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Research Article

Efficacy of leaf extracts of apple of Sodom (*Calotropisprocera*) for the control of subterranean termite *Odontotermesobesus*

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Abstract

Despite serious safety concerns, *Calotropisprocera* is used for gastrointestinal disorders including constipation, diarrhea and stomach ulcers, even toothache, painful joint, cramps, joint pain and control parasitic infections like worms' elephantiasis. The subterranean termites *Odontotermesobesus* is extremely invading wood pests that cause direct economic losses worth billions of dollars throughout the world particularly in humid and subtropic regions. Leaf extract of *Calotropisprocera* in two solvent hexane and acetone, is used against workers of subterranean termites, mortality. LC_{50} and tunneling behavior of termites was observed at different concentration of 3%, 7% and 10% respectively and distilled water applied a control. Two solvent hexane and acetone is used for extraction. Data have been analyzed using software Minitab (version 16) at 5% level.

Introduction

Calotropisprocera commonly called milkweed and a belong to family Apocynaceae. It is low growth weed frequently originate in arid, dry, uncultivated soils. *Calotropisprocera* leaves rubber and flowers have been used in Ayurvedic and Unani medicines. Latex of *Calotropis. procera* has also been shown to have pesticidal property against termites. The latex fillings have been investigated by many scientists and it is a list of mechanisms, include, proteolytic enzymes, gutta-percha rubber, alkaloids, amino acids, carbohydrates, and hydrocarbons resembling with petroleum etc. The latex contains proteolytic enzymes.

Milkweed plant belongs to the family Asclepiadaceae a common harsh environment unwanted plant dispersed in humid and sub-tropic section of Asia (Moen Ahmed et al., 2005). In native system of Ayurvedic parts of the plant are used as medicine. Similarly, in the recent years milk wild plant gained importance as a potential pesticidal source in contrast to insect pests, (Mesh ram, 1995). It is insecticidal to known have antifeedant (Pari et al., 1998), antitermiticidal (Badshah et al., 2004), antiseptic (Philip et al., 1993), nematocidal and antifungal properties (Anil Srivastava et al., 2000). The extract plant of leaves *Calotropisprocera* had shown effectiveness against lepidopterous and sucking pests of some cultivated crops (Muhammad et al., 2003). Extracts of milkweed plant containing insect toxic principles such as cardenolides, cardiac glycosides, flavonoids, giganticness (a novel nonprotein amino acid) and other cytotoxic principles which are effective against an arrangement of insect pests.

Some botanical insecticides, such as Asadirachtaindica oil, severally effect the immune system of various insect species (Duarte et al., 2020). This help protect agro-farms and furniture from termite invasion in tropical communities. In addition, the helpfulness of these plant peels found in the area was appreciated. Termites are among the most catastrophic insect pests; they belong to the isopteran order of insects. So named because of the equal shape and venation (An arrangement or system of vein) of the fore and hind wings (Ito and Ighere, 2017).

Out of 300 species of termites known so far from Africa, about 35 species. Such as East Africa, in some part of Africa, Macrotermes basis yield of loss 30-60%. Severely damaging agricultural crops and urban organization (Verma et al., 2016).

Termites are dominant pests of tree plantation and agriculture crops products of wood in warm temperate, tropical and subtropical areas of the world. Infestation of termites may be controlled or prevented by several ways in different countries (Howick and Staunton, 2017). Current methods of termite's management for building include discriminating soil treatments (Interior and exterior perimeter treatment or spot treatment), non-repellent chemical and baiting with slow acting (Iqbal and Evans, 2018).

The higher termite's species *Odontotermesobesus*, is fungus growing and a scavenging termite. There are various field and laboratories studies that have been conducted against this specific species which are involving in different insecticides but with different ways of methodologies and purpose (Rasib et al., 2018).

Protozoa population was directly related to the filter paper area consumption treated with plant extract. And these are found that after two weeks of experience of termites to treated filter paper in no-choice bioassay at the concentration of 20mg/ml of plant leaf extract of *Calotropisprocera* reduced the protozoa population by 62.90% as compared to the untreated negative control (Afzal et al., 2019).Now a days, compared pyrethroids and organophosphate insecticides, imidacloprid and fipronil as newer chemical gaining acceptance as termicides. For long term safety imidacloprid is popularized in terms of perimeter protocol owing to reduction of active ingredients utilization for this purpose and innately provision of advantage to bring down potentials (Henderson et al., 2016).

Materials and Methods

Collection of plant leaves Calotropisprocera

The plants leave *Calotropisprocera* were collected the different area from The University of Lahore (Hazrat et al., 2007) the fresh and healthy leaves of plant *Calotropisprocera* were collected for the experiment. Subsequently, plant leaves of *Calotropisprocera* were carried in The University of Lahore laboratory in the Pakistan the leaves were cleaned with distilled water to remove dust and other undesirable soil particle.

Grinding of leaves

The cleaned leaves shade down in the 15 days. The dry leaves were grinded in an electric grinder and kept in the container. Finally, fine powder was collected in the bag to avoid in moisturize.

Collection of termites

The test termite species were collected from The University of Lahore and brought in the Entomological Research laboratory. Termites were kept in plastic bag along with trays in the laboratory. Water and tissue paper will be used as the food source/substrate (Ahmad et al., 2006). Termites were maintained in the laboratory condition under dark environmental using black cloth.

Experimental treatment

The arrangement was complete treatment three replicates in each concentration. Treatment comprised in extract of N-hexane *Calotropisprocera* and extract of acetone *Calotropisprocera* applied at the different concentration for the control of termites. Untreated the three replicates petri dishes to apply the distilled water for the control of termites. Different concentration of termicides were applied on the toxicity test. The termite's mortality data were recorded every 30-minute interval until 8 h after experience. The mortality was calculated using percentage termite's:

$$\text{Mortality (\%)} = \frac{\text{Number of dead termites}}{\text{Total number of termites}} \times 100$$

Filtration process

The solvent mixture shakes in every two hours then I brought Buchner funnel, carbon filter paper and vacuum flask 1000 g in the laboratory of University of Lahore. The filter papers are double coated to wrap in funnel to fit in vacuum flask. The mixture is pass through the filter paper in which liquid fluid are removed but retains the waste solid particle in the *Calotropisprocera* of leaves. Either the clarified mixture, and waste particle removed from the mixture may be the clear mixture are formed.

Preparation of plant extracts

The dried leaf (800 g) powder used were grounded and the extracted was prepared using n- hexane (1400 ml) and acetone (1000 ml) solution. And heated at 60–80 Co) for 8 hrs (Ito and Ighere, 2017). The extracted crude mixture was stored at refrigerator temp (- 4 to - 60 Co).

Antitermitic bioassays

Antitermitic bioassays using leaf extracts were performed in Petri dishes at 10 g soil. Every treatment with 3%, 7% and 10% concentration of plant extracts N-hexane and acetone with control were repeated thrice (Kang et al., 1990). Data for mortality were recorded after an interval of 30 minutes up to 8 hours in a toxicity test.

Effect of plant extracts on galleries formation

Termitidae members of the family make galleries during foraging. This shows the termites in the activity of the soil. The termites in progress making tunnel along the bottom of each Petri dish. The galleries formation termites' response towards for each extract at each concentration after 2 hours was determined.

Statistical analysis

ANOVA test in statistical of Minitab (conversion 16, 19) was carried at significance level $P<0.05$ (Reddy et al., 1998).

Results

Toxicity test

Toxicity test indicated that only one concentration 3% take time more than 8hour to kill the all termites. But other two concentration take less time more than 8hour to kill the all termites. The 7% concentration take less time than 3%concentration to kill the termites. Whereas the 10% concentration take more less time than 8hour in 3%concentrations to kill the all termites. The percentage mortality for subterranean termite in extract of acetone *Calotropisprocera* and N-hexane *Calotropisprocera* treated with soil, within 8hours was, 100%, 90%, 85%, 43% in 3%, 7%, 10% and 0% concentration in control group respectively. The lowest percentage mortality was observed in low concentration in0% is 43%. And the highest percentage mortality was noted in highest concentration 10% is 100%. In control treatment, soil is treated only with distilled water and lowest percentage mortality was recorded is 43%. The different in toxicity of each concentration of acetone and N-hexane was significantly different ($p<0.05$).

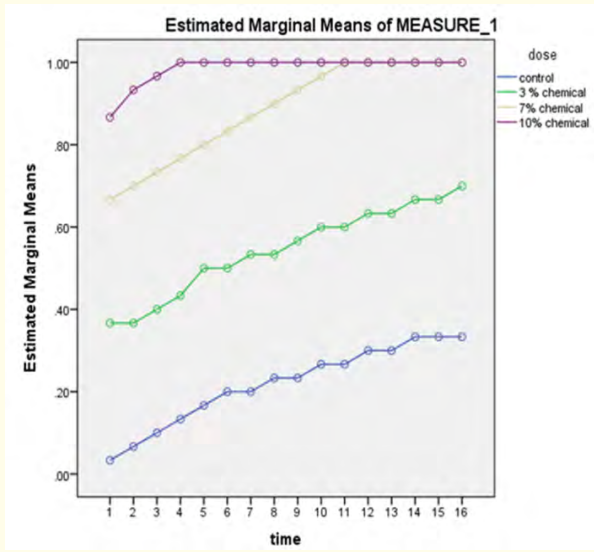
Toxicity test of N-Hexane

Data (%) were transformed before the analysis of variance; means (-standard error) are values three replicates; means with the same lower case alphabet column are not significantly different from each other ($p>0.05$); in different concentration (3%,7% and 10%); p=probability value.

Percentage mortality of termites. *Calotropisprocera* using the N-hexane as solvent in the Calotropisprocera in toxicity test. Values with percentage mortality rate at every 30-minute shows percentage standard error at each time points.

Concentration	*1hour*	*2hour*	*3hour*	*4hour*	*5hour*	*6hour*	*7hour*	*8hour*
control	.066±.057	.133±.057	.200±.000	.233±.057	.266±.057	.300±.100	.333±.057	.333±.057
3 %	.366±.057	.433±.057	.500±.000	.533±.0577	.600±.000	.633±.570	.666±.057	.700±.000
7 %s	.700±.000	.766±.057	.833±.083	.900±.1000	.966±.057	1.00±.000	1.00±.000	1.00±.00
10 %	.933±.057	1.00±.000	1.00±.000	1.00±0.00	1.00±.000	1.00±.000	1.00±.000	1.00±.000
P value	.000	.000	.000	.000	.000	.000	.000	.000

Table 4.1.5: Time in 8 hours N-hexane.



N-hexane *Calotropisprocera* of toxicity test for the mortality rate with N-hexane.

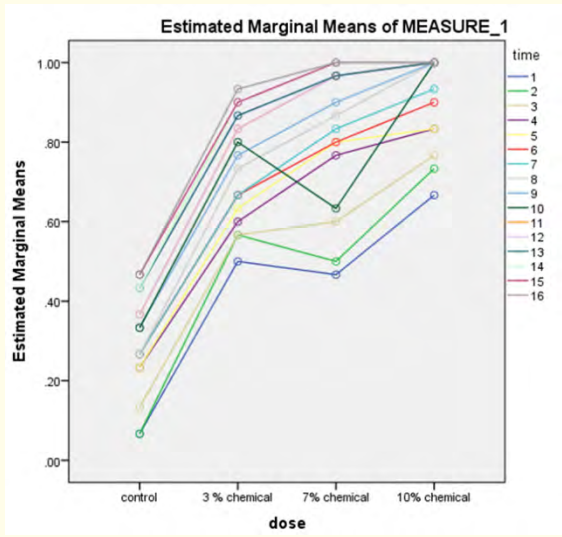
Toxicity test of Acetone

Concentration	*1hour*	*2hour*	*3hour*	*4hour*	*5hour*	*6hour*	*7hour*	*8hour*
control	.0667±.05774	.1333±.05774	.2000±.000	.266±.057	.333±.577	.366±.057	.433±.115	.466±.152
3 %	.3667±.577	.433±.0774	.5000±.0000	.733±.057	1.000±8.00	.833±.057	.9000±.000	.933±.057
7 %	.7000±.000	.7667±.057	.833±.057	.866±.0577	.633±.461	.966±.057	1.00±0.00	1.00±0.00
10 %	.933±.0577	1.000±.000	.900±.000	1.000±.000	1.00±0.00	1.000±0.00	1.000±0.00	1.000±0.00
P value	.000	.000	.000	.000	.000	.000	.000	.000

Table 4.2.3: Time in 8 hours acetone.

Time	*Doses*	*X2*	*df*	*Mean square*	*F*	*Sig*
4 hours	Acetone	3.269	15	.218	24.262	.000
8 hours	Acetone	.380	45	.008	.940	.000

Table 4.2.4: Toxicity test to recorded the data of mortality rate 4 h and 8 hours.



4.3 Comparison of LC50 values (hrs) of different concentration of leaf extracts of using *Calotropisprocera* in different solvent.

Comparison of LC50 values (hrs) of different concentration of leaf extracts of using Calotropisprocera in different solvent

In the case of using the leaf extract of N-hexane *Calotropisprocera* at high mortality of termites *Odontotermsobesus* at higher concentration 10% after 8 h of treatment was observed 91.7 % and 81.13% with N-hexane and acetone based on extracts, respectively. At 7% concentration after 8h of treatment mortality was observed 76.0% and 66.1% with N-hexane and acetone based on extracts, respectively. At 3% concentration after 8 h of treatment mortality was observed 54.1% and 43.1% with N-hexane and acetone based on extracts, respectively. Therefore, lowest mortality was observed 43.1% in acetone of extracts of *Calotropisprocera* at the lowest dose 3% after 8 h of exposure time.

All concentration (3%, 7% and 10%) of two solvent *Calotropisprocera* significant values. N-hexane extracts was comparatively more effective than the extracts of acetone *Calotropisprocera*. Therefore, mortality response was influence by exposure time and concentration of the plant extracts of leaves *Calotropisprocera*. Probit analysis of N-hexane and acetone extracts of *Calotropisprocera* showed LC50 for 8 h (3%, 7% and 10%) was respectively. Table show the significance level of different concentration. All the solvent same the concentration shows significance value in acetone and N-hexane the extracts of *Calotropisprocera*.

Time	*Regression Equation*	*Significance values*	X^2	*LC50*	*Lower bound*	*Upper bound*
4 hours	-1.383+403	.000	6.726	3.298	2.658	3.98
8hours	-1.488+675	.000	8.679	2.550	1.678	2.478

Table 4.3.1: LC50 values calculated of N-hexane using the extract of *Calotropisprocera*.

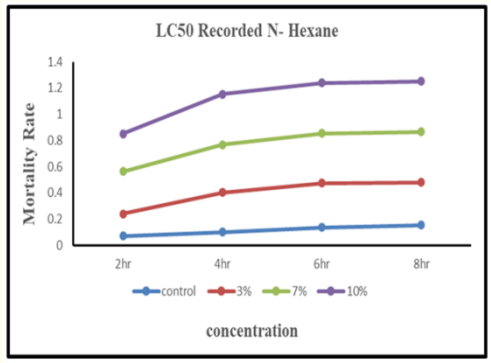


Figure: LC50 recorded of N-hexane.

Time	*Regression Equation*	*Significance values*	*X^2*	*LC50*	*Lower bound*	*Upper bound*
4 hours	-1.163+231	.000	7.409	5.425	4.349	7.893
8hours	-689+336	.000	8.065	2.243	1.069	2.678

Table 4.3.2: LC50 values calculated of acetone using the extract of *Calotropisprocera*.

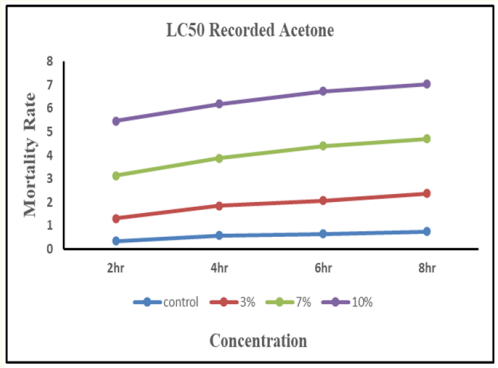


Figure: LC50 recorded of Acetone.

Discussion

Discussion control of termites was dependent upon on synthetic insecticides and persistent organochloride, organophosphate insecticides (Anonymous 2000). The application of pesticides/insecticides triggered pest resistance, environmental degradation and health concerned forced an alternative solution of the problem to more precise and environmentally friendly biorational program on plant of leaves extracts against pest species (Coats, 1994). Since biorational application of insecticides is accepted worldwide (Loganet al.,1990) and biorational option are much better strategy using plant extracts along with their bioactive compound in managing termites. Since the extracts of leaves plants are investigated in other regions of globe with multi-dimensional parameters comprising of LC50, and other properties (Zhu et al., 2001; Isman (2006). In the previous studies, biorational plants insecticides get impeutus in term of claim besides with termite's control. The Antitermitic activity is seem to different plants like *Cinnanomum cassia*, *Cymbopogan,*

Citratus, *Vetiveriazizanoides, Eucalyptus citrodora, Calotropisprocera, Eucalyptus globules, Cedrusatlantica, Syzgumaromaticum* and *Coleus amboinicus* (Singh 2002; Blake et al., 2003).

The plant extract along with oil of *Calotropisprocera* with many steroids. The use of plants extracts has also been reported by several authors to be effectives in the management of termites and serve as an alternative to synthetic insecticides (Sen 2001; Sbeghen et al, 2002; Sohail et al, 2005; Abdullah et al, 2014). Furthermore, the non-significance chi square values for both *j. curcas* and *A, indica* oil shows that toxicity model generated from the Probit regression was similar to the theoretical model which described the observed mortality of *Calotropisprocera* on treated wood sample as an outcome of the toxic effect of the botanical oil extracts.

The significant negative association between termites' mortality and wood wight loss further shows that the toxicity of the termicides may be responsible for the significance reduction in termites wood consumption mortality, and it was similar to the mortality caused by 10 and 15ml of solignum.

Terpene alcohol may contribute to termite mortality as a result of bioactive compound (Ogunsinaet al., 2009 Upadhyay et al., 2010; Manzoor et al., 2011; Elango et al, 2012) and termite protection comprising *O. obesus Microtermbissoni*, Reticulitermessantonensis and R. virginicus. Termites' researchers in other portion of globes highlighted and leaves extracts *Calotropisprocera* using the solvent acetone and N-hexane used against the control of termites *Odontotermsobesus* to detect tunneling behaviors of termites and mortality rate (Elsayed, 1997). Ganapatyet al (2004) described those results 10%, 7% and 3% concentration to show the result 93.1%, 83.1% and 71.3% mortality in 8 h application. Similarly, the *larixkeptoleplepis* crude extracts in water holding flavonoids in great amount showed effectively termites feeding detereent functions (Chen et al., 2004). Extracts of *Lanatanacamara*, *Calotropisprocera*, *Ocimum sanctum, E.helioscopia* and *S.incanum* and *W.somnifera* was found effective against termites workers (Manzooret al. 2011).

Wood is known as differ in their susceptible to termite's attack; this difference may be attribute to factors such as chemical composition wood density and availability of susceptibility species of termites (Gerardet al, 2019). However, all the wood tested showed significant susceptibility to *C, sjostedi* attacks. Particularly, the untreated wood samples from all the tested plants species recorded the highest weight loss when compared with treated samples. Based on the results, wood consumption by *C, sajostedti seems* to be higher in *Ceibapentandra* wood than other wood types tested. These results agree with report of Faruwaet al., (2015) who observed a significant impact on bio-based preservatives as control measures against damage caused by fungi and termites in *Triplochitonscleroxylon*, *Gmelinaarborea*, *Ceibapantandra* wood samples.

The harmful impacts of plants chemical constitutes or crude extracts on insects are established in many methods, involving growth retardation, suppression of calling behaviors, toxicity feeding inhibition, oviposition deterrence, reduction of fecundity and fertility (Zhaoet al. 1998; Muthukrishnan and Pushpalatha 2001). Searching for anti termitic activity of plants extracts with medicinal attributes could lead to the discovery of new agents for termite's control. So, we can conclude our study that acetone and N-hexane extraction of *Calotropisprocera* be used as a potential natural termicides against termites, Isolation of Antitermitic constituents to find out their mode of action against termites. However, the results of our study revealed that leaf extracts of *Calotropisprocera* have amazing toxic effect against the termite's species *Odontotermsobesus*.

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