

Toxicity of Birbira (*Milletia ferruginea*) Seed Crude Extracts To Some Insect Pests As Compared To Other Botanical and Synthetic Insecticides

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Abstract

Toxicity effect of polar and non polar extracts of “Birbira”, *Milletia ferruginea* seed powder were evaluated at different concentration levels (10- 40% w/v) against the different castes of adult *Macrotermes* termites and sorghum chaffer (*Pachnoda interrupta*) and compared with other plant extracts and standard insecticide. The study was conducted under laboratory condition on filter paper and soil sawdust mixture. In the filter paper bioassays, water extract of *M. ferruginea* caused higher toxicity to all the castes of termites in which 93 to 100% mortality was recorded at all concentration levels. Water extract the Birbira seed (filtered with cheese cloth) caused 45-60% mortality sorghum chaffer within 24-48 hrs and this was significantly higher than mortality caused by the standard insecticide, carbaryl, applied at recommended rate (1.5 kg/ 400lit of water).

Keywords /phrases: Extract toxicity, chlorpyrifos, *Macrotermes*, *Pachnoda interrupta*, insect growth regulator, Carbaryl.

1. Introduction

Birbira, *Milletia ferruginea* (Hochst.) Baker; Leguminaceae) is a large shady tree which grows up to length of 35 m high. It is endemic to Ethiopia and widely distributed in the country and performs well in moist lowland as well as dry, moist and wet semi-highland agro climatic zones of 1000-2500 m above sea level. There are two sub-species known to occur in Ethiopia. These are: *M. f. ferruginea* which is confined to the northern part of the country and *M. f. darasana* which occurs in southern provinces, particularly Sidamo region. Trees from central and western Ethiopia show mixture of the two species (Azene *et al.*, 1993). The Birbira tree is used for fish poisoning where mature pod and seed are ground to fine powder and is spread over the surface of water (Siegenthaler, 1980). Furthermore, the tree is extensively used as shade for coffee (*Coffea arabica*) in Hararge region, Eastern Ethiopia (Teketay and Tegineh, 1991).

To date over 25 flavonoids, 50 isoflavonoids, 12 chalcones and miscellaneous compounds have been reported from *Milletia* genus alone (Bekele, 1988). Rotenone is one of the dominant compounds found in the seed and stem bark of *M. ferruginea* (Bekele, 1988). George (1980) reported that rotenoids have been used as insecticides since 1848, when they were applied to plants to control leaf eating caterpillars. It has oral LD₅₀ of 350 mg/kg and has been used as ideal general garden insecticide. It has both contact and stomach poison to insects and is sold as spray concentrates and ready to use dust. Taking for granted that the plant is composed of rotenone this experiment was initiated with the objectives of investigating the toxicity potential of the plant against one of the most important insect pests of sorghum, sorghum chaffer (*Pachnoda interrupta*) (Oliver) (Coleoptera: Scarabaeidae) and mound-building termites (*Macrotermes subhyalinus* and *Macrotermes herus*) (Isoptera: Termitidae). Birbira has been observed to be very effective in controlling insects pests (Sabitii, 2002; Jembere, 2002; Damte and Chichaybelu, 2002) and the present study also

focused on how effective can the Birbira plant materials could be compared to other plant materials.

2. MATERIALS AND METHODS

2.1 Plant material collection

For the experiment on sorghum chaffer: Birbira (*M. ferruginea*) seeds were collected from Addis Ababa, air dried and ground to a fine powder using grinding machine. Neem (*Azairacta indica*) seeds collected from Melkawerer Research Center were depulped, air dried and ground to fine powder with grinding mill. The root of Rungia (*Rungia grandis*) was collected from North Shoa Rasa village, air dried and grounded using mortal and pestle. Similarly Aloe (*Aloe debrana*) plant was obtained from Rasa village and the leaves were chopped into smaller pieces using knife and grounded with mortal and pestle. Carbaryl insecticide was obtained from Crop Protection Department of Ministry of Agriculture.

For the experiment on termites, mature green whole leaves and fruits of *Datura stramonium* L. and leaves and stem bark of *Croton macrostachys* Hochest used for the laboratory test were collected from Melkassa Agricultural Research Center. Stem bark of *Croton* used for the field application was collected from Mukiye forest found in Mukiye Peasant Association, west of Meki town. Mature pods of *M. ferruginea* 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 were obtained from the Agricultural Bureau of Dire Dawa Administrative Council. A synthetic insecticide, Chlorpyrifos 48% EC (Dursban®) was purchased from Chemtex PLC, Addis Ababa.

2.2. Extraction

2.2.1. For bioassay on sorghum chaffer

Different polar and non-polar solvents were used for the extraction of the different botanicals. The powders of the Birbira and Neem seeds, and Rungia roots, and chopped and ground leaves of Aloe were dissolved in different solvents (distilled water, acetone, hexane, ethanol and chloroform) at the rates of 5, 10, 15, 20,30g per 100ml of each solvent. The solution was allowed to stand for 24 hours for extraction. After 24 hrs the mixture was filtered with cheese cloth and filter paper (Jembere, 2002) and the filtrate was used for the different treatments.

2.2. 2. For bioassay on termites

Fresh fruits and leaves of *D. stramonium*, stem bark and leaf 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). Ten, 25 and 40 grams of each ground plant materials were soaked in 100ml of water to obtain crude extracts of three concentration levels of 10, 25 and 40%, (w/v). Each mixture was filtered with clean cheese cloth after 24h. The three concentration levels were determined based on preliminary extraction and toxicity evaluation studies. Chlorpyrifos was diluted with water to make 0.21% based on the standard field application rate.

For soil sawdust mixture test, based on the results of filter paper bioassay, three plant materials which caused the higher mortality rates , i.e., cheesecloth filtered 40% stem bark water extract of *C. macrostachys*, seed extract of *A. indica* and 10% seed extract of *M. ferruginea* were used.

2.3. Insect collection

Adult beetles of sorghum chaffer were collected from Melkaworer Agricultural Research Center and from Shoarobit, Rasa village. These beetles were kept in the Entomology Laboratory of Department of Biology and fed with banana until the experiment was carried out.

Mature workers, major and minor soldiers of *Macrotermes* termites were collected from mounds at Melkassa Agricultural Research Center. Based on the methods of Gitonga *et al.* (1995) and Umeh and Ivbijaro (1997), 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 a plastic box (22 x 17 x 7cm). The boxes containing soil and moist cotton wool were provided with fine aeration holes in the lid, (used to maintain the necessary humid conditions). Similarly, live alates were collected during actual flights based on the procedures of Ochiel *et al.* (1995).

2.4. Bioassay

2.4.1. Bioassay on sorghum chaffer

Two milliliter of the filtrates of *Milletia*, *Neem*, *Rungia* and *Aloe* extracts were applied to What Man No.1, 9cm diameter filter paper contained in a Petri dish and in the case of organic solvents the treated filter papers were exposed to open air to allow the solvent evaporate for 30 minutes (acetone, chloroform and hexane) and for 60minutes for ethanol, depending on the evaporative property of the solvent. After the solvents have evaporated completely 1ml of distilled water was applied to the treated filter paper to moisten it and 5 beetles were introduced and mortality was recorded after 24 and 48 hours. The different solvents were used as a control and each experiment was replicated three times.

2.4.2. Bioassay on termites

2.4.2.1. Filter paper bioassay

Whatman No.1 filter papers of 9cm diameter were placed in Petri dishes and treated separately with 2ml 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 the *Macrotermes* termites were selected and placed into the Petri dishes containing the 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, 9 dia.). 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 24h after treatment. The experiments were conducted under room conditions ($25 \pm 3^{\circ}$ C and 60 - 70% r.h.).

2.4.2.2. Soil/ sawdust bioassay

A plastic box (24 x 10 x 6cm) containing 450g of soil and 10g of sawdust added as food (Gurusbramanian *et al.*1999) was treated with 150ml of each of the extracts. Then, twenty workers, minor soldiers and major soldiers, and five healthy alates of the termites were placed in each box and covered with a black plastic sheet. The same volume of 0.21% Chlorpyrifos EC 48% and water were used as a standard check and control, respectively. The treatments were replicated three times. Mortality was recorded once after 24h of treatment.

2.5. Data analyses

The mortality data obtained in the different treatments were analyzed using the SPSS computer software (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 percent mortality were transformed where necessary (arc sine) before analysis based on the method of Gomez and Gomez (1984).

3. Result and discussion

3.1. Toxicity of Birbira extract to sorghum chaffer as compared to different plant extracts

In all cases of the different solvents lower doses (5-15g/100ml of solvent) caused no mortality of the insect 24 and 48hrs after treatment. However, when the 20g/100ml dose was used mortality rates were 40 and 53.33% for water extract 24 and 48 hrs after treatment, respectively (Fig.1). Furthermore, when 30gm/100ml water extract was used mortality increased to 46.67 and 66.67% after 24 and 48hrs of treatment, respectively (Fig.2). From probit analysis the LD₅₀ of *Milletia* when applied as contact was found to be 24.54mg/filter paper. There was no mortality due to acetone extract at the dose of 20gm/100ml however, at 30gm/100ml, 13.33 and 26.67% mortality was obtained 24 and 48hours after treatment, respectively (Fig. 2). From the different solvents used water was found to be most effective as compared to all other solvents.

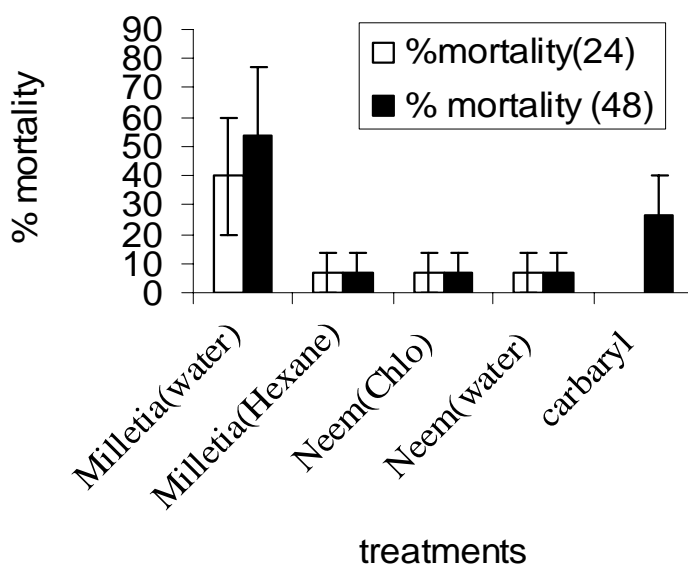


Fig.1. Percentage mortality of *P. interrupta* due to different solvents extract of different botanicals at 20g/100ml and carbaryl after 24 and 48 hours of treatment.

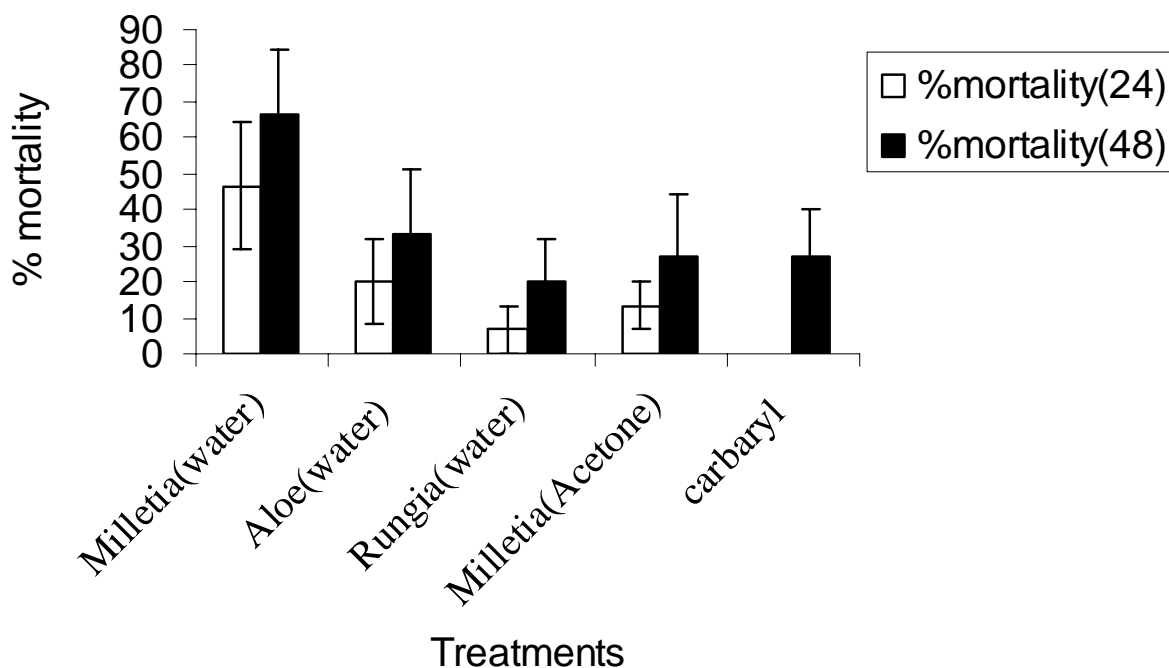


Fig 2. Percentage mortality of *P. interrupta* due to water and acetone extract of different botanicals at 30g/100ml and 1.5kg/400L of carbaryl after 24 and 48 hours of treatment.

At lower doses the beetles were found to be highly tolerant to the different tested botanicals (Milletia Neem, Rungia and Aloe) including the insecticide carbaryl at the recommended rate. These doses of Milletia were highly effective to other insects (Jembere, 2002). Thus, higher doses were required to kill the beetle as compared to other insects. This may be because the beetles are highly sclerotized. The low mortality of *Pachnoda* treated with carbaryl may be associated with the development of resistance by the beetle to the insecticide, since carbaryl is the only insecticide in use for the control of the pest in the locality where the beetle was collected (personal observation and communication).

3.2. Toxicity of Birbira extract to termites as compared different plant extracts

3.2.1. Filter paper bioassay

All extracts which were further filtered with filter paper did not show significant mortality of all the casts of the termites. However, there was a significant difference ($P < 0.05$) among the different botanical treatments, extracted with cheese cloth only, at all levels of concentration. From all the 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 casts (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 mortality of worker termites after Milletia seed at 10% (Table 1a). From all the botanical treatments extracted at 25%, 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 than the rest of the treatments except milletia seed and chlorpyrifos.

Table 1. Mean percent mortality of termite castes due to water extracts of different botanicals.

a: at 10% concentration

Treatment	Mean % mortality \pm SE			
	Alates	Major soldiers	Minor soldiers	Workers
DF	6.67 \pm 6.67c	3.33 \pm 1.67cd	8.33 \pm 1.67d	6.67 \pm 1.67e
DL	0.00 \pm 0.00c	6.67 \pm 1.67bc	8.33 \pm 4.41d	15.00 \pm 0.00d
CL	13.33 \pm 6.67c	3.33 \pm 1.67cd	18.33 \pm 7.26cd	11.67 \pm 1.67de
CB	33.33 \pm 6.67b	13.33 \pm 3.33b	83.33 \pm 4.41b	38.33 \pm 8.82c
NS	0.00 \pm 0.00c	10.00 \pm 5.0bc	23.33 \pm 1.67c	76.67 \pm 1.67b
MS	93.33 \pm 6.67a	100.00 \pm 0.0a	100.00 \pm 0.0a	100.00 \pm 0.0a
CP	100.00 \pm 0.0a	100.00 \pm 0.0a	100.00 \pm 0.0a	100.00 \pm 0.0a
C	0.00 \pm 0.00c	0.00 \pm 0.00d	0.00 \pm 0.00e	0.00 \pm 0.00f

b. at 25% concentration

Treatment	Mean % mortality \pm SE			
	Alates	Major soldiers	Minor soldiers	Workers
DF	20.00 \pm 11.55bc	3.33 \pm 1.67cd	21.67 \pm 6.67d	10.00 \pm 0.00d
DL	20.00 \pm 11.55bc	8.33 \pm 1.67c	16.67 \pm 3.33d	36.67 \pm 14.24c
CL	13.33 \pm 6.67bc	5.00 \pm 2.89c	40.00 \pm 2.89c	13.33 \pm 1.67d
CB	33.33 \pm 6.67b	21.67 \pm 4.41b	88.33 \pm 4.41b	65.00 \pm 5.00b
NS	0.00 \pm 0.00c	8.33 \pm 1.67c	56.67 \pm 14.53c	96.67 \pm 1.67a
MS	100.00 \pm 0.0a	100.00 \pm 0.0a	100.00 \pm 0.0a	100.00 \pm 0.0a
CP	100.00 \pm 0.0a	100.00 \pm 0.0a	100.00 \pm 0.0a	100.00 \pm 0.0a
C	0.00 \pm 0.00c	0.00 \pm 0.00d	0.00 \pm 0.00e	0.00 \pm 0.00e

c: at 40% concentration

Treatment	Mean % mortality \pm SE			
	Alates	Major soldiers	Minor soldiers	Workers
DF	20.00 \pm 0.00c	0.00 \pm 0.00e	21.67 \pm 3.33d	8.33 \pm 3.33c
DL	20.00 \pm 0.00c	11.67 \pm 1.67c	38.33 \pm 9.28d	68.33 \pm 16.67b
CL	80.00 \pm 0.00b	5.00 \pm 0.00d	66.67 \pm 8.82c	13.33 \pm 3.33c
CB	93.33 \pm 6.67a	21.67 \pm 3.33b	86.67 \pm 4.41b	83.33 \pm 1.67b
NS	6.67 \pm 6.67d	5.00 \pm 2.89d	73.33 \pm 6.01bc	100.00 \pm 0.00a
MS	100.00 \pm 6.67a	100.00 \pm 0.00a	100.00 \pm 0.00a	100.00 \pm 0.00a
CP	100.00 \pm 0.00a	100.00 \pm 0.00a	100.00 \pm 0.00a	100.00 \pm 0.00a
C	0.00 \pm 0.00d	0.00 \pm 0.00e	0.00 \pm 0.00f	0.00 \pm 0.00d

Means within a column followed by the same letter are not significantly different at 5% level of significance, SNK. DF = Datura fruit, DL = Datura leaf, CL = Croton leaf, CB = Croton bark, C = Chlorpyrifos NS = Neem seed, MS = Millettia seed, C = Control

Except *Datura* fruit on major soldiers, all the extracts at 40% concentration showed significant mortality of the termite castes compared to the control. *Milletia* seed and *Croton* bark at 40% caused high mean % mortality to alate termites similar to that of chlorpyrifos which caused 100% mortality. *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 level of concentration, alate termites were more susceptible to *Croton* bark and *Croton* leaf at 10% and 20% and to *Croton* bark, *Datura* fruit and *Datura* leaf at 40% 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 to workers and minor soldiers than to other castes.

3.2.2. Soil/sawdust bioassay

Significant differences ($P < 0.05$) were observed among the treatments for mean % mortality of the termite castes. *Milletia* at 10% caused the highest mortality (100%) of all the castes and this was similar to the effect of the synthetic insecticide, chlorpyrifos. Second to *Milletia* seed at 10%, *Croton* bark at 40% induced high mortality of the castes except for alates, which were not significantly different from neem seed and control (Table 2).

Table 2. Mean percent mortality of termite castes due to soil treated with water extracts of NS 40%, CB 40% and MS 10%

Treatment	Mean % mortality \pm SE			
	Alates	Major soldiers	Minor soldiers	Workers
NS	6.67 \pm 6.67b	11.67 \pm 3.33c	11.67 \pm 1.67c	23.33 \pm 6.01c
CB	13.33 \pm 6.67b	25.00 \pm 5.00b	58.33 \pm 9.28b	63.33 \pm 7.24b
MS	100.00 \pm 0.00a	100.00 \pm 0.00a	100.00 \pm 0.00a	100.00 \pm 0.00a
CP	100.00 \pm 0.00a	100.00 \pm 0.00a	100.00 \pm 0.00a	100.00 \pm 0.00a
C	0.00 \pm 0.00b	0.00 \pm 0.00d	0.00 \pm 0.00d	0.00 \pm 0.00d

Means in a column followed by the same letter are not significantly different at 5% level of significance, SNK. NS = Neem seed, CB = *Croton* bark, MS = *Milletia* seed, CP = Chlorpyrifos, C = Control

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, chlorpyrifos. Almost all the termites treated with this botanical died within a few hours of application. Jembere (2002) evaluated the toxicity of *Milletia* seed against *Sitophilus zeamais* and reported higher mortality of the weevil within 48 hours after treatment. The toxicity of the plant can be attributed to rotenone which 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; George, 1980). Damte and Chichaybelu (2002) also tested the toxicity of *Milletia* seed against Adzuki bean beetle, *Callisobruchus chinunesis*, and found that it gave complete protection of stored chickpea for six months in the laboratory. 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.

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