Evaluation of Toxicity of Crude Extracts of Some Botanicals on Different Castes of *Macrotermes* Termites

Daniel Getahun, Bekele Jembere[1]

Addis Ababa University Department of Biology, P. O. Box 1176, Addis Ababa, Ethiopia:
Tel. + 251-11-1239471 + 251-911-645640; Fax: (+251) - 11 -1239469;
E- mail: bekeleja@sc.aau.edu.et or bekeleja@yahoo.com

Abstract

The effects of water extracts of seed powder of *Milletia ferruginea* and *Azadirachta indica*, fresh stem bark and leaf of *Croton macrostachys*, and fresh leaf and fruit of *Datura stramonium* were evaluated at three concentration levels (10, 25, and 40% w/v) against different castes of adult *Macrotermes* termites. The study was conducted under laboratory and field conditions at Melkassa Agricultural Research Center and Ziway, respectively. One conventional synthetic insecticide, chlorpyrifos, was used as a standard check. Extracts of seed powder of *M. ferrugina* and *A. indica* and stem bark of *C. macrostachys* that showed higher toxic effects in filter paper bioassays were used to treat termite castes placed in a mixture of soil and sawdust in plastic boxes. *M. ferruginea* and *C. macrostachys* were further tested in the field to poison mounds. In filter paper bioassays, water extract of *M. ferruginea* caused higher toxicity to all castes in which 93–100% mortality of alate termites was recorded at all concentration levels. The effect of the extract was equal to chlorpyrifos which caused 100% mortality to all castes. Croton stem bark extract at 40% concentration caused relatively higher (> 83%) mortality of workers, alates and minor soldiers than the control. Neem seed powder extract caused low mortality (0–10%) of alates and major soldiers at all levels of concentration; whereas, the toxicity of the extract on other castes was high. The toxic effects of datura leaf and datura fruit extracts were relatively low (0–20%) on all castes, except that datura leaf extract at 25 and 40% concentration showed relatively higher mortality (37% and 68%, respectively) on minor soldiers and workers. In mound poisoning, cheesecloth filtered water extract of croton stem bark at 40% concentration showed no effect, and there was no significant difference from with that of the control ($p > 0.05$). *Milletia* seed powder extract at 10% concentration caused low mean percentage mortality of major soldiers, minor soldiers and worker termites; the toxic effect of the extract was significantly lower than that of chlorpyrifos, but higher than that of the control.

Key words: botanicals, chlorpyrifos, crude extracts, *Macrotermes* termites, mortality

Introduction

Termites are polymorphic social insects that live in colonies consisting of a few thousand to several million individuals (Abdurahman 1990). Termites live all their lives in dark galleries, either under-ground, in wood or in special nests. Their cuticle is thin and delicate, and only matured males and females can withstand exposure to dry air, and then only for a short period (Matheson 1951).

Different termite species are serious pests of agricultural, horticultural and plantation crops, forest trees, structural timbers, various textile products (Gurusubrmanian et al. 1999), and rangelands (EECMY-WS 1997). Losses in crops and trees due to pest species are often over 15%, and in serious cases it can reach 100%. In addition, thatching in African houses can be expected to last 5–6 years (Pearce 1997). Although Gauchan et al. (1998) reported that wood-made houses in the west Wollega area of Ethiopia, which used to least over 10 years in the past, can only last 2–3 years. The frequent rebuilding of wood straw thatch houses leads to excessive clearing of native woodland and forest. Most of the damage to crops, forest trees, rangelands and wooden buildings in rural areas of Ethiopia is caused by several species of Macrotermitinae. The most important termite pests are the mound-building *Macrotermes* (*M. subhyalinus* and *M. herus*) and *Pseudocanthotermes militaris* (Hagen). Many *Macrotermes*, *Odontotermes* and *Pseudocanthotermes* cause damage to plants by penetrating the larger roots and excavating within the stem (Wood 1986).

Generally, termite damage is most prevalent where plants are under stress; whereas, vigoros crops and trees are rarely attacked by termites. Therefore, improvement of the soil, particularly greater use of compost and green manure, may reduce crop damage by providing an alternative source of food to termites. High levels of organic matter, good moisture retention and nutrient maintenance in the soil can benefit root growth and plant production while at the same time reducing the risk of termite damage (Stoll 2000).

Termite control in both agriculture and forestry previously relied on persistent organochlorine insecticides like Aldrin, Dieldrin, Heptachlor and Chlordane, which are known to protect crops throughout the season. However, due to the persistence of such insecticides in the environment and their negative effects on non-target organisms, research has been centered in developing alternative methods of termite control (Gurusurbamanian et al. 1999, Gethi et al. 1995, EECMY-WS 1997). As the use of chemical insecticides imposes health hazards and ill-effects on the environment and the average peasant farmer cannot afford for the steadily increasing cost of synthetic insecticides, research efforts should be geared to the development of plant-derived insecticides (Cobbinah and Osei-Owusu 1988). Thus, economic and environmental considerations demand an integrated termite control strategy in which environmentally friendly termiticides replace hazardous ones. The aim of the current study was to evaluate the toxicity of crude water extracts of different plant materials on termites and evaluate the possible use of the effective plant materials for termite control under field condition.

Materials and Methods

Plant material collection and extraction

Mature green whole leaves and fruits of *Datura stramonium* L. and leaves and stem bark of *Croton macrostachys* Hochest used for laboratory test were collected from Melkassa Agricultural Research Center. Stem bark of *Croton macrostachys* used for the field application was collected from Mukiye forest which is found in Mukiye Peasant Association, west of Meki town. Mature pods of *Milletia ferruginea* (Hochest) Baker were collected from Addis Ababa and dried in a well-ventilated area under shade until they exploded to release the seeds. Seeds of the latest harvest of neem tree (*Azadirachta indica* A. Juss) were obtained from the Agricultural Bureau of Dire Dawa Adminisrative Council. A synthetic insecticide, Chlorpyrifos 48% EC (Dursban®), was purchased from Chemtex PLC in Addis Ababa.

Fresh fruits and leaves of *D. stramonium*, stem bark and leaves of *C. macrostachys* and dried seeds of *M. ferruginea* and *A. indica* were ground manually with mortar and pestle as described by Tekie (1999) and Jembere (2002). About 10, 25 and 40 g of each ground plant material were soaked in 100 ml of water to obtain crude extracts of three concentration levels of 10, 25 and 40% (w/v). Each mixture was filtered with clean cheesecloth after 24 hours. The three concentration levels were determined based on preliminary extraction and toxicity evaluation studies. Chlorpyrifos was diluted in water to make 0.21% based on the standard field application rate.

Test insect collection

Mature workers and major and minor soldiers of *Macrotermes* termites were collected from mounds at Melkassa Agricultural Research

Center. Mounds were dug up and soil containing termites was spread on a plastic sheet. The termites were then collected using camel brush and fine forceps and placed into plastic boxes (22 x 17 x 7 cm each) as described by Gitonga et al. (1995) and Umeh and Ivbijaro (1997). The boxes were then provided with fine aeration holes in the lid, contained soil and moist cotton wool (used to maintain the necessary humid conditions). Similarly, live alates were collected during actual flights based on the procedures of Ochiel et al. (1995).

Filter paper bioassay

Whatman No.1 filter papers of 9 cm diameter each were placed in Petri dishes and treated separately with 2 ml of the water extracts of each of the three concentration levels filtered with cheesecloth only. Twenty mature workers, minor soldiers, and major soldiers, and five healthy alates of *Macrotermes* were selected and placed into Petri dishes that contained treated filter papers. Similar groups of termites were treated with the same volume of the extracts that were further filtered with filter paper (Whatman No.1). In all experiments, 0.21% of chlorpyrifos EC 48% and water served as a standard check and control, respectively. All the treated petri dishes were then covered with a double ply of black plastic sheet to simulate the dark galleries of termites. The treatments were replicated three times. Mortality was recorded 24 hours after treatment. The experiments were conducted under room conditions (25 ± 3 °C and 60–70% r.h.).

Soil/sawdust bioassay

Based on the results of filter paper bioassay, three plant materials which caused the highest mortality were used in the soils/sawdust bioassay. The materials were: 40% stem bark cheesecloth filtered water extract of *C. macrostachys*, seed extract of *A. indica* and 10% seed extract of *M. ferruginea*. A plastic box (24 x 10 x 6 cm) containing 450 g of soil and 10 g of sawdust added as food (Gurusbramanian *et a.*1999) was treated with 150 ml of each extract that thoroughly wetted the mixture. Twenty workers, minor soldiers and major soldiers, and five healthy alates of *Macrotermes* were placed in each box and covered with a black plastic sheet. In the experiments, 0.21% Chlorpyrifos EC 48% was used as a standard check and water as a control. The treatments were replicated three times. Mortality was recorded once 24 hour after treatment.

Mound poisoning (field application)

The field application was conducted around Meki town, 135 km south of Addis Ababa, where dense *Macrotermes* mounds were very common. The top portions of *Macrotermes* mounds were dug until the main exit/ventilation galleries that lead to the central part of the hive were exposed (Abdurahman1990). Twenty litters of 10% (w/v) of *M. ferruginea* seed extract and 40% (w/v) of *C. macrostachys* stem bark extract filtered with cheesecloth were applied into the nest through the main galleries using a plastic pipe. Chlorpyrifos and water were used as a standard check and control, respectively. Each mound was dug from top to bottom until the hive (nest) was reached 24 hours after treatment. Soil samples from the top portion of the hive (near the point of treatment application), the center of the hive and around the royal chamber of the hive were collected using a plastic box (15 x 10 x 10 cm). Dead and live termite castes found in each soil sample were counted and recorded. Each treatment was replicated three times.

Data analysis

The mortality data obtained in the different treatments were analyzed using the SPSS computer software (SPSS 1989). One-way analysis of variance (ANOVA) was used to compare treatment effects and mean comparison was done using Student-Newman-Keuls (SNK) method at 5% level of significance. Data on percentage mortality were transformed where necessary (arc sine) before analysis based on the method of Gomez and Gomez (1984).

Table 1. Mean percentage mortality of termite castes due to water extracts of different botanicals at three concentration levels

Treatment	Mean % mortality ± SE*			
	Alates	Major soldiers	Minor soldiers	Workers
Table 1a: Mortality at 10% concentration				
Datural fruit	6.67 ± 6.67c	3.33 ± 1.67cd	8.33 ± 1.67d	6.67 ± 1.67e
Datura leaf	0.00± 0.00c	6.67 ± 1.67bc	8.33 ± 4.41d	15.00 ± 0.00d
Croton leaf	13.33 ± 6.67c	3.33 ± 1.67cd	18.33 ± 7.26cd	11.67 ± 1.67de
Croton bark	33.33 ± 6.67b	13 33 ± 3.33b	83.33 ± 4.41b	38.33 ± 8.82c
Neem seed	0.00 ± 0.00c	10.00 ± 5.0bc	23.33 ± 1.67c	76.67 ± 1.67b
Milletia seed	93.33 ± 6.67a	100.00 ± 0.0a	100.00 ± 0.0a	100.00 ± 0.0a
Chlorpyrifos	100.00 ± 0.0a	100.00 ± 0.0a	100.00 ± 0.0a	100.00 ± 0.0a
	0.00 ± 0.00c	0.00 ± 0.00d	0.00 ± 0.00e	0.00 ± 0.00f
Table 1b. Mortality at 25% concentration				
Datural fruit	20.00± 11.55bc	3.33 ± 1.67cd	21.67 ± 6.67d	10.00 ± 0.00d
Datura leaf	20.00± 11.55bc	8.33 ± 1.67c	16.67 ± 3.33d	36.67 ± 14.24c
Croton leaf	13.33 ± 6.67bc	5.00 ± 2.89c	40.00 ± 2.89c	13.33 ±1.67d
Croton bark	33.33 ± 6.67b	21.67 ± 4.41b	88.33 ± 4.41b	65.00 ± 5.00b
Neem seed	0.00 ± 0.00c	8.33 ± 1.67c	56.67 ± 14.53c	96.67 ±1.67a
Milletia seed	100.00 ± 0.0a	100.00 ± 0.0a	100.00 ± 0.0a	100.00 ± 0.0a
Chlorpyrifos	100.00 ± 0.0a	100.00 ± 0.0a	100.00 ± 0.0a	100.00 ± 0.0a
	0.00 ± 0.00c	0.00 ± 0.00d	0.00 ± 0.00e	0.00 ± 0.00e
Table 1c: Mortality at 40% concentration				
Datural fruit	20.00 ± 0.00c	0.00 ± 0.00e	21.67 ± 3.33d	8.33 ± 3.33c
Datural leaf	20.00 ± 0.00c	11.67 ± 1.67c	38.33 ± 9.28d	68.33 ± 16.67b
Croton leaf	80.00 ± 0.00b	5.00 ± 0.00d	66.67 ± 8.82c	13.33 ± 3.33c
Croton bark	93.33 ± 6.67a	21.67 ± 3.33b	86.67 ± 4.41b	83.33 ± 1.67b
Neem seed	6.67 ± 6.67d	5.00 ± 2.89d	73.33 ± 6.01bc	100.00 ± 0.00a
Milletia seed	100.00 ± 6.67a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
Chlorpyrifos	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
Control	0.00 ± 0.00d	0.00 ± 0.00e	0.00 ± 0.00f	0.00 ± 0.00d

*Means within a column followed by the same letter are not significantly different at 5% level of significance, SNK

Results

Filter paper bioassay

There was a significant difference ($p < 0.05$) among the different botanical treatments at all levels of concentration. From all botanical extracts, milletia seed extract caused a statistically comparable mortality to the synthetic insecticide chlorpyrifos at all levels of concentration. Milletia seed extracts at all concentration levels and chlorpyrifos caused 100% mortality of all castes (tables 1 a–c). Croton bark at 10% had more toxicity next to milletia seed on alates and soldiers. Neem seed at 10% caused very high mean percentage mortality of worker termites after milletia seed at 10% (Table 1a). Among the botanical treatments extracted at 25%, concentration, croton bark had similar effect with milletia seed and chlorpyrifos on worker termites, followed by croton bark. The effect of croton bark at 25% on major and minor soldiers was significantly higher ($p < 0.05$) than the rest of the treatments except milletia seed and chlorpyrifos. Neem seed at 25% had no significant effect ($p > 0.05$on alate termites, while it was as toxic as milletia seed and chloropyrifos. Other treatments had very lower effect at 25% concentration (Table 1b).

Except datura fruit on major soldiers, all botanical extracts at 40% concentration caused significant mortality of termite castes compared to the control. Milletia seed and croton bark at 40% caused high mean percentage mortality to alate termites similar to that of chlorpyrifos (100%). Croton bark caused 100% mortality of worker termites (Table 1c). With the exception of milletia seed, which was highly toxic to all castes at all levels of concentration, alate termites were more susceptible to croton bark and croton leaf at 10% and 20% concentration and to croton bark, datura fruit and datura leaf at 40% concentration, while major soldiers were susceptible to croton bark only. Similarly, minor soldiers were susceptible to croton bark, neem seed and croton leaf at all levels of concentration. Neem seed was observed to be more toxic ($p < 0.05$) to workers and minor soldiers than to other castes.

Soil/sawdust bioassay

Significant differences ($p < 0.05$) were observed among the treatments for mean percentage mortality on the termite castes. Milletia at 10% inflicted the highest mortality

Table 2. Mean percentage mortality of termite castes due to water extracts of different botanicals in the soil/sawdust

Treatment	Mean % mortality ± SE*			
	Alates	Major soldiers	Minor soldiers	Workers
Neem seed 40%	6.67 ± 6.67b	11.67 ± 3.33c	11.67 ± 1.67c	23.33 ± 6.01c
Croton bark 40%	13.33 ± 6.67b	25.00 ± 5.00b	58.33 ± 9.28b	63.33 ± 7.24b
Milletia seed 10%	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
Chlorpyrifos	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00± 0.00a
Control	0.00 ± 0.00b	0.00 ± 0.00d	0.00 ± 0.00d	0.00 ± 0.00d

*Means in a column followed by the same letter are not significantly different at 5% level of significance, SNK.

Table 3. Mean percentage mortality of termite castes due to mound poisoning

Treatment	Mean % mortality ± SE*		
	Major soldiers	Minor soldiers	Workers
Milletia seed at 10%	22.96 ± 12.25b	22.89 ± 12.56b	19.35 ± 4.60b
Carton bark at 40%	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00c
Chlorpyrifos	94.79 ± 1.12a	99.07 ± 0.93a	96.83 ± 0.42a
Control	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00c

*Means within a column followed by the same letter are not significantly different at 5% level of significance, SNK

(100%) of all the castes and that was similar to the effect of the synthetic insecticide, chlorpyrifos. Second to milletia seed at 10%, croton bark at 40% induced high mean percentage mortality of the castes except to alates, which were not significantly different from neem seed and control (Table 2).

Mound poisoning

Even though mortality due to milletia was not very high, there was a significant difference ($p < 0.05$) between extracts of milletia and the control. From all the treatments, only milletia and chlorpyrifos inflicted mortality of the observed castes, i.e., major soldiers, minor soldiers, and worker termites (Table 3). Alate termites were not found in the soil samples though few of them were found in the nest of all the treated mounds. From the mounds treated with chlorpyrifos, the queen and the king found in two mounds were dead. Only one died from the mounds treated with milletia, while no queen or king died due to croton and control treatments.

Discussion

The results of the present laboratory experiments indicated that all concentration levels of *M. ferruginea* seed extract filtered with cheesecloth caused very high mortality of all the termite castes similar to the synthetic insecticide, chloropyrifos. Almost all the termites treated with milletia died within a few hours of application. According to the present finding, the killing power of milletia seed extract was different from a similar work by Sabitti (2002) on *Chilo partellus*, in which milletia seed at 15% applied topically did not inflict 100% mortality of the insect even after several days. But similar to the present finding, milletia seed caused more mortality than neem seed extract. The difference in the effect of milletia on both insects may be due to the differential susceptibility of the insects to milletia seed extract. Jembere (2002) evaluated the toxicity of milletia seed against *Sitophillus zeamais* and reported higher mortality of the weevil within 48 hours after treatment. Rotenones is one of the dominant compounds found in the seed and stem bark of birbira, and is a well-known botanical insecticide through contact and stomach poisoning (Jembere 2002, Saxena 1983). It is also highly toxic to fish and soluble in polar solvents (Jembere 2002). Damte and Chichaybelu (2002) also tested the toxicity of milletia seed against Adzuki bean beetle (*Callsobruchus chinunesis*) and found that it gave complete protection of stored chickpea for six months in the laboratory, even though it was not effective in controlling this storage pest when used by farmers. It deterred egg-laying. Mulatu and Gebremedhin (2000) reported from their laboratory study that the oils of *M. ferruginea* and *A. indica* were able to effectively control Adzuki bean beetle infestation of faba bean by partially or completely preventing egg-laying, and no bruchids emerged from the few eggs laid.

All levels of concentration of *D. stramonium* fruit and leaf extracts filtered with cheesecloth did not show appreciable effect on all the treated castes except datura leaf at 40% which caused higher mean percentage mortality of the worker termites (tables 1a and b). The mortality caused by datura leaf was generally higher than that of datura fruit. This is in agreement with the work of Eticha and Tadesse (1998) on maize weevil (*Sitophilus zeamais*). The authors reported that datura leaf caused more adult weevil mortality (78%) than datura seed (56%) seven days after treatment.

The toxicity effect of *C. macrostachyus* leaf at 10 and 25% on the alate termites was not significantly different from the control, and all levels of concentration had no significant effect on major soldiers. However, croton stem bark at all rates had a significant effect on all the castes. The difference between the mean percentage mortality caused by the two botanical parts may be that the bark contains stronger chemical compound(s) toxic to termites than leaf part. Jenson (1981) reported that *C. macrostachyus* has various uses in Ethiopia, among which the pulverized bark is used together with dried *Hagenia abssynica* (Kosso) flowers as a very purgative antihelmenthic, and the seeds and the resin are toxic and used as a fish poison.

The prevention of harvested crops from termite attack by placing them on cut leafy branches of the *C.macrostachyus* tree is common in Wollega, Shewa, and Borena (Southern Oromia region). Termites do not pass through these cut branches and attack crops (personal observation). This may show that the leaves and bark of croton have substances which are toxic and/or repellent to termites. It may also suggest that spreading of croton leaves on the soil in the field of growing crops as mulches or incorporating them in the soil can act as repellents to keep away termites and reduce yield loss. In line with this, Logan et al. (1990) stated that sawdust or wood chip from trees containing repellent chemicals may provide some protection if incorporated into soil or used as mulch round crops or trees. The simplest method of application is as mulch. Water extracts of plants are mixed with irrigation water, sprayed on to plants or mixed with soil to protect trees and crops. The authors list many plant species reported to be toxic or repellent to termites. In Ethiopia, croton plant can easily be grown on farmers' field, and thus farmers can get the leaves and bark easily.

The mean percentage mortality of minor soldiers and worker termites increased with increased level of concentration of neem seed unlike that of major soldiers and alate termites in which mortality was not significantly different from the control. The absence or very less percentage mortality of alates and major soldiers as compared to minor soldiers and worker termites could be attributed to the higher sclerotization and larger body size of the former castes. Pearce (1997) stated that some termites are more tolerant to environmental factors which can depend on the size and degree of sclerotization of the cuticle as well as adaptation linked to their normal habitat.

From all the botanical treatments, croton bark showed higher mean percentage mortality after milletia seed on alates and both types of soldier. But in the case of worker termites, neem seed caused significant effect than croton bark at all levels of concentration. The difference in mortality response could be due to differences in physiology and susceptibility of the termite castes to different botanicals.

In all the botanical extracts treated with filter papers, the worker termites did not eat the filter papers unlike those treated only with water (control). The avoidance of the botanically treated filter papers may suggest the escape of toxic substances or allelochemicals that affect insects. It is possible that termites are less susceptible to the botanical extracts or the effect could be slow, not just toxicity, lacking a 'knock-down' effect unlike chlorpyrifos and *M. ferruginea*, affecting, for instance, feeding behavior and reproduction in which the effects are seen over a long time. According to Kareem et al. (1989), plant materials may not necessarily be toxic; but they may affect the pest by altering its behavior and physiology. Schmutterer (1989) stated that application of neem-based insecticides against adult insects, for instance, bugs and beetles, does not normally lead to mortality, but may result in a substantial reduction in the fecundity of the insects, so that the following generation may be reduced below economic threshold level. Jembere et al. (1995) reported that the effect of the different plant materials on insects may depend on several factors such as chemical composition and species susceptibility.

Both filter paper and soil/sawdust laboratory bioassays indicated that milletia seed extract at 10% was found as effective as the standard chemical insecticide Chlorpyrifos. Croton stem bark at 40% also inflicted high mortality of minor soldiers, alate and worker termites. But under field condition, the mean percentage mortality due to milletia was low and no mortality was recorded due to croton stem bark. In the laboratory bioassays, termites were in continuous contact with the extracts, while under field conditions they could leave the treated soil and move to soil where the extracts did not reach, as the soil of the mound could not be uniformly treated.

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