International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 19, No. 1, p. 55-64, 2021

INNSPUB

RESEARCH PAPER OPEN ACCESS

Termicidal activity of *Dalbergia sissoo, Heterophragma adenophylum, Grewia asiatica* and *Punica granatum* against subterranean termite *Heterotermes indicola*

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Key words: *D. sisoo, H.indicola.* Mortality, *termite gut.*

http://dx.doi.org/10.12692/ijb/19.1.55-64 Article published on July 31, 2021

Abstract

The present research was planned to assess the toxic nature of leaves extracts of Dalbergia *sissoo*, *Heterophragma adenophylum*, *Grewia asiatica* and *Punica granatum* against subterranean termite *Heterotermes indicola*. Which is very destructive termite and is widely distributed in Pakistan and all over the world. It caused loss of billions of dollars throughout the world every year. It mainly attacks on wood and wooden material including furniture and timber. It also destroys paper, cloths and buildings throughout the world. Different chemicals used in termite control have harmful effects on human health and environment. So alternative methods should be used. Replacement of chemical insecticides to bio-pesticides is great revolution as they are nontoxic and eco-friendly. Purpose of experiment was to explore termiticidal and protozoidal activity of leaves extracst of *D.sissoo, H.adenophylum*, *G. asiatica* and *P. granatum* as they are eco-friendly. The extract was prepared by making fine powder of leaves. Soxhlet apparatus was used for extraction. All of the three formulations tested were found to be effective in termite mortality and protozoa count, *D.sissoo* leaves extract was found to be very effective than other plants extracts used in study. So the toxicity of these plants can be expressed in following order: *Dalbergia sissoo>Heterophragma adenophylum >Grewia asiatica >Punica granatum* Leaves extracts against mortality and protozoans of *H.indicola. and D. sisso* was found to be more effective against termite and caused loss of protozoans' number in termite gut as compare to control group.

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Introduction

Termites being social insects live in form of colonies (Peterson *et al.,* 2006). Termites are social insects having complete developed linage system. It has been reported about 2600 species of termites are spread in all over the world, and among them 300 species are considered economically important (Ibrahim and Adebote, 2012).

Being ability to digest wood termite is economically most important in some major parts of world (Eggleton *et al.,* 1996; David and diana, 2015). Regional generic and the species of local diversity are highly affected by the climate and habitat. The highest diversity of termite is found in flat land tropical forests. Latitudinal and longitudinal (inter-tropical) gradients are highly influenced termite diversity (Eggleton, 2000). According to studies, ethiopian biogeographic region is mostly termite free while Indo-Malayan regions have less diversity of termite. This diversity of termite is based on theory of net primary productivity (NPP) but later on this theory was rejected. It was hypothesized that different climatic conditions of different regions were responsible for difference in termite diversity. Wet tropical rain forest of south East Asia and America is richest in termite diversity (Groombridge, 1992). Alternating period of climate change had effect the diversity of termite throughout the geographical regions. As the latitude increase, diversification of taxonomic species decrease of temperature with altitude is main reason of low diversity of termite species (Willing *et al.*, 2003 and Hillebrand, 2004).

Heterotermes indicola (Wasmann) and *Reticulitermes flavipes* (Kollar) are the most important termite and considered to be pest in South East Asia and United States (Maiti, 2006). Subterranean termites are considered the most damaging annoyance of wood products and wood structures. Most of the areas with high temperatures are considered vulnerable to the attack of termites (Peterson *et al.,* 2006).They mostly damage agricultural crops, home timber books, post, hurdles, clothes, earth dams and canals of irrigation (Abe *et al.*, 1979; Ibrahim and Adebote, 2012). In short cases masses of loses subterranean termites cause the more or less 80% of this damage (Ahmed *et al.*, 2016).

Annually millions of dollars are paid to restraint the termites worldwide (Tsunoda, 2003). Non-natural chemicals used to control the destructive effects of termite cause ecological, environmental problems and badly affect the most useful insects and many organisms (Abudulai *et al.,* 2001). Extract of naturally repellent plants and heartwood with biologically active ingredient give another possible option to control the pest. Furthermore extract of plants are perishable and they aid to solve many ecological issues related to non-natural insecticide (Kim *et al.*, 2006; Ahmed *et al.*, 2007 or Rodrigues *et al.*, 2011).

Studies show that even a single species of termite possess hundreds of species of microbes in gut which is peculiar to termites, and this biota of termite is specific in a termite species of host (Hongoh, 2010). Termites being most abundant in land ecosystems play a most important role in recycling of lignocelluloses by biological way. Termites with their gut microbiota symbiosis degrade the lignocelluloses most effectively. Subterranean lower termite posses double decaying systems i.e they contain their enzymes for cellulose degradation and protozoan of their gut, was illuminated at molecular stage. Higher termites e.g.*Odonto obesus* decompose plant cellulose by the use of their solely enzymes, as they lack of symbiotic protozoan or protists (Ohkuma, 2003).

In gut of lower termites microbial population of both protists and prokaryotes with flagella are present, it has captivating most of the scientists as it has mutual association of gut microbes for proper and complete decomposition of lignocelluloses (Ohkuma, 2008).

Digestions of lignocelluloses by termites feeding on wood rely on symbiotic relationship of protists with flagellates which are present in their hind gut. The lignocelluloses digesting ability depend on physiology of the digestive tract but also on symbiotic association of termite and their gut and intestinal microbiota.

Effect of extract various heartwoods (*Tectona grandis*, *Dalbergia sissoo*, *Cedrus deodara* and *Pinus roxburghii*) were checked to see the mortality, feeding rate and protozoan population in two lower termites, *Reticulitermes flavipes* and *Heterotermes indicola*. All of these wooden extracts checked lowered protozoan numbers in the gut of termite workers and soldiers, which was almost closely correlated with worker death rates (Hassan *et al.,* 2017).

The objective of current study was to determine the termicidal effects of different plants extract (*Dalbergiasisso*, *Hetrophragma adenophylum*, *Gravia asiatica* and *Punica granatum*) on gut protozoans of *Heterotermes indicola*.

Materials and methods

Collection and maintenance of termites

The termites Wasmann (*Heterotermes indicola*) were collected from the botanical garden of University of the Punjab Lahore. The healthy colony of workers and soldiers were arranged separately and placed in entomology laboratory of department of Zoology University of the Punjab Lahore. These animals were provided normal laboratory conditions for a period of almost one week. The humidity was maintained through wet filter papers and sterilized soil. Temperature, light and darkness were maintained. Physically active and healthy termite workers were preceded for further experiment.

Collection of plants

The leaves of the plants *Dalbergia sisso, Heterophragma adenophylum, Gravia asiatica, Punica granatum* were obtained from botanical garden University of Punjab Lahore.

These leaves were then brought to Taxonomy laboratory of Botany department, University of the Punjab, Lahore, Pakistan for further identification and proceedings. The fresh soils were obtained from Zoological garden, Department of zoology, university of Punjab. This soil was sieved through a thin net and sterilized in an oven at 70°C overnight.

Preparation of plants extracts

The extracts of plants were prepared by using the Soxhlet apparatus.

Extracts and its dilutions

10ml of plant extract was taken with 90 ml of solvent to make 100 ml stock solution. Further dilutions were made with solvent used in extraction. The dilutions were of 10%, 5%, and 3%.

Anti termitic assay

Kang *et al.,* (1990), bioassay for termicidal effect of four plants (sheesham, berri patta, phalsa and anar) was adopted. Four experimental and one control groups were made to assess the anti-termite activity of plants extracts. Each plant extract experimental group is with further 10%, 5% and 3% dilutions. 10µl of each dilution of plant extract was applied on filter paper. Sterilized soil of 5mg in each Petri dish was kept beneath filter paper. A blank filter paper and filter paper treated with solvent merely were used as control. Solvent was evaporated at room temperature with control of 105 termites; 100 workers and 5 soldiers were kept in each Petri dish. Black piece of cloth was used to cover the Petri dishes at 28°C.Periodic shedding of water at bottom edge of Petri dish was done to keep the optimum moisture level for termites. Just those termites were considered dead whose appendages were not moved when touched with needle. It was examined that filter paper with solvent had no distinct effect on mortality of termite. Solvent was air dried at room temperature. To keep the termite live water and filter paper were used as food for termites. Three replicates were made for each test sample and rate of mortality was determined daily for 14 days. The mean mortality was calculated by following formula:

$$\text{Mortality rate \%} = \frac{\text{Number of dead termites}}{\text{Number of initial termites}} \times 100$$

Isolation of termite gut

Three termites from each Petri dish were taken for dissection to isolate the gut after two weeks of treatment. *H. indicola* were treated with absolute ethanol to sterilize for 1 mint and then they were allowed to air dry for 1 mint.10 µl of distilled water was taken in cavity block for dissection in cavity block. Fine tip needle and forceps were used for dissection of termite. Hindgut was isolated and placed in 100µl liter of PBS in a sterilized appendorf tube. With the help of sterilized needle the gut content was gently mixed. Following procedure was used to count the protozoan of hind gut.

Protozoans count

For counting of protozoans heamocytometre (Neubauer counting chamber) was used as described by Lewis and Forschler, 2006.

Statistical analysis

Data were analyzed by using the *SPSS 13* to determine difference in percent mortality for germicidal tests by using one way ANOVA. Results with P≤O.O5 were considered significant. All results were brought from three independent experiments and expressed as mean ±SEM.

Results

Antitermitic potential of plants extracts

Four plants (*H.indicola*. *D.sisso, H.adenophylum, G.asiatica* and *P.granatum*) were used to find the antitemitic properties on *H.indicola*. All of them had termicidal properties. The data, represent the termite mortality on different plants extracts had shown in table 4. Highest mortality of termite was shown on *D.sisso* plants extract. Average mortality was on treatment of *H. adenophylum* and *G. asiatica* and on treatment of *P. granatum* least mortality of termite was observed. Comparison between mortality of termite at 10%, 5% and 3% dilution of plants extract is given in table and figure.1.

Table 1. Effects on mortality of termite (±SEM) with different plants extracts.

	DS		HA		GA		PG	
Control	16±1.85a		16±	1.86a	16±	1.86a	16±	1.86a
10%	90±	5.03b	85±	3.61b	75±	3.61b	44±	14.73a
5%	89±	2.65c	82±	3.53b	72±	2.00b	52±	5.51a
3%	87±	4.36c	77±	1.53b	43±	25.87a	48±	5.69a

Statistical observations

Doses and percentage mortality results was compared by analyzing the one-way ANOVA. To investigate group comparison, Tukey test was applied. Results indicated that experimental four groups were significantly different from each other and from control (Table. 1).

Protozoacidal activity of plants extracts

The number of protozoans was observed from the gut of workers termite. For this purpose, thirty workers termite were collected from treated groups after two weeks. Protozoans in the gut region of termite were counted, their quantity show that these plant extracts have termicidal properties.

A notable reduction of protozoans has been noted (*D.sisso*. *H.adenophylum*. *G.asiatica* and *P.granatum*) plants extracts (at their 10%, 5% and 3%) compared to control group (Table and Figure. 2).

Discussion

In present study the effect of plants extract on mortality and gut protozoans was determined against subterranean lower termite, *H. indicola*. Extracts of four plants were used for experimentation, extracts of *P. granatum* (Anaar), *G. asiatica* (Phalsa), *H. adenophylum* (Berri patta) and *D. sisso* (Sheesham).

The plants extracts caused greatest termiticidal and protozoacidal potential against test termite's workers. According to data the *D. sissoo* plants extracts cause highest average termiticidal potential of about 90% which is correlated with reduction of protozoan's number in termite gut at its 10% of extract dilution.

It has highest termiticidal potential when it was compared with other plants extracts.

Table 2. Plants extract effect on protozoans' number per ml from termite.

Serial no	Plants extracts	Protozoans count per ml $\times10^3$
1	*D.sisso* 10%	140
2	*D.sisso* 5%	180
3	*D.sisso* 3%	200
4	*H.adenophylum* 10%	300
5	*H.adenophylum* 5%	320
6	*H.adenophylum* 3%	380
7	*G.asiatica* 10%	420
8	*G.asiatica* 5%	460
9	*G.asiatica* 3%	540
10	*P.granatum* 10%	680
11	*P.granatum* 5%	400
12	*P.granatum* 3%	460
13	Control	820
14	Solvent control	650

According to Sharma *et al.,* (2014) *Aloe Vera* possesses antitermitic potential which are in an agreement of our findings. Abbas *et al.,* (2013) reported is significant increase in termicidal activity of termites as the concentrations increase. These results are correlated with our findings because in our study highest mortality of termite was seen in 10% of plant extract which was about 96%. *H.adenophylum* showed good degree of mortality of termites which was about 85%.*G.asiatica* extract has well antitermitic activity with average mortality of 75% and 72% and 43% at different dilutions of extracts of 10% 5% 3% respectively. Zia- ul-Haq *et al.,* (2013) reported *G.asiatica* possess high rate of nutrients and other bioactive constituent such as anthcyanin, and flavonoids and its leaves have antimicrobial, anticancer, anti-platelet and antiemetic activities and can be used against microorganisms which are in agreement of present study.

P. granatum exhibit less degree of %mortality of about 44% at 10% of its extract. Our results are not in accordance with Mishra *et al.,* (2017) who reported its antitermitic potential against *Microcerotermes beesoni*, bioactive component is chlorpyriphos. This plant can be used as termite managing agent. Protozoacidal activities of these plants extracts also correlated with termite mortality. *D. sissoo* has Greater effect of extract against protozoans and cause significant loss of number of protozoans. Termites digest wood containing lignocelluloses with the help of symbiotic microbes present in their gut. After treatment of plants extracts gut protozoans reduce in number up to 99% in termites *Reticilitermes flavipis* (Hassan *et al.*, 2017).

Termites usually rely on the interstinal symbionts to assimilate and breakdown organic substance but the Macrotermitinae domesticated *Termitomyces* fungi to manufacture their own food. This change was accompanied by a shift in the composition of the gut microbiota, but the complementary roles of bacteria have remained a problem in the symbiotic process. We obtained high-quality annotated draft genomes of the termite *Macrotermes natalensis*, Glycoside hydrolases are present in symbiosis and has maximum capability to tackle complex carbohydrates and final digestion of oligosaccharides (Poulsen *et al.*, 2014).

The plants extracts of Neem, capsaicin and gleditschia cause significant loss of flagellated protozoans in Formoson subterranian termite *Coptoterms heimi* (Dolittle *et al.*, 2007).

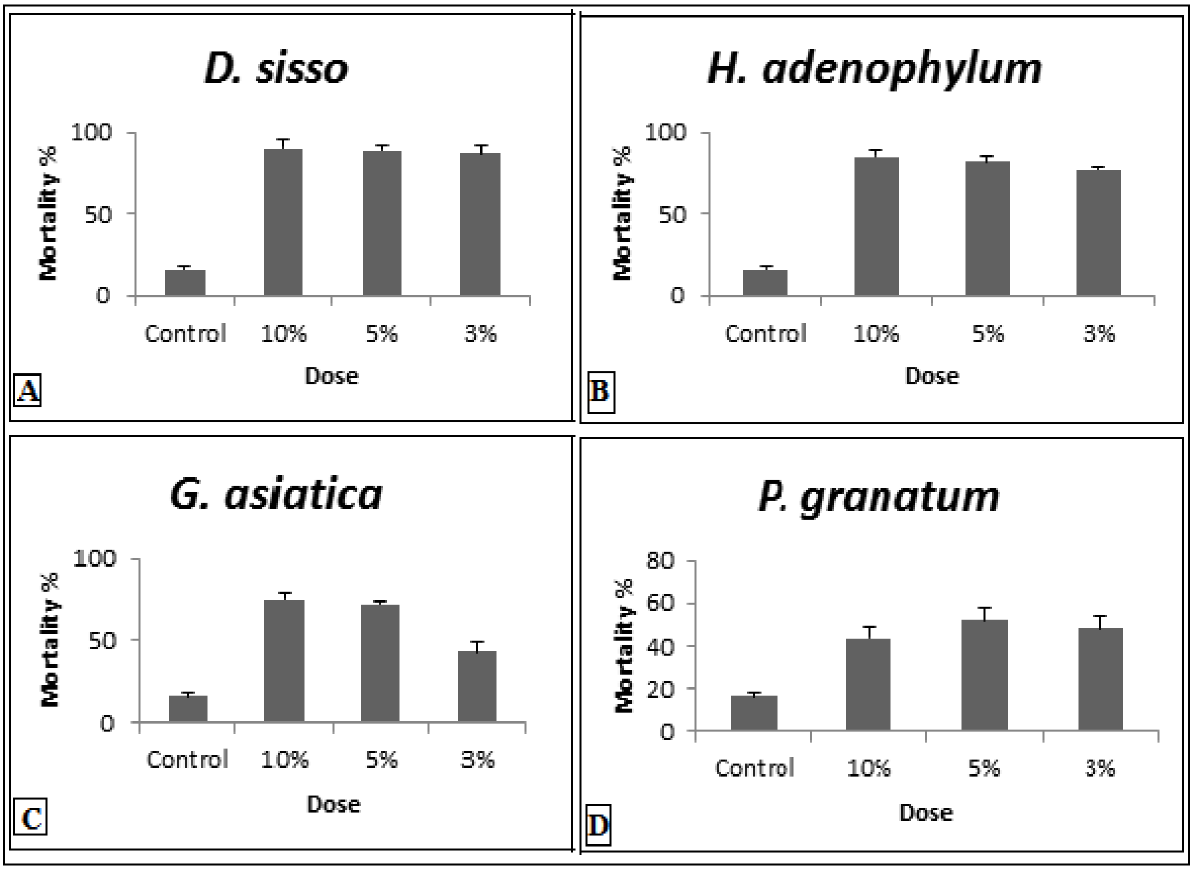

Fig. 1. Graphical representation of average % mortality of *H. indicola* workers in different dose groups.

These results agree with the present study. A worse worthy increase in termite mortality and death of gut flagellates was anlyzed due to activity of plants extract of *Mantha arviensis* leaves extracts on *Coptotermes heimi* and *H. indicola* (Qureshi *et al.,* 2012). Maisterello *et al.,* (2003) reported that vetiver oil cause reduction of symbiotic protist in Formosan subterranean termite, *Coptotermes formosanus.* Three species of protozoans had been investigated in gut of *Coptotermes formosanus*. The investigated three species are *Holomastigotoides hartmani, Pseudotrichonympha gressi* and *Spirotrichonympha leidi* (Ohkuma *et al.,* 2000). In *H.indicola* protozoan species of *Holomastigotoides hartmanni, Spirotrichonympha leidi, Microjoenea pyrifomis* and *Trichonympha agilis*. The most commonly and abundantly found protozoan in *H. indicola* is *Trichonympha agilis*.

Termites are considered as world champions to digest the compounds of lignin and cellulose, gratefulness for association among their own enzymes and the enzymes from exogenic enzymes of microorganisms, Small microorganisms which are prokaryotes cause maximum lignocelluloses metabolism. Bacteria are present in lower and higher termites and enzymatic activity associated had been described in termite gut (Matteotti *et al.,* 2012). *Heterotermes spp* (Wasmann) are largely distributed and most of the ill famous subterranean, wood damaging termites in Pakistan. The galleries of feeding adopted by these termites were large, compressed with brown dirty color droppings (Chaudhry *et al.*, 1972).

Six species of termite Heterotermes are recognized on base of a soldier based- key of South American species of Heterotermes. Six species are recognized *H. assu* sp. n., *H. convexinotatus, H. crinitus, H. longiceps, H. sulcatus and H. tenuis. H. assu* sp. is described from the Brazilian Atlantic forest, including the imago, soldier and worker castes. *H. assu sp.* is also dicovered from urban areas as an annoying pest. The imago of *H. longiceps* is reported and demonstrated for the first time. The soldiers of all species are demonstrated, and their known dispersion mapped, with respective new records (Constantino, 2000). Termites cause most of the loss of buildings and timber. Subterranean termites, consisting mound buildings and arboreal species, are 147 (80%) of the economic important species (Su and Scherahn, 2000).

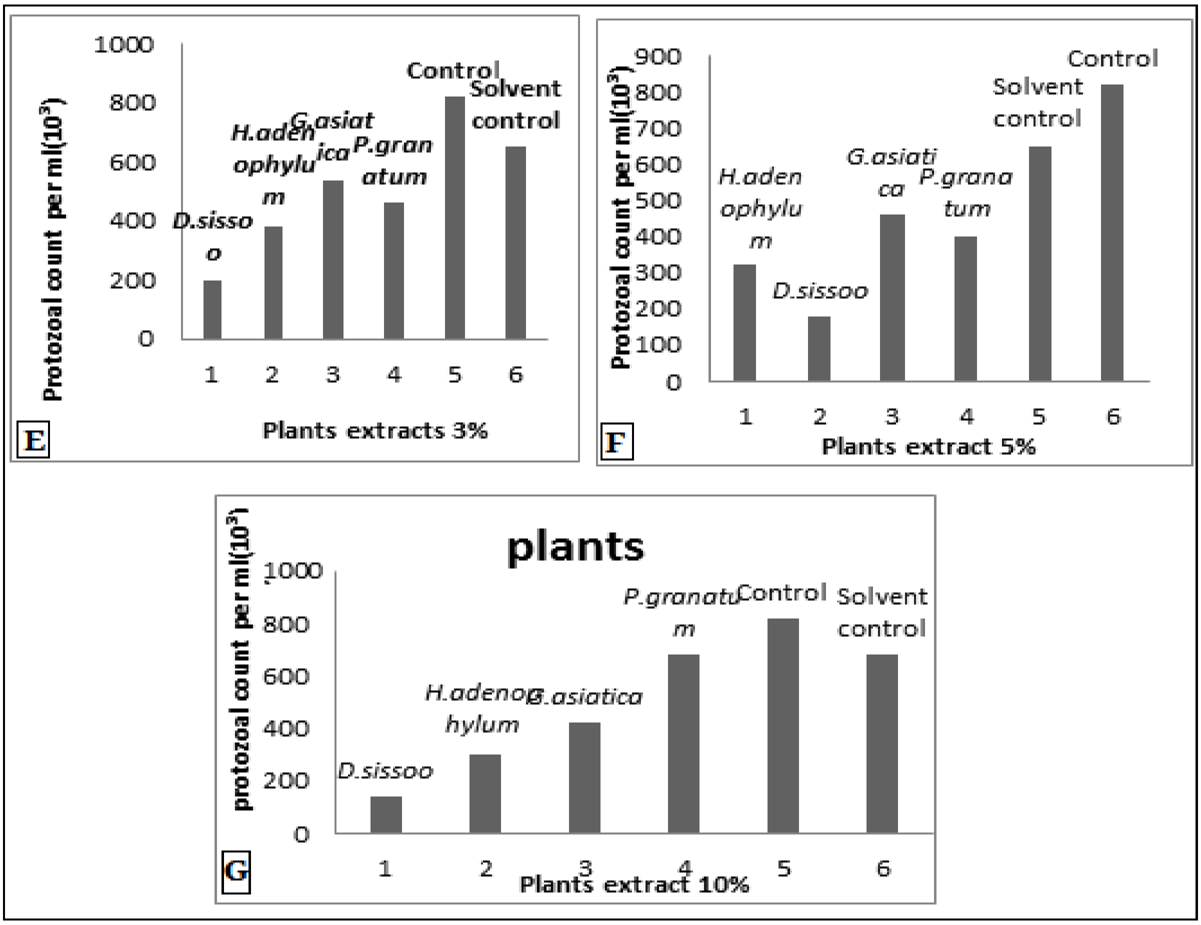

Fig. 2. Graphical representation of protozoans in gut of *H. indicola* in 3%, 5% 10% dose groups.

Symbiotic microbiota is present in highly dense population in hind gut of termites which feed on wood. All the symbiotic microbiota is well established as the flagellates of eukaryote cause most important role in digestion of lignin and cellulose. There is little knowledge about the identification and work of cooperation of symbionts of prokaryotes with the flagellated symbionts (Strassert *et al.,* 2010). Use of synthetic pesticides cause the environmental problems. These environmental problems caused chemical pesticides generally lead to development of an alternate method to termite control including non-chemical method, use of bio-pesticides. (Verma *et al.*, 2009 and Rust, 2014). Plants showed great termiticidal properties. The active components of the plants can be used to control termite as termiticidal (Verma *et al.,* 2009). The plants extracts have toxic properties and regarded as the alternative of chemical insecticides to control the termites (Isman, 2006; Dhang and Sanjayan, 2014). A wide range of plants are toxic, repellent, or have some anti-feeding properties several of which were considered as pesticide or termiticides (Boulogne *et al.*, 2012; Raina *et al.*, 2012; Addisu *et al.,* 2014).

Conclusion

Termites are annoying pest all over the world which widely affects the lifestyle of rural and urban areas by damaging wood, wooden structures, timber, standing crops in growth cycle, juvenile forest plantation. In this study it has been concluded that the extract of *D.sisso* has maximum antitermitic potential which is associated with loss of protozoan's number in termite gut. Further work and characterization of these plants extracts is recommended in near future.

Acknowledgement

The author very humbly thanked the Institute of Zoology, University of Punjab, Lahore, Pakistan.

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