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Research article

EVALUATION OF REPELLENT AND TOXIC PROPERTIES OF *MILLETTIA FERRUGINEA* (HOCHST) BAKER (LEGUMINACEAE) AQUEOUS EXTRACT AGAINST TERMITE, *MACROTERMES* (ISOPTERA: TERMITIDAE) SPECIES

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ABSTRACT
Aqueous extract of *Millettia ferruginea* leaves, pods and seeds were tested for repellent and toxic properties against termite *Macrotermes* (Isoptera: Termitidae) species which causes enormous damage on agricultural crops and domestic materials. Aqueous extracts were prepared at 10,000 ppm stock concentration from the residues collected after the removal of water. Repellent and toxic properties in concentrations 50, 100, 150, 200 and 250 ppm were tested in Petri plates using Whatman no.1 filter paper. The Whatman no 1 filter paper was treated individually with each concentration and kept in the Petri plates and 20 worker termites were released. The experiment was replicated three times. In general, repellent and toxic activity of the plant extracts was based on concentration and exposure period. In repellency test, after 60 min observation, 96.6% repellency was observed in seed extracts followed by 86.6% in leaf extract. In toxicity test at 250 ppm concentration of seed extract, 100% mortality was observed after 96h exposure period. Chi-square analysis showed statistically significant difference within the concentration tested. The study shows that aqueous extract of leaves, pods and seeds proved to have repellent and toxic principle compound against *Macrotermes* species. These plants are endemic to Ethiopia and it will be a good source for resource-poor farmers to control termite.

Keywords: Aqueous extract, *Millettia ferruginea*, repellent, toxicity, termite.

INTRODUCTION
Termite belongs to the family Termitidae genus *Macrotermes* causes extensive damage to many agricultural crops and domestic materials. The estimated global damage in general and Ethiopia in particular caused by termite species was 100% (Michael, 2000; UNEP and FAO, 2000; Sekamatte, 2001; Nyeko *et al.*, 2010). In East Africa and in general, the estimated loss in various agricultural crops and tree species was ranged from 50-100%

(Sekamatte, 2001; Nyeko *et al.,* 2010). In Ethiopia, up to 100% loss was observed in one or two-year-old *Eucalyptus* transplantation, 45-50% on maize, 50-100% on teff and 25% on sorghum (Abdurahman, 1990, 2000). The estimated yield reduction due to termite attack in hot pepper and maize was 62% and 36% respectively (Abdurahman, 1983; Abraham, 1990; Devendra *et al.,* 1998). Some of the crops damaged by the termite species are sugarcane, maize, wheat and fruits etc. (Salihah *et al.,* 1986; Sattar and Salihah, 2001). In the Western part of Ethiopia many farmers abandon their farmland and migrated to other places due to economic loss caused by termite in various agricultural crops (Abraham, 1990; Abraham and Adane, 1995; Devendra *et al*., 1998). To control damage caused by these pests and also to replace the use of hazardous chemical pesticides safer alternative measures are needed in recent time. The utilization of plant products to pest control program was considered as one of the safer alternative methods.

Millettia ferruginea belongs to the family Leguminaceae is commonly called as Birbira in Amharic. It is a large shady tree growing up to the length of 35 m height and 1000-2500 meter above sea level. In Ethiopia, sub-species *Millettia ferruginea ferruginea* is confined to northern part and *Millettia ferruginea darasana* occurs in southern provinces, particularly Sidamo region. However, trees from central and western Ethiopia are the mixture of these two species (Azene *et al.*, 1993). In Eastern Ethiopia, these trees are extensively used as a shade tree for coffee plantation and traditional fishermen use pulverized mature pod and seeds for fish catch due to toxic effects (Legesse Negash, 1995; Azene Bekele, 2007 and Banouzi *et al.,* 2008). The toxic properties of different plant parts extracted with acetone were proved against *Sitophilus zeamais* and also seed powder mixed with 10% w/w reduced reproductive rate of the insect significantly (Bekele, 2002). Bekele *et al.* (2007) reported that water, acetone, chloroform and petrol extracts of *Millettia ferruginea* seeds and dry leaf powder was toxic against bean bruchid, *Zabrotes subfaciatus*. Debella *et al*. (2007) observed dose depended on larval mortality against three species of mosquito larvae by using the lyophilized amorphous power of *Millettia ferruginea* aqueous extract. According to Shiberu *et al.* (2014) aqueous extract of *Millettia ferrugiena* seeds showed 100% termite mortality. From the *Millettia* genus so far over 25 flavonoids, 50 isoflavonoids, 12 chalcones, and miscellaneous compounds have been isolated. Among the various compounds isolated and reported, rotenone was the dominant one found in the seed and stem bark of *Millettia ferruginea* (Dagne and Bekele, 1990; Yankep *et al.,* 1997). George (1980) reported that rotenoids have been used as insecticides since 1848. The rotenone ingestion was effective in killing the insects due to stomach poison (Isman, 2006). Therefore, the present study was aimed to evaluate repellent and toxic properties of aqueous extracts of leaves, pods, and seeds of *Millettia ferruginea* against termite, *Macrotermes* species.

MATERIALS AND METHODS

Processing of the plant material

Repellent and toxic properties of aqueous extract of *Millettia ferruginea* leaves, pods and seeds were tested at Entomology laboratory, Maraki campus, Department of Biology, College of Natural and Computational Sciences, University of Gondar, Ethiopia from February 2017 to May 2017.

One kg of leaves and mature pods of *Millettia ferruginea* were collected from Bata Mariam church, Gondar town, Ethiopia (Fig. 1). Each part was thoroughly washed with tap water to remove unwanted debris attached from their natural environment. The seeds were removed from mature dry pods. The leaves, pods, and seeds were shade dried and powdered by an electric blender (RRH-A200, Shanghia Yuanya Industries and Trade Co Ltd China) at 28000 rpm. Then 100 g powder was dissolved in 500 ml of tap water individually and kept in a shaker for 16 h for homogenous mixing. Later, the aqueous layer was filtered using a muslin cloth and the water was removed using rotary evaporator by maintaining the water bath at 60°C. After complete evaporation, the residue was collected and used to prepare different concentrations for subsequent experiments.

Insect collection

Termite, *Macrotermes* species were collected from dry wood in and around Tewodros and Maraki campus, University of Gondar. The worker termite was collected from the dry wood by using fine camel brush and kept in a plastic container. From their natural environment, pieces of dry wood were collected and added to the container as a food source. The plastic container was covered with black cloth to provide a dark condition for insects to acclimatize in the laboratory.

Preparation of concentration

The aqueous extract residue of leaves, pods, and seeds was used to prepare 10,000 ppm stock concentration by mixing 1 g with 100 ml tap water. From the stock solution, working concentration of 50, 100, 150, 200 and 250 ppm was prepared for the subsequent experiment.

Repellency test

Repellent and toxic properties of aqueous extract of *Millettia ferruginea* plant parts were tested following the protocol of Addisu *et al.* (2014). The experimental design was completely randomized block design with three replications. For repellency test, half part of Whatman no. 1 (4.5 cm diameter) filter papers were treated with 10,000ppm concentration of plant extract and the half part left untreated. The treated and untreated filter paper was kept in Petri plates (4.8 cm diameter). The distance maintained between treated and untreated filter paper was 1 cm. Totally 20 termites were released in each Petri plates and number of termite on treated and untreated half was observed 1 h with 20 min interval. The percentage repellency was calculated using the following formula. Repellency (%) = (C-T)/(C) X 100, whereas C= number of termite observed on untreated filter paper; T = number of termite observed on treated filter paper.

Toxicity test

For toxicity test, 20 termites were released into 500 ml plastic container. The Whatman no 1 filter paper treated with 2 ml of different concentration of plant extracts was kept at the bottom of the container. The plastic container was covered with black paper to create a conducive environment for termite. The Whatman filter paper treated with water was used as negative control. The experiment was replicated three times for all the five concentrations tested. The number of dead termites was recorded continuously four days with 24 h interval. The mortality rate was corrected and calculated using Abbott's formula (Abbott, 1925).

Corrected % mortality = (% mortality in test - % mortality in control)/ (100-% mortality in control) X 100.

Data analysis

The data collected from each experiment was subjected to statistical analysis using SPSS version 16 software for windows. The descriptive statistical analysis was carried out to calculate percentage mean and standard deviation. The statistically significant difference for repellency test, within the plant part, tested and observation time was confirmed by two-way ANOVA. Profit analysis was used to calculate LC_{50} and LC_{90} concentration and also the range of upper confidence limit (UCL) and lower confidence limit (LCL) for LC_{50} and LC_{90} concentration. The Chi-square analysis was carried out to confirm statistical significance at 5% level ($p<0.05$).

RESULTS

Repellent activity of *Millettia ferruginea* extracts

Table 1 shows repellent activity of *Millettia ferruginea* aqueous extract tested at 10,000 ppm concentration. After 20 min observation 73.3% repellent activity was observed in seed extract followed by leaves 66.6%. After 40 min both seed and leaf extracts showed 73.3% repellency. After 60 min observation, 96.6% repellency was observed in seed extract followed by leaves 86.6% and pods 76.6%. The two way ANOVA results clearly demonstrate the statistically significant difference ($p<0.05$) within the plant parts tested ($F=12.58333$; $p<0.000381$) and within the observation time ($F=25.08333$; $p<6.24E-06$). However, interaction among the samples was statistically not significant ($F=0.583333$; $p> 0.678683$).

Table 1. Repellency of *Millettia ferruginea* extracts against *Macrotermes* sp.

Plant parts tested	Observation time (min)		
	20	40	60
Leaves	66.6 ± 5.77	73.3 ± 11.54	86.6 ± 5.77
Pods	56.6 ± 5.77	63.3 ± 5.77	76.6 ± 5.77
Seeds	73.3 ± 5.77	73.3 ± 5.77	96.6 ± 5.77

Values are mean ± standard deviation of three replications.

Toxicity of *Millettia ferruginea* leaf extracts against *Macrotermes* species

Table 2 reveals toxicity of *Millettia ferruginea* leaf extracts tested against *Macrotermes* species. Among the concentration tested, after 24 h exposure period, 83.3% mortality was observed at 250 ppm. After 48, 72 and 96 h exposure period percentage mortality was 86.6, 88.3 and 91.6% respectively at 250 ppm. In general, percentage mortality was observed above 50% except for 50 ppm concentration after 24 hr exposure period. The calculated LC_{50} values were 76.29, 36.3, 37.5 and 22.4 ppm for 24, 48, 72 and 96 h respectively. The calculated LC_{50} and LC_{90} concentration, upper and lower confidence limit range for LC_{50} and LC_{90} concentration were decreased from 24 hr to 96 h exposure period. The Chi-square analysis showed that the percentage mortality was statistically significant ($p<0.05$) within the concentrations tested.

Toxicity of *Millettia ferruginea* pod extracts against *Macrotermes* species

Table 3 shows toxicity of *Millettia ferruginea* pod extracts tested against *Macrotermes* species**.** Among the concentration tested, after 24 h exposure period, 86.6% mortality was observed at 250 ppm concentration and 91.6% mortality was observed after 72 and 96 h exposure period. The percentage mortality ranged from 70 to 91.6% at 150 ppm and above concentrations tested after 48 h. The calculated LC_{50} values were 66.1, 48.0, 44.4 and 29.9 for 24, 48, 72 and 96h exposure period respectively. In general, LC_{50} and LC_{90} concentration, upper and lower confidence limit range for LC_{50} and LC_{90} concentration decreased from 24 h to 96 h exposure period. The Chi-square analysis showed statistically significant ($p<0.05$) percentage mortality in 48, 72 and 96 h exposure period. However, percentage mortality after 24 h exposure period was statistically not significant ($\chi2 =21.6$ $p>0.05$).

(A). *Milletia ferruginea* tree with immature pods

(B). Leaves **(C). Pods** **(D). Mature Seeds**

Fig. 1. *Milletia ferruginea* tree and parts

Table 2. Toxicity of *Millettia ferruginea* leaf extracts against *Macrotermes* species.

Concentration in ppm	Exposure period (h)			
	24	48	72	96
50	40.0 ± 5.00	56.6 ± 2.88	56.6 ± 7.63	66.6 ± 2.88
100	58.3 ± 2.88	60.0 ± 2.88	70.0 ± 5.00	70.0 ± 5.00
150	65.0 ± 0.00	68.3 ± 5.77	73.3 ± 2.88	75.0 ± 5.00
200	66.6 ± 7.63	70.0 ± 5.00	75.0 ± 5.00	76.6 ± 5.77
250	83.3 ± 2.88	86.6 ± 2.88	88.3 ± 2.88	91.6 ± 2.88
LC_{50} value	76.29	36.3	37.5	22.4
LCL-UCL	59.4-90.7	12.1-56.61	18.5-53.6	3.0-42.7
LC_{90} value	570.8	757.5	478.8	499.9

LCL-UCL	404-3791	412.1-3628.4	320.1-1070.8	286.8-2745.1
χ2	22.3	28.2	24.2	35.2
p-value	0.05	0.007	0.029	0.001

Values are mean ± standard deviation, LC – Lethal concentration; LCL- Lower confidence limit; UCL-Upper confidence limit; x^2- Chi-square

Toxicity of *Millettia ferruginea* seed extracts against *Macrotermes* species.

Table 4 ishows toxicity of *Millettia ferruginea* seed extracts tested against *Macrotermes* species. Among the concentration tested, after 24 h and 48 h exposure period, 95% mortality was observed at 250 ppm. After 72 and 96 h exposure period, percentage mortality was 98.3% and 100% respectively at 250 ppm. The calculated LC_{50} values were 46.0, 29.8, 42.2 and 38.7 ppm for 24, 48, 72 and 96 h exposure period respectively. In general, LC_{50} and LC_{90} concentrations, upper and lower confidence limit ranges for LC_{50} and LC_{90} concentration decreased from 24 h to 96 h exposure period. The Chi-square analysis showed statistically significant ($p<0.05$) percentage mortality within the concentration irrespective of the exposure period.

Concentration in ppm	Exposure period (h)			
	24	48	72	96
50	45.0 ± 5.00	53.3 ± 7.63	56.6 ± 7.63	63.3 ± 7.63
100	60.0 ± 5.00	60.0 ± 5.00	65.0 ± 5.00	68.3 ± 5.77
150	66.6 ± 2.88	70.0 ± 5.00	71.6 ± 2.88	73.3 ± 5.77
200	70.0 ± 0.00	75.0 ± 5.00	76.6 ± 5.77	76.6 ± 5.77
250	86.6 ± 2.88	88.3 ± 2.88	91.6 ± 2.88	91.6 ± 2.88
LC_{50} value	66.1	48.0	44.4	29.9
LCL-UCL	49.6-80.0	28.3-64.1	22.7-61.9	6.6-50.8
LC_{90} value	507.4	471.1	404.9	464.2
LCL-UCL	366.2-871.3	322.5-974.8	275.9-894.6	277.7-1983.8
χ2	21.6*	26.9	35.4	41.0
p-value	0.061	0.013	0.001	0.000

Table 3. Toxicity of *Millettia ferruginea* pod extracts against *Macrotermes* species.

Values are mean ± standard deviation, *indicates statistically not significant ($p>0.05$); LC – Lethal concentration; LCL- Lower confidence limit; UCL-Upper confidence limit; x^2- Chi-square

Table 4. Toxicity of *Millettia ferruginea* seed extracts against *Macrotermes* species.

Concentration in ppm	Exposure period (h)			
	24	48	72	96
50	58.3 ± 10.41	58.3 ± 10.41	61.6 ± 10.4	65.0 ± 5.00
100	65.0 ± 13.22	68.3 ± 15.2	70.0 ± 10.00	71.6 ± 7.64
150	71.6 ± 7.64	73.3 ± 5.77	75.0 ± 5.00	75.0 ± 5.00
200	85.0 ± 5.00	86.6 ± 2.88	88.3 ± 2.86	90.0 ± 8.66
250	95.0 ± 5.00	95.0 ± 5.00	98.3 ± 2.88	100.0 ± 0.00
LC_{50} value	46.0	29.8	42.2	38.7

LCL-UCL	18.4-67.0	6.1-50.8	15.7-62.6	11.6-59.8
LC_{90} value	288.2	285.3	227.8	209.4
LCL-UCL	198.9-704.0	191.2-845.5	163.8-484.0	149-465.4
$\chi 2$	75.2	59.5	85.1	94.2
p-value	0.000	0.000	0.000	0.000

Values are mean ± standard deviation, LC – Lethal concentration; LCL- Lower confidence limit; UCL-Upper confidence limit; x^2- Chi-square

DISCUSSION

Botanicals are played important role in pest control program due to the presence of a mixture of different bioactive secondary metabolites in their parts. In the present study, aqueous extract of *Millettia ferruginea* leaves, pods and seeds showed dose and time-dependent percentage repellent and toxicity against termite, *Macrotermes* species. Repellent activity was observed maximum in seed extracts compared to leaves and pods. The mean percentage mortality of 100% was observed in seed extracts at 250 ppm concentration after 96 hr exposure period. Earlier Shiberu *et al*. (2014) observed soldier and worker termite mortality was 100% exposed to seed extracts after 24 h exposure period. The time taken for killing insects may be associated with the concentration of the plant extracts and the methodology adopted for experiments and also laboratory conditions. The concentration used to obtain 100% in their study was 25 g ground seed powder mixed with 100 ml of water. However, the concentration used in the present study was much lower compared to their study.

Percentage mortality observed in the present study varied significantly within the plant parts, the concentration of the extracts tested and exposure period. In general, maximum percent mortality was observed in 250 ppm concentration. These findings are corroborated by the report of Bekele *et al*. (2007) but they have observed 100% mortality against bean bruchids exposed to acetone extract of *Millettia ferruginea* seeds after 24 h. Choudhary and Shiferaw (2016) also observed 100% tick mortality exposed to acetone extract of seeds after 90 minutes. Earlier reports and present findings clearly demonstrate that the percentage mortality was associated with dissolving nature of active ingredient to solvent. The dissolving nature of rotenone may be greater in acetone extract compared to aqueous extract. The mortality rate was maximum in seed extracts compared to leaves and pods. The earlier report confirmed the presence of rotenone in the seeds (Bekele, 2002). George (1980) reported rotenone was one of the bioactive molecules used to prepare insecticides. Therefore, higher percentage mortality in the seed extracts compared to leaves and pods clearly indicates accumulation of rotenone in each part. This may be the reason for higher mortality in seed extract.

CONCLUSION

The present study confirmed repellent and toxic potential of *Millettia ferruginea* leaves, pods, and seeds against the termite *Macrotermes* species. These plants are endemic to Ethiopia and it will be one of the valuable resource materials for farmers who are unable to afford the cost of chemical pesticides to control termites. The development of a simple technique for isolation and formulation of bioactive molecules will be useful to produce eco-product to control termites.

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