Journal of Science and Sustainable Development (JSSD), 2018, 6(2), 51-61

ISSN: 2304-2702 (print); 2414-4479 (online)
DOI: https://doi.org/10.20372/au.jssd.6.2.2018.089

Investigation and chemical composition of plants used as anti-termite infestation and repellant in eastern Tigray, Ethiopia

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Abstract

Development of insect resistance to synthetic chemical, the high operational cost, environmental pollution, toxicity to humans and harmful effect on non-target organisms have created the need for developing alternative approaches to control insect pest. Botanical survey and extraction of top three plants was conducted to identify the plants used against termites' infestation and to screen chemical composition. The present research reports a total 15 plants used to control termites' infestation as the information collected and recorded from the study area (table-2). Phytochemical analysis was conducted for top three plants depends on informants' ideas and extracted by Maceration with acetone (Nicotiana glauca Graham, Euphorbia cactus Boiss and Aloe vera L) showed that presence of various active ingredients (table-5). The highest repellency was induced by positive control called diazinon (98.33%). Acetone extract of Nicotiana glauca Graham was found to be more repellency at 1.00gm/mL (90.48%) at all concentrations than Euphorbia cactus followed by Aloevera L however, no repellence was observed for negative untreated control. Acetone extract of Nicotiana glauca Graham test has shown best level of mortality than Euphorbia cactus Boiss and Aloe vera L extracts.

Keywords: Ethno-botanical, Termites, Mortality, Repellency

Introduction

The environmental problems caused by overuse of pesticides have been the matter of concern for both scientists and public in recent years (Opender et al., 2008). Currently different kinds of preventive and curative control measures are practiced to get protection from insect pests (Benhalima.et al., 2004). Plants offer an alternative source of insect-control agents because they contain a range of bioactive chemicals, many of which are selective and have little or no harmful effect on non-target organisms and the environment (Asgar, 2013). They are safe and eco-friendly. They are more compatible with the environmental components than synthetic pesticides (Cavoski et al., 2011).

Botanical insecticides degrade rapidly in air and moisture and are readily broken down by detoxification enzymes. This is very important because rapid breakdown means less persistence in the environment and reduced risks to non-target organisms (Isman, 2008). 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 (Isman, 2008).

Repeated applications of synthetic insecticides have disrupted natural enemies in the biological control system and led to outbreaks of insect pests, widespread development of resistance and environmental and human health concerns (Sagheer et al., 2013). Termites are a group of insects consisting of 2,500 species of which 300 are considered pests. Termites have long been recognized as important agricultural and domestic pests. Termites attacks on trees and field crop can also cause significant losses (Sagheer et al., 2013;

Delate and Grace, 1995). In protecting plants as well as structures from termites attack, soil insecticides are currently the principal tool (Tapondjou et al., 2005; Akutse, 2012).

The floras found in Ethiopia are diverse and expected that, there are varieties of botanical insecticides. However, there are only few studies made so far on plants used anti-termite infestation in the country and there was no study has been conducted and documented plant that act as anti-termites in eastern Tigray. Therefore, this study was intended to investigate the plants used as anti-termite infestations traditionally as well as bioactive constituents of plants were investigated.

Materials and methods

Description of study area

The study was conducted in Eastern Tigray, North Ethiopia. Eastern Tigray is found in Northern part of Ethiopia, which is about 818 km far from Addis Ababa and about 35 km from Mekelle town, the capital city of Tigray regional state. It is located at altitudinal ranges from 2000-3000 m.a.s.l and geographically located 14°16"34' N latitude and 39°27 "52' E longitudes. It has a unimodal rain fall distribution with the highest rain falling from June to early September. Annual average rain fall of this zone ranges from 450mm-600mm and the minimum and maximum temperature is 6 and 21^{o}C. The income of most inhabitants of the study area is depending on agricultural activities and termites causes the economic loss to many crop pants and tree species. However, farmers use different plants for controlling the termite attack as the result of finding in study area indicate.

Field Survey and Ethno botanical Data Collection

An ethno-botanical survey was conducted to gather information on the traditional usage of plants in anti-termite Infestation and repellant using a semi-structured interview, observation and field guided walks with local farmers and people in study area who had willing to share their indigenous knowledge. Among the seven woreda, four were selected randomly. Those Woredas are Wukura, Gulomekeda, Fatsi and Atsibi. Interviewed was undertaken appropriately and all information regarding plants under investigation were recorded based on respondents' knowledge on these traditional medicine and information forwarded by them. The interview was conducted with two hundred interviewees. During collection of information regarding habitat data, part of the plant used as anti or repellency, effective of the plant relative to the other and traditional ways of use were recorded.

Plant sample collection

Top three plants were selected and collected for chemical information, based on the information provided by the local farmer and people in study area during the survey. Healthy parts of these plants were collected locally from the areas. After collection, samples were transported to chemistry department of Adigrat University. The leaves of the sample were washed with water and dried under shaded area at room temperature.

Extraction

After dried, each plant was grinded by mortar and pistol, weighed and stored at room temperature. Maceration method was used for this extraction. 100gm plant materials were extracted using acetone for one day for each sample. The same method was repeated until enough crude residues were collected. The solvent was evaporated to dryness. The remaining extract was dried in vacuum oven to remove any residual solvent. The crude residues were then

weighed and kept in a refrigerator (-4 ^{o}C) until Phytochemical test was done

Phytochemical analysis of acetone extract

The screening and study for phytochemical constituents was performed using generally accepted laboratory technique for qualitative determinations. Screening was carried out for crude extract to identify the active chemical constituents. The tests were done for saponins, Terpinoids, flavonoids glycosides, steroids, Anthraquinone, Phenolics, Tannin and Carotenoids (Harbone, 1973; Gibbs, 1974; Ayoola, 2008; Horwitz, 2000; Treare and Evans, 2008).

Test for Glycosides

2mL of concentrated H_2SO_4 was added carefully to 1gm of each crude extract in test tube and shaken gently. A reddish-brown color was observed for all extracts. This indicated the presence of glycone portion of the glycoside

Test for Phenols

1gm of each Crude extract was mixed with 2mL of 2% solution of $FeCl_3$ in separate test tube. A bluish-black was observed for each extract

Test for Saponins

1gm of each crude extract was mixed with 5mL distilled water in a test tube and it was shaken vigorously. Stable foam was formed for all the extracts. This taken as an indication for the presence of saponins.

Test for Tannins

1grm each crude extract was boiled in 10mL of water in a test tube and then filtered. 3drops of 0.2% ferric chloride were added and a blue–green was observed.

Test for Terpenoids

1grm of each crude extract was dissolved in 2mL of chloroform and evaporated to dryness. To each 2ml of concentrated H_2SO_4 was added and heated for 2 minutes. A reddish-brown colour was observed (Bohnenstergel, 1999).

Test for Flavonoids

1grm of each Crude extract was mixed with 2mL of 2% solution of NaOH in separate test tubes. An intense yellow color was formed for all the three samples which turned colorless on addition of 3drops of dilute HCl which indicated the presence of flavonoids

Test for Steroids

1grm each crude extract was mixed with 2mL of chloroform and concentrated H_2SO_4 was added sidewise. A red color was produced in the lower chloroform layer. This result was indicating the presence of steroid.

Test for Anthraquinone

Each 0.5gm of the extract was boiled with 10mL of sulphuric acid and filtered while hot. The filtrate was shaken with 5mL of chloroform. The chloroform layer was pipette into another test tube and 1mL of dilute ammonia was added. The resulting solution was observed for colour changes.

Test for Carotenoids

Sulphuric acid was added to 0.5gm extract and the color change was observed.

Repellency and antitermitic activities

Experiments were performed at chemistry department, Adigrat University. The Practical applications of plant extract testing against termites'. Plant extracts test were checked for both insects' repellent and insecticidal activities.

Insect pests' collection

Termites were collected from Adigrat area by digging up their mound with spade by the help of local farmers and soil contain termites was collected on plastic sheet. Termites were collected from plastic sheet and placed in to polyethylene plastic box. Grinded dry leaf, wood plants and some soil with moist were added to plastic box and covered by muslin cloth and transported to chemistry laboratory and experiment was done without consuming time.

Repellent test

Method described by Tapondjou was used in this experiment (Bohnenstergel, 1999). Test solutions were prepared by dissolving plant extracts in distilled water (0.25, 0.5, 0.75 and 1.00 w/v). Each Whatman filter paper circle (9cm in diameter) was cut into two halves to fit into glass petri dish and each plant extract was applied to a half filter paper as uniform as achievable using a pipette. The other half was treated only with distilled water and it served as control. A filter paper was placed on the bottom of the glass Petri-dish. 20 termites were released separately at the center of each filter paper disc. The Petri-dishes were covered and placed in dark place to minimize the effect of light on the termites. Three replications were performed for each concentration of extract and positive control. The number of termites on treated and untreated filter paper was counted for each dish starting from 30mi after treatment exposure. Percentage repellency (PR) for a given treatment time was obtained using the formula:

$$PR = [(Nc-Nt) / Nc+Nt] \times 100,$$

Where Nc= the number of termites in the untreated (control) and Nt = treated areas, respectively with positive values of PR indicate attractancy (Huang, 2002). Along with this standard repellent 0.20% Diazinon was used as a positive control (Huang, 2002; Bekele and Hassanali, 2001). the same experimental conditions were utilized as the extracts.

Mortality bioassay

For percentage mortality calculation, different concentrations (0.25, 0.5, 0.75 and 1.00) were obtained by distilled water. Solution of Diazinon standard was also prepared by dissolving in distilled water at the same concentration. Both the solutions were applied on to the Whatman filter circle in each of the Petri-plate (9 cm diameter). Control was prepared by treating the filter paper with distilled water only. 20 termites were released separately into each treated and control Petri-plate. Dead insects were counted after 24 hours of exposure. The mortality (%) was calculated in the treated filter papers and compared with control. Percentage insect mortality was calculated using Abbott formula (Tapondjou et al., 2005; McGovern, 1977)

$$\% \text{ mortality} = (1-Nt/Nc)*100$$

Where: Nt = number of dead insects in treated, Nc= number of insects in control

Data analysis

Percentage and tables were used to summarize botanical plants data, phytochemical result, repellency and mortality data. Phytochemical, repellency and mortality analysis were done in triplicate.

Table: 1. Description of botanical plants used for the experiments and their concentration (g/ml)

Botanical name	Vernacular name	Part used	Conc.(g/mL)
Nicotiana glauca Graham	Teklay Tegegne(T)	Leaf extract	0.25
			0.5
			0.75
			1.00
Euphorbiacactus Boiss	Qulqual /hamat(T)	Cladode extract	0.25
			0.5
			0.75
			1.00
***Aloe vera* L.**	Era(T)	Cladode extract	0.25
			0.5
			0.75
			1.00
Control	*Distilled water(mL)*		3
	Diazinon (%)		0.21

Results

Traditional remedies against termites

Different crop types are cultivated in study areas, as the woreda people lead their life based on crop production. According to information collected from the informants, individual farmer production is used for household consumption and local markets. Farmers in these woredas used different plants to control termites' infestation. During the survey, 15 traditional plants remedies were identified and recorded from respondents in the control of termites from crops. Table: 1 presents an overview of these remedies, with their respect families and way of use according to the informants.

Table 2 Traditional medicinal plants used as ant termites and repellency by local farmers from Eastern Tigray, Northern Ethiopia **(**T=Tigrigna)

Botanical name	**Family name**	**Localname (T)**	**Habit**	**How local people use**
Nicotianaglauca Graham	Solanaceae	TeklayTegegne(T)	Shrub	-Fresh leaves are collected and placed on the place where termites found and used as repellent
Maesa lanceolata	Maesaceae	Saoria	grass	- used as repellent
Chenopodium album L	Amaranthaceae	Hamedmado	herb	-used as repellence
Croton macrostachyus	Euphorbiaceae	Tunbako	Tree	-used as repellency and make soil fertile
Tagetes minuta	Asteraceae	Etsefaruos	Annual	-used as repellence and

			herb	make soil fertile
Datura stramonium	Solanaceae	Mestenagr	annual herb	-used as repellence
Euphorbia cactus Boiss	Euphorbiaceae	Qulqual /hamat	clumping	-used as repellency and also protect the soil from erosion
Nicotiana tobaccum.l	Solanaceae	Tunbako	herb	- expect it as repellence
Schinus molle	Anacardiaceae	Tselim berbere	tree	-used as repellence
Ficus vasta	Moraceae	Daero	Tree	-used as repellence
Dodonaea viscose.l	Sapindaceae	Sonkuah.	shrub	- people expect it as repellence
Euphorbia petitiana **A. Rich.**	Euphorbiaceae	Demaito demu	herb	-used as repellence
Laggera tomentosa	Asteraceae	Kasho	herb	-used as repellence
*Aloe vera.***l**	Aloaceae	Era	herb	-used as repellency and also protect the soil from erosion
Ficus palmata Forssk	*Moraceae*	beless	Shrub/small tree	-used as repellence

According to respondents, local farmers used plants either to repel termites from their farm land to other or they belief that some plants are used as killer of termites. Depending on informants' ideas, around 93.3% of recorded plants used as repellency and around 6.7% of the total identified plant used as killer (*Nicotianaglauca Graham)* (Table 2).

Diversity of plants used to control termite infestation by local farmers from Eastern Tigray, Northern Ethiopia

The present research work reported total, 15 traditional plant remedies used by local farmer and people to control termite infestation. These traditional plant remedies belong to 10 families with Solanacea, Maesaceae, Amaranthaceae, Euphorbiaceae, Anacardiaceae, Moraceae, Sapindaceae and Aloaceae (table:3)

Table 3: Families of plants used among farmers and local people to control termite's infestation in the study areas.

	Family	Number of species	Proportion (%)
1.	Solanaceae	3	**20**
2	Maesaceae	1	**6.7**
3	Amaranthaceae	1	**6.7**
4	Euphorbiaceae	3	**20**
5	Asteraceae	2	**13**
6	Anacardiaceae	1	**6.7**
7	Moraceae	2	**13**
8	Sapindaceae	1	**6.7**
9	Aloaceae	1	**6.7**

Habitat, growth forms and threats of medicinal plants

Habitat

The Study reveals that, around 60% of identified Plants were found around house and land farm however, 40% takes long time and distance to collect necessary data. Regarding to the growth forms, the research identify that the herbs were the widely used for the termite control with largest percentage (47%) followed by tree (27%) and shrubs (20%) with minimum of grass and clumping.

Phytochemical analyses of acetone extract of Nicotiana glauca Graham, Euphorbia cactus Boiss and Aloe vera L.

Top three plants were selected for phytochemical screening depends on respondents and presences of deferent active ingredients were assured and organoleptic evaluation of acetone extracts (color) was take place during extraction and observations color for each extract was recorded (Table 4)

Table 4 Organoleptic evaluation of acetone extracts of *Nicotiana glauca Graham*, *Euphorbia cactus Boiss and Aloe vera*

acetone extract	Colour	Review literature
Nicotiana glauca Graham	black	-
Euphorbia cactus Boiss	White	-
***Aloe vera* L.**	Orange(lighter)	-

Table 5 Phytochemical analysis of acetone extracts of *Nicotiana glauca Graham*, *Euphorbia cactus Boiss and Aloe vera* L.

Constituents	***Nicotiana glauca Graham***	**Observation**	*Euphorbia cactus* ***Boiss***	**Observation**	*Aloe vera* **(L.) Burm.f**	**Observation**
Flavonoids	+	Yellow color	+	Yellow color	+	Yellow color
Glycosides	-	black	+	A reddish-brown color	+	reddish brown color
Carbohydrates	-	No colour change	+	blue colour	+	blue colour
Anthraquinone	-	No colour change	-	No colour change	+	Formation of cloudiness
Tannin	-	yellow	+	Blue-Green color	+	Blue-Green
Saponin	+	Formation of foam	+	Formation of foam	+++	Formation of foam
Terpinoids	+	A reddish brown colour	+	A reddish brown colour	+	A reddish brown
Steroids	+	A red color	+	A red color	-	No colour change
Phenol	-	No colour change	+	A bluish-black	+	A bluish-black
Carotenoids						

Table 6 Mean percentage repellency of termite due to acetone extract of *Nicotiana glauca Graham*, *Euphorbia cactus Boiss and Aloe vera* L.

Concentrations (g/mL)	***Nicotiana glauca Graham***	***Euphorbia cactus Boiss***	***Aloe vera* L**
0.25	66.67±0.32	60.00±0.35	42.86±0.50
0.50	73.91±0.3	66.67±0.15	48.20±0.32
0.75	81.82±0.15	73.91±0.2	60.00±0.29
1.00	90.48±0.1	81.82±0.32	66.67±0.15
Water(3mL)	0.00		
Diazinon (0.20)	98.33±0.00		

Table 7 Mean percentage mortality (%) effect of worker termites after 24hr exposure of acetone extract of *Nicotiana glauca Graham*, *Euphorbia cactus Boiss and Aloe vera* L.

Concentrations (g/mL)	Time (hrs)	*Nicotiana glauca Graham*	*Euphorbia cactus Boiss*	*Aloe vera* L
0.25	24	71.67±0.5	68.33±0.5	58.33±0.21
	48	83.33±0.2	81.67±0.3	78.33±0.1
	72	98.33±0.1	93.33±0.1	91.67±0.2
0.50	24	81.67±0.29	76.67±0.41	68.33±0.12
	48	93.33±0.3	88.33±0.32	83.33±0.15
	72	100±0.00	98.33±0.5	96.67±0.2
0.75	24	91.67±0.1	85.00±0.03	73.33±0.52
	48	98.33±0.32	98.33±0.1	88.33±0.3
	72	100±0.00	100±0.00	98.33±0.35
1.00	24	96.67±0.21	93.33±0.1	83.33±0.5
	48	100±0.00	100±0.03	98.33±0.29
	72	100±0.00	100±0.00	100±0.00
Diazonin(0.21)	24	100±0.00		
	48	100±0.00		
	72	100±0.00		
Water(3mL)	24	0±0.00		
	48	0±0.00		
	72	0.00		

Discussion

Different people who are indigenous for different local have their own knowledge on plant use, management and conservation (Cotton, 1996). The people of this study area have a long history of using medicinal plants (Abebe and Ayehu, 1993). Termites have been regarded as serious insect pests that attack a wide range of crops, trees and different objects in eastern Tigray. People in this study area use different plants to Control termites' infestation; this is one part of this knowledge whether they think as repellency, antifeedant or anti activities of these animals. The present research reports a total 15 plants used to control termites' infestation as the information collected and

recorded from the informants (Table 2). This study similar to research conducted by Aschalew and Ahimad (Aschalew, 2008) reported the use of Tagetes *minuta* , *Chenopodium album L*, *Nicotiana tobaccum* and *Datura stramonium* as pesticidal plants against termites in Bako, Oromia regional state, Ethiopia.

This study shows that, around 40% of the plants are found away from house and takes long time and distance to collect data, however, 60% of the plants are found around house and land farm and take short time and distance to collect necessary data and samples.

Phytochemical analysis was conducted for top three plants depends on informants' ideas and extracted by Maceration with acetone (*Nicotiana glauca Graham*, *Euphorbia cactus Boiss and Aloe vera* L) showed that presence of various active ingredients like Flavonoids, Glycosides, Carbohydrates, Anthraquinone, Tannin, Saponin, Terpinoids, Steroids, Phenol and Carotenoids(table-5). Furthermore, isolation and characterization of these plants will be needed by the help of advanced spectroscopic instruments and others to isolate new candidate drugs which against termites.

Repellent responses were observed by using serial concentrations 0.25, 0.50, 0.75 and 1.00gm/mL. The repellency effect of the plant extracts on termite is presented in (table-6). Repellency test of acetone extracts of three plants at different concentrations (0.25, 0.5.0.75 and 1.00gm/mL) on termites showed different repellency effect. The highest repellency was induced by positive control called diazonin (98.33%). Among the all extracts, acetone extract of *Nicotiana glauca Graham* was found to be more repellency at 1.00 gm/mL (90.48%) and also for all concentrations than *Euphorbia cactus* followed by *Aloevera* L however, no repellence was observed for negative untreated control. There is correlation between concentrations of acetone extracts and repellency that means as concentration of the botanical extract increase repellency increase.

Result obtained showed that, the extracts of these tested plants were effective against termites. As concentration of botanical extract (dose) and time increase, percentage mortality increase. At every interval of time, exposure of termites to different botanical extracts, there was highly significance difference in mortality. Acetone extract of *Nicotiana glauca Graham* test has shown best level of mortality than *Euphorbia cactus Boiss and Aloe vera* L extracts. The effect of *Nicotiana glauca Graham* extract was very spectacular even at lower temperature. The Maximum percentage mortality was recorded from diazinon followed by *Nicotiana glauca Graham.*

Conclusion

The Present finding revealed that people in study area have different knowledge of controlling termite infestation. One of the good methods is using ethno-botanical which has multiple uses for farmers. The results demonstrate that all the three botanical extracts tested against termite possess termiticidal properties that can be used in controlling termites. This toxicity is an implication of presence of insecticidal compounds in all the extracted plants. And also, phytochemical analysis showed that presence of various bioactive organic compounds. This indicate that these plant extracts could serve as an alternative method as chemical control.

Recommendations

1. Documentation of plants used to control termite infestation should be continued from all part of Ethiopia to

save indigenous knowledge and transfer to the next generation as well as to prepare precondition for extraction.

2. The study assures that botanical extract of the three plants becoming power full and control termite infestation instead of chemicals that have side effects, so, specific organic compound responsible for this resistance should be identified by NMR, IR and GC-MS.

Acknowledgment

We are thankful to Adigrat University Aacademics, Research and Community Services for giving the opportunity in terms of financial supports for this research work.

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