-killed termites, together with hormonal resistance may limit the spread of disease in the colony (Rath, 2000).

2.5.4 Resistant plant varieties and genetic engineering

The development of resistant tree varieties against termites through breeding programmes and genetic engineering is still at an early, exploratory stage, and is hampered by the long rotation period of forest plantation trees in comparison to annual agricultural crops. However, it may become an important means of protection against termites in the future. Genetic engineering for resistance usually involves the introduction of genes from insect pathogens, such as bacteria, into the tree, which then expresses pathogenic characteristics towards insects. The environmental implications of such genetically modified organisms in agriculture and forestry are controversial and still largely unstudied. Some studies indicate that they may carry a set of environmental problems of their own (UNEP/FAO/Global IPM Facility Expert Group, 2000).

3. Materials and Methods

3.1. Description of the Study area

The investigation was carried out at Ziway, 163 Km south of Addis Ababa. It is located in the rift valley, $07^0 56^’$ North and $38^0 43′$ East. It is found in the hot to warm sub-humid low land agro-ecological zone and has an altitude of 1725 m above sea level, total annual rainfall of 642 mm and mean annual minimum and maximum temperature of 12.4 and 26.2 ^{0} C, respectively (Getachew Desalegn *et al*., 2003). The study site was dominated by subterranean and mound building termite species, which belonged to genus *Macrotermes* (Gtachew Desalegn *et al*., 2003). All the laboratory works were done in Insect Science Laboratory at the Department of Biology, Addis Ababa University.

3.2 Termite collection for laboratory experiments

Test insects (*Macrotermes* termites) were collected from the study area by digging active mounds with spade and putting soil and combs infested with termite worker into the plastic cans. Saw dust of susceptible plants (*Croton macrostachyus* & *Pinus radiata*) was added to the can as feed for the termites. Then the top part was covered with a mesh cloth so that air can easily enter into the can and the moisture would not increase. The insects were then brought to the insectory and kept at a temperature of 27^0C and relative humidity of 60-70%. Later, they were used for different experiments.

3.3. Plant material collection and preparation for the study

Wood materials of *H. abyssinica*, *C. macrotstachyus*, *J. procera* and *Pinus radiata* were bought from Ziway town and stakes having a volume of 160 ml were prepared for field and laboratory tests. Saw dust of the different plant woods were also prepared at the workshop of Science faculty, Addis Ababa University.

3.4. Choice and repellency test

For this test, Y- tube olfactometer which had one stem and two arms was used. Three gas washing bottles, each having volume of 250 ml, (one containing activated charcoal for filtering pumped air which comes from air pump connected to it, the other two for placing different treatments from which the different odours were taken to the arms of the Y-tube). Flow meter was used to regulate the amount of air passing from the gas washing bottle containing activated charcoal into the other gas washing bottles (Figure 1). For this experiment, air was pumped into the bottles then to the Y-tube arms at a rate of 1.5 litres per minute. The set up was covered with black plastic to simulate the actual darkness in which worker termites forage. After each test the "Y" glass tube and the gas washing bottles were washed, rinsed with acetone and dried at 250^{o}C for 8 hours and then used for the different experiments.

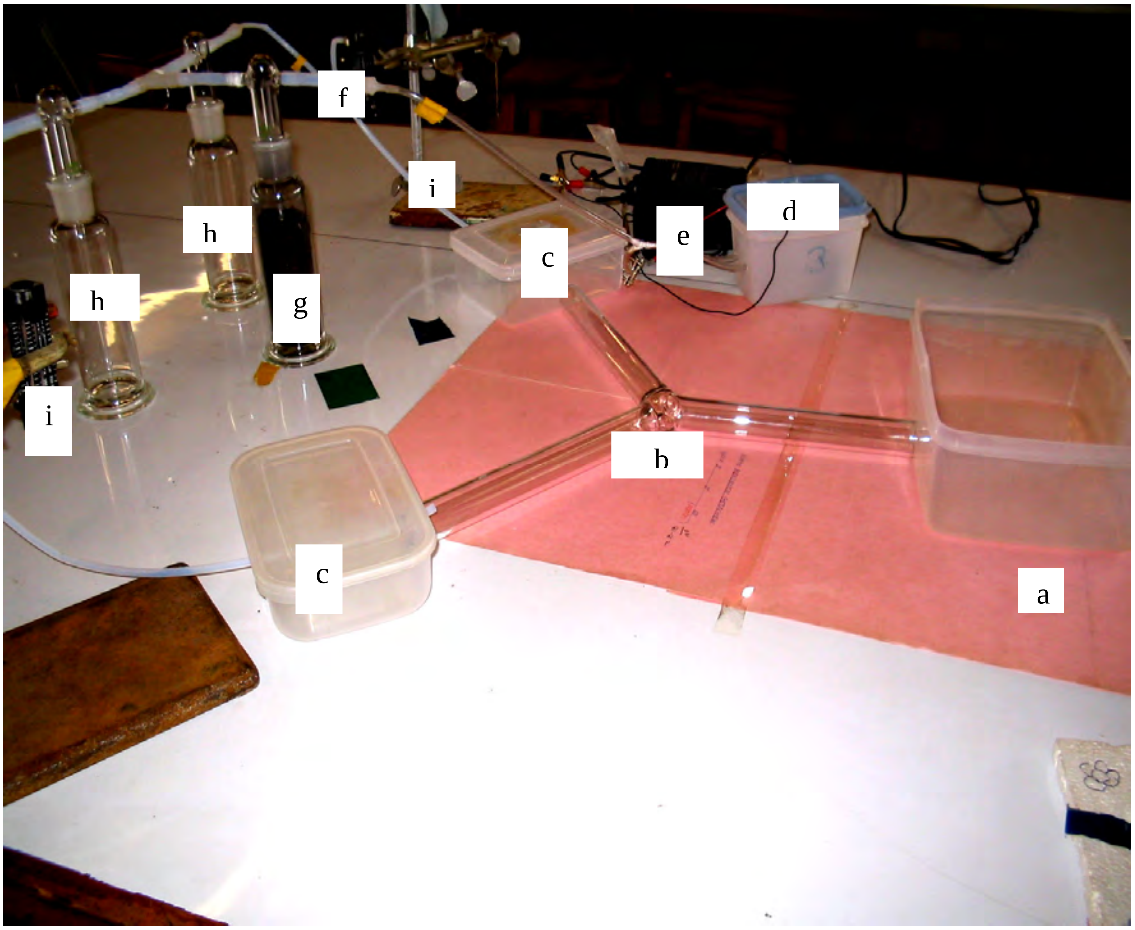

Figure1. Y-tube olfactometer set up (**a**-release box, **b**-Y-tube, **c**- insect collection boxes, **d**-air pump, **e**- power source, **f**- Teflon tube, **g**-wash bottle with activated charcoal, **h**- wash bottles for different treatments, **i**-flow meter, **j**- iron stand)

3.4.1. Choice test

In this test, stakes of susceptible plant (*C. macrostachyus*) and resistant plants (*J. procera* and *H. abyssinica*) (Getachew Desalegn *et al*., 2005) weighing 60 gm were placed in the gas washing bottles described above. Then, air was pumped at a rate of 1.5 litres per minute with regulated air pump into the gas washing bottles containing activated charcoal for filtration through Teflon tube. The filtered air then passed into the gas washing bottles having different treatments (*C. macrostachyus*, *H. abyssinica* and *J. procera* as compared with air and soil). Then, ten worker termites were released into a plastic box having an opening through which they move into the stem of the Y-tube. After fifteen minutes the number of insects that moved into different treatments was counted and each treatment was replicated ten times for each test. Finally, the insects were counted and the attraction was determined statistically by using chi square (χ^2).

3.4.2 Repellency test

For this test, wood stakes from resistant and susceptible plants weighing 100 gm and 500 gm of soil from the termite infested area were included. In addition to this, sawdust of resistant plant and susceptible plants were used. Soil alone from the termite infested area is also used as control. During the experiment the stakes and sawdust were mixed with the soil and placed in a plastic box having opening at one end and infested with 100 worker termites. Then, openings of the stems of the Y-tube were connected to the openings of the plastic boxes. Finally, the setup was covered with black plastic sheet and left for 30 minutes. Then, the number of worker termites that moved away from the treatments was counted and each treatment was replicated five times. Mean % of worker termites moved away was determined as follows:-

% moved away = N released in the plastic – N remained / N released. Then, the relative repellence was analysed statistically.

3.5. Volatile collection and bioassay

Volatiles were collected from both resistant and susceptible plants. Head space sampling technique was employed for the collection of volatiles (Zhang *et al*., 2000). Stakes and leaves of *C. macrostachyus, H. abyssinica*, and *J. procera* plants were enclosed in Teflon bag and a pump that contains a super Q filter plugged with polypropylene wool and nylon stoppers in its both ends was attached(Zhang *et al*., 2000). The collection was conducted for two hours. Then, the filter was rinsed with 200µl of hexane to obtain extracts of volatiles emitted by the plant material. In this way, sufficient volatiles for the test were collected. Then 200 µl of the extracted volatiles were applied to Whatman No. 1 filter paper and exposed to air to allow the solvent evaporate for 10-15 minutes. Then the filter paper was placed into the gas washing bottles and evaluated for their attractivity by using the Y-tube olfactometer described above.

3.6. Toxicity and antifeedant test

Ten, fifteen and twenty grams of the saw dust of *H. abyssinica* and *J. procera* prepared and soaked in 100 ml of different solvents (acetone, ethanol, petroleum ether and water) following the method of Bekele Jembere (2002). The same amount of aqueous extracts of *Azadirachta indica* (neem) and *Melletia ferrruginea* (Birbira) *s*eed and *H. abyssinica*'s female flower powder, were also prepared in the same manner. After 24 hours of soaking, the mixtures were filtered with cheesecloth. Then, the filters were used for the tests.

3.6.1. Toxicity test

One, two and three milliliters of each of the 10%, 15% and 20% concentration of the different plant extracts (*H. abyssinica, J. procera, M. ferruginea* and *A. indica* were applied onto the Whatman No.1 filter paper in the Petri dish. Then the organic solvents were allowed to evaporate at room temperature for 30-60 minutes. Then, 1ml of distilled water was added as a carrier. This makes the extracted active ingredient to adhere to the body of the insects. Finally, 10 worker termites were added to each Petri dish. For each toxicity test, three replicates were made. In addition to the application of each extract, 1 ml, 2 ml and 3 ml of each solvent was used for each experiment as control for comparison. Then mortality was observed 24 hours after application of treatments.

3.6.2. Antifeedant test

The test was done at the actual habitat of the insects at Ziway. Stakes of susceptible plant (*C. macrostachyus*) having relatively equal volumes (160 ml) were prepared. About 500 ml of extracts of resistant plants (*H. abyssinica* and *J. procera*) were also prepared in the lab. From each plant 9 stakes were immersed into the extracts for 3 minutes. Similar activities were done for all extracts. After the solvents were evaporated, the stakes of susceptible plant that were immersed in the extract and other untreated stakes of susceptible plant, resistant plant stakes (*H. abyssinica* and *J. procera*) as controls were taken to the field and buried around termite mounds. After a month, stakes were dug out and brought to the laboratory and their volumes were measured. This was done by pouring water into a measuring cylinder of 700 ml in volume. Then each stake was immersed into the cylinder, the difference in volume of damaged and undamaged stake was measured and the percentage of the plant material eaten was calculated as follows:-

(Volume of undamaged stake-Volume of damaged stake)/Volume of undamaged stake *100.

3.7 Data Analysis

Data entry and analysis were done using Microsoft Excel and SPSS Version 10 (SPSS Inc., 1999). Data were transformed by Arcsine transformation when necessary. χ^2 analyses were used for attractivity test and one-way analysis of variance ANOVA was used for repellence and antifeedant tests (Gomez and Gomez, 1984). In cases where significant results were obtained, mean comparisons were conducted using Tukey's Studentized Range test at 5% level of significance.

4. Results

4.1. Attractivity of plant materials to worker termites

4.1.1 Plant materials compared with soil

In most of the treatments significant differences were not ($P>0.05$) shown in attractivity. However, more number of worker termites moved to soil than *H. abyssinica* and Croton stakes. In the contrary, when attractivity of *J. procera* and soil was compared, more number of insects moved to *J. procera* than soil. Significant differences ($P<0.05$, $\chi^2 = 10.798$ and 9.449, respectively) were seen in comparison of *C. macrostachyus* together with soil versus *J. procera* with soil and *H. abyssinica* with soil versus *J. procera* with soil (Table 1).

Table1. Mean % of worker termites attracted to soil, stakes of *C. macrostachyus, H. abyssinica, J. procera* and their mixtures

Treatments	Mean % worker termites attracted	χ^2-value	P-value	Mean % non responding worker termites
H. abyssinica vs soil	30 37	0.731	0.392	33
Croton C. macrostachyus vs soil	44 50	0.383	0.536	6
J. procera vs soil	54 40	2.085	0.149	6
C. macrostachyus & soil vs *H. abyssinica* & soil	41 34	0.653	0.419	25
C. macrostachyus & soil vs *J. procera* & soil	29 60	10.798	0.001	11
H. abyssinica & soil vs *J. procera* & soil	30 59	9.449	0.002	11

4.1.2 Wood stakes compared with air

No significant differences (P> 0.05) were observed when wood stakes were compared with air for their attractivity to worker termites. However, the number of termites that moved to *J. procera* were significantly higher (P=0.05) than those that moved to air (Table 2).

Table2. Mean % worker termites attracted to wood stakes of *C. macrostachyus, H. abyssinica, J. procera* and their mixtures as compared to air

Treatments	Mean % attracted worker termites	χ^2- value	P- value	Mean % non responding worker termites
C. macrostachyus vs air	48 37	1.424	0.233	15
J. procera vs air	55 33	3.857	0.050	12
H. abyssinica vs air	45 38	0.590	0.442	17
C. macrostachyus & *H. abyssinica* vs air	37 33	0.229	0.633	30
C. macrostachyus & *J. procera* vs air	47 39	0.744	0.388	14

4.1.3 Plant leaves compared with air

When the leaves of *C. macrostachyus, H. abyssinica* and *J. procera* were compared with the air for their attractivity, significant differences (P < 0.05, χ^2=29.391 and 42.250, respectively) were observed between *H. abyssinica* and *J. procera* leaves against air. 6% and 72% worker termites moved to *H. abyssinica* and *J. procera* leaves, respectively. Though not significant statistically the number of termite workers moved to *C. macrostahcyus* was also larger than those which moved to the air (Table 3).

Table 3. Mean % worker termites attracted to leaves of *C. macrostachyus, H. abyssinica* and *J. procera* as compared to air

Treatments	Mean % worker termites attracted	χ^2- value	P- value	Mean % non responding worker termites
C. macrostachyus vs air	56 41	2.320	0.128	3
H. abyssinica vs air	6 58	29.391	0.000	36
J. procera vs air	72 20	42.250	0.000	8

4.1.4 Plant materials compared to plant material

In all the three treatments significant differences ($P<0.05$, $\chi^2=5.232$, 25.19 and 4.651) were observed in attractivity between stakes prepared in varying combinations (*C. macrostachyus* versus *H. abyssinica*, *C. macrostachyus* versus *J. procera* and *H. abyssinica* versus *J. procera*) respectively. More number of termites was attracted to *J. procera* in combinations made with it (Table 4).

Table 4. Mean % worker termites attracted to different wood stakes of *C. macrostachyus, H. abyssinica* and *J. procera*

Treatments	Mean % worker termites attracted	χ^2- value	P- value	Mean % non responding worker termites
C. macorostachyus vs *H. abyssinica*	44 25	5.232	0.022	31
C. macorostachyus vs *J. procera*	19 65	25.19	0.000	16
H. abyssinica vs *J. procera*	33 53	4.651	0.031	14

4.2. Repellency of the different plant materials

Mean % repelled worker termites in all four treatments, different plant materials plus soil (*C. macrostachyus, H. abyssinica, J. procera* plus soil) and soil alone showed no significant differences (P>0.05). However, there was difference in the number of termites left at the site of release after 30 minutes (Figure 2) (Annex 1).

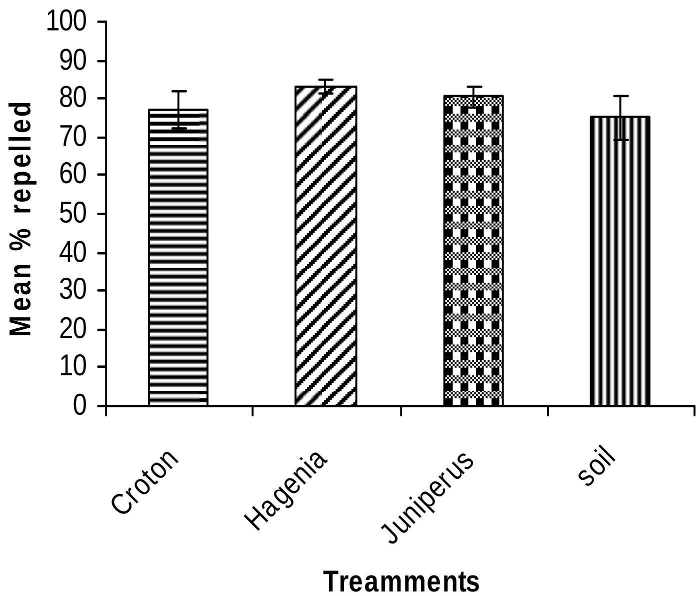

Figure 2. Mean % of worker termites that moved away (repelled) from different plant materials and soil

4.3. Response of the worker termites to different plant volatiles

No significant differences ($P>0.05$) were observed in attractivity of different volatiles extracted from the wood and leaves of *C. macrostachyus, H. abyssinica* and *J. procera*. Relatively, equal number of insects moved to different treatments. The comparison of each treatment with air also showed no significant difference (Table 5).

Table5. Mean number of worker termites attracted to wood and leaf volatiles of *C. macrostachyus, H. abyssinica,* and *J. procera* versus air

Treatments	Mean % worker termites attracted	χ^2- value	P-value	Mean % non responding worker termites
C. macrostachyus leaf vs air	47 50	0.093	0.761	3
C. macrostachyus wood vs air	44 49	0.269	0.604	7
H. abyssinica leaf vs air	48 43	0.275	0.600	9
H. abyssinica wood vs air	44 52	0.667	0.414	4
J. procera leaf vs air	50 48	0.041	0.840	2
J. procera wood vs air	43 47	0.178	0.673	10

4.4. Toxicity of different plant extracts to termite workers

There was no mortality of worker termites due to acetone, ethanol and water extracts of *H. abyssinica* and *J. procera* fine saw dust extracted at 10%, 15% & 20% and applied at 1, 2 &3 ml per filter paper. On the other hand, all worker termites released to filter paper treated with aqueous extract of neem seed powder remained alive after 24 hours of observation. No mortality was also observed in all of the control treatments. However, those termites released to filter paper treated with *Milletia ferruginea* seed and *H. abyssinica* female flower powder extract were killed at 100% rate within the time of observation while no mortality was observed in the control (Table 7).

Table 6. Mean % mortality of worker termites due to water extracts of *Milletia ferrugenia* and *Azadirachta indica* seed powder and ground dry flower of *H. abyssinica* 24 hrs after treatment

Plant materials	Level of extraction g/100ml	Dose ml/filter	% mortality ± S.E after 24 hours
Birbira seed extract	10	1ml	100±0.000
		2ml	100±0.000
		3ml	100±0.000
	15	1ml	100±0.000
		2ml	100±0.000
		3ml	100±0.000
	20	1ml	100±0.000
		2ml	100±0.000
		3ml	100±0.000

Neem seed extract	10	1ml	0±0.000
		2ml	0±0.000
		3ml	0±0.000
	15	1ml	0±0.000
		2ml	0±0.000
		3ml	0±0.000
	20	1ml	0±0.000
		2ml	0±0.000
		3ml	0±0.000
Hagenia flower powder extract	10	1ml	0±0.000
		2ml	0±0.000
		3ml	100±0.000
	15	1ml	0±0.000
		2ml	0±0.000
		3ml	100±0.000
	20	1ml	0±0.000
		2ml	0±0.000
		3ml	100±0.000

4.5. Antifeedant effects of *C. macorostachyus, H. abyssinica* and *J. procera* test

Different damage levels were observed on the wood stakes of different plants in this test. Untreated stakes of *C. macrostachyus* were damaged significantly (82.93%) compared to *H. abyssinica* (18%) ($P< 0.05$). There was no damage by worker termites on the stakes of *J.*

procera. Significant differences (P<0.05) were observed as the damage on *C. macrostachyus* stakes was compared with all other treatments. *C. macrostachyus* stakes treated with chloroform extract of *H. abyssinica* and *J. procera* were significantly (P<0.05) damaged than those treated with acetone and ethanol extracts. *C. macrostachyus,* treated with ethanol extract of *H. abyssinica* were less damaged than those treated with ethanol extract of *J. procera,* but not significantly different (P>0.05). However, the damage levels of *C. macrostachyus* stakes treated with ethanol extracts of *H. abyssinica* were less compared to that of acetone extracts of *J. procera* and *H. abyssinica*, but still there was no significant difference in the extent of damage (P>0.05) (Table 6).

Table 7. Mean % damage levels of *C. macrostachyus*, *H. abyssinica*, *J. procera* and *C. macrostachyus* treated with acetone, chloroform and ethanol extracts of *H. abyssinica* and *J. procera* due to worker termites.

Treatments	Mean % damaged ± SE** after a month
C. macrostachyus (control)	82.93± 2.00a
H. abyssinica	17.78± 4.25d
J. procera	0.00± 0.00e
H. abyssinica (acetone)	39.00± 6.37bc
J. procera (acetone)	45.66±7.62bc
H. abyssinica (choloroform)	51.63± 5.78b
J. procera (chloroform)	48.15± 7.68b
H. abyssinica (ethanol)	25.00±3.93cd
J. procera (ethanol)	41.48± 8.45bc

Means with in a column followed by different letters are significantly different, P<0.05, Tukey Studentized Range test (HSD).

5. Discussion

The results of investigations made for comparing attractivity of different plant materials against soil showed significant differences among treatments. *J. procera* attracted considerable % of termites than *H. abyssinica* and C. *macrosatchyus* within 15 minutes time. This is may be due to the odour *J. procera* has unlike the other two.

Comparison of plant materials (dry wood & fresh leave) with the clean air indicated *J. procera* leaves attracted greater number of insects followed by *C. macrostachyus* leaves and *Juniperus* wood stakes. This enables us to conclude that termites were attracted to the odor of plants irrespective of the antifeedant property the plants have. Although the toxicity of *C. macrostachyus* plant to termites was reported by Daniel Getahun (2003), this study indicated that its fresh leaves attracted large number of insects.

Among *C. macrostachyus*, *H. abyssinica* and *J. procera* leaves and wood volatiles, there appears no significant difference in termite workers attractions or repulsion. Where as, in the case of test done for attractivity, it was observed that significant number of insects were attracted and repelled to fresh leaves of *C. macrostachyus* and *H. abyssinica*, respectively. Therefore, the absence of significant difference in attraction among different volatiles described above could be, the small amount of active ingredient extracted which the worker termites were not able to detect. Hence, the movement of insects into the arms of Y-tube could be random.

As it is shown in the result, no significant difference was observed in insects which moved away from the release box in all treatments including the control (soil alone). The released insects moved away in more or less the same rate in all treatments within 30 minutes time. It seems that all plant materials and soil repelled the insects. The effect of repellency was not clearly seen by the treatments. Therefore, their movement can not be explained in terms of repellence rather it might be due to disturbance of natural environment that caused them to randomly move away. Though, some plants are important in repelling termites away from crops (Pearce, 1997), the plant materials used in this study did not show difference in repellence.

Some plants and plant extracts can provide a simple means of control that can be used by farmers. Tree resins such as sesquiterpenes especially of primary forest trees, are effective temiticides. Various trees and leaves in India have shown to be effective against termites. The commercial preparation of neem, Margosan (0.3% azadirachtin with 14% neem oil), at 100 parts per million (ppm) can cause significant mortality of *Coptotermes* but lasts only for a short time (Grace and Yates, 1992). Taking the above idea into consideration, toxicity test done on *Macrotermes* termite workers was not effective. Different levels of extraction (10%, 15% & 20%) and doses (1ml, 2ml & 3ml) of acetone, ethanol and water extracts of *H. abyssinica* and *J. procera* saw dust showed no toxicity to termites similar to the control within 24 hours. Aqueous neem seed powder extract applied to the insects in similar manner to the above test also showed no toxicity which is different from the report made by Grace and Yates (1992). This could be due to the environmental conditions the plants grew. However, aqueous seed powder extract of *Milletia ferruginea* caused 100% mortality at all levels of applications (1 ml, 2 ml and 3 ml) and doses 10%, 15% & 20% levels of extraction. This is agrees with the result obtained by Daniel Getahun (2003). Furthermore, aqueous extract of *H. abyssinica* which is commonly used by the local people of Ethiopia as antihelimenth, caused 100% mortality at 10%, 15% and 20% dose and rate of 3ml. This may indicate that the antihelimenthic ingredient is effective also in killing termites not only worms. This may be due to the higher concentration of the active ingredient in the flower. Further investigation is needed to find out the reason why insects were killed.

Different plants show different level of resistance to termites. The more durable timber species owe their resistance mainly to their extractives, which serve as natural preservatives (Getachew Desalegn *et al*., 2005). Preference and resistance of plants to termites rely on many factors such as: hardness, lignin content or chemical constituents of wood. Many researchers have examined the effect of wood extracts on the behavior of termites. Some parts of wood may not be eaten due to their hardness. Hardness is less important for some species, such as *Cryptotermes* and especially *Neotermes,* which can eat one of the hardest wood, teak trees.

Wood containing saponins which are toxic to insects including termites has been found intact over 1000 years (Pearce, 1997). Dry wood termites tend to have definite wood preferences. Some termites prefer fast growing tissues. e.g. spring growth, which produces large cells with thin walls and fewer fibers. Susceptibility of trees is also dependent on different factors such as moisture content of the wood, attack by fungi or other decomposers and even the age of the wood which determine compactness (Pearce, 1997).

The presence of organic chemical, e.g. phenols, quinones, terpenoids and high concentration of lignin may also affect the areas where feeding takes place. The PH of wood content might be important. Sap wood which has more starch and sugar, is generally preferred to heart wood. *Trinervitermes* have been known to eat wood when grass availability was reduced. Neem mulches are somewhat deterrent to *Coptotermes* but may not be effective for all termites (Pearce, 1997).

Different plants have differing antifeedant properties based on various factors such as the area where the plant grows, the type of existing insect species, and the strength of the plant and environmental conditions (moisture, temperature). In this study, stakes of the most susceptible plant, *C. macrostachyus,* dipped into 10% solvent extracts of *H. abyssinica* and *J. procera* were less damaged by the worker termites. *C. macrostachyus* was highly consumed compared to all others; on the other hand no damage was observed on *J. procera*.

However, Getachew Desalegn *et al*. (2005) reported that *J. procera* was damaged by the worker termites in thirteen years time of the study though the damage was less. Hence, *J. procera* resists the attack of worker termites followed by *H. abyssinica*. Generally, in this study, it was found that *J. procera* had better antifeedant property against termites than other *C. macrostachyus* and *H. abyssinica.*

6. Conclusion and Recommendations

6.1 Conclusion

- Results of these and earlier studies suggest that different plants and plant parts have varying degrees of attraction to worker *Macrotermes* termites. Stakes of *J. procera* tree and its leaves attracted relatively larger number of insects followed by *C. macrostachyus*. However, fresh leaves of *H. abyssinica* repelled the insects more than its own stakes. These depicted that, different concentrations of repellent substances exist in different parts of the same plant.

- Acetone, ethanol and aqueous extracts of *H. abyssinica* and *J. procera* saw dust, were not toxic to worker termites as compared to aqueous extract of *M. ferruginea* which was 100% lethal to termites. Similarly, aqueous extract of fresh leaves of *H. abyssinica* was toxic at higher doses to the termites.

- *C. macrostachyus*, *H. abyssinica* and *J. procera* and C. *macrtostachyus* treated with acetone, chloroform and ethanol extracts of the latter two have varying degrees of susceptibility and resistance to termite attack.

- Resistance of *J. procera* to worker termites was not due to extractable compounds as extracts of the plant did not protect the treated *C. macrostachyus* stakes as compared to *H. abyssica* extracts.

- *H. abyssinica* wood stakes were less resistant to termites attack than *J. procera* while their ethanolic extract gave some degree of protection.

6.2 Recommendations

- In this study *J. procera* was observed to be highly resistant to worker termites' damage followed by *H. abyssinica*. But, the reason for their resistance was not identified. Therefore, it would be better if further study is carried on identifying the protective substance from these plants especially from *J. procera* and make available for the end users.
- In the absence of other options people use commercial insecticides to control termite attack. These insecticides are costly and are not easily obtained moreover, harmful to non target organisms and to the environment at large. Hence, the use of resistant plants and natural insecticides in termite infested area should be encouraged and research should be pressed forward to identify and formulate such natural insecticides and use.

- *J. procrera* leaves and wood stakes attracted more insects compared to other treatments. However, it is the most resistant plant to worker termites attack. Therefore, attractant plant should not be considered as susceptible.

- Solvent extracts of *H. abyssinica* and *J. procera* did not kill termite workers. But the plants showed antifeedant properties. Hence, the absence of toxicity should not lead researchers to the conclusion that the plant can not be used for control purpose.

- In termite infested parts of our country farmers protect their harvested crops from termites attack by placing fresh *C. macrostachyus* branches under the harvest though in this study the plant did not show repellency, toxicity and antifeedance when dry. Accordingly, properties of the plants in terms of their effect and chemical composition when they are fresh (alive) or dry (dead) should be investigated differently.

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Annexes

Annex 1. Analysis of variance (ANOVA) for mean % of worker termites moved away from the plastic box treated differently.

Source of error		Sum of Squares	df	Mean Square	F	Sig.
Repellence of worker termites by *C. macrostachyus*, *H. abyssinica*, *J. procera* and soil	Between Groups	181.200	3	60.400	.728	.550
	Within Groups	1326.800	16	82.925		
	Total	1508.000	19			

Annex 2. Analysis of variance (ANOVA) for mean % damage of stakes of *C. macrostahcyus, H. abyssinica, J. procera* and *C. macrostachyus* treated with acetone, ethanol and water extract of *H. abyssinica* and *J. procera.*

Soruce of errror		Sum of Squares	df	Mean Square	F	Sig.
Damage levels of different stakes by worker termites	Between Groups	39513.716	8	4939.215	16.463	.000*
	Within Groups	21601.915	72	300.027		
	Total	61115.631	80			

* P<0.05 significant difference

Declaration

I, the undersigned, declare that this thesis is my original work and that it has not been presented in any other University for any Degree. All sources of materials used in the thesis are duly acknowledged.

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