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Anti-termitic Activity of Aqueous Extracts from Saharan Toxic Plants Against *Anacanthotermes ochraceus*

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ABSTRACT

In Algeria Sahara, *Anacanthotermes ochraceus* is among the most devastating termite pests, it cause great damage to house hold materials and agriculture in oasis. The objective of this study was to evaluate Saharan plants extracts for their termiticidal activities against *Anacanthotermes ochraceus* in a research programme for effective, environment and eco-friendly termite control agents. This study investigate, the anti-termitic activity of aqueous extracts from different part of four Saharan plants (*Calotropis procera, Hyoscyamus muticus, Pergularia tomentosa* and *Datura stramonium*) against termite workers using direct contact application. All the crude extracts showed significant anti-termitic activity in a different doses and the mortality, among various aqueous extracts were statistically different ($p<0.05$). The highest termite mortality (50%) was found in leaves extract of *Calotropis procera*. This results is the first report on the search of termiticidal natural compounds extracts against the Saharan *Anacanthotermes ochraceus*, it can open the possibility of further investigations on eco-friendly termite control agents.

Key words: Termite, plants extract, *Anacanthotermes ochraceus*, TL_{50}, Algerian Sahara

INTRODUCTION

Termites are highly destructive insects; a termite can cause significant economic damages from paper fabrics to wood constructions. It also constitutes a serious menace to agriculture and forestry (Verma *et al*., 2009). Although, termites are part of a long "Superorder" that includes cockroaches, they are classified separately in a group called "Isoptera" and a new family called "Termicidae". In another hand these pest insects are a large and diverse group consisting of over 2500 species worldwide (Elango *et al*., 2012).

It is well know that excessive uses of pesticides for termites control is detrimental to environment and create several problems such as phytotoxicity, mammalian toxicity, insect resistance, pesticides residues in soil and water (Badshah *et al*., 2004).

A great number of plants over 1000 species have been studied to search the insecticidal phyto-compounds from their leaves, stems, flowers, seeds and roots. However, only a few of them have been used for practical insect control on commercial scale in the past. The chemical poisons of plants are mostly alkaloids. Various Scientists have studied different plant extracts for their toxicity, attractancy and repllency in various natural products against different termites and insect spp. (Badshah *et al*., 2004).

In fact, Natural anti-termitic compounds may be very useful. They have different types of activity against different species of termites (Haouas *et al.*, 2011). Toxic plants constitute a source of many natural compounds presenting pesticidal activities (Sakasegawa *et al.*, 2003; Jembere *et al.*, 2005).

Due to the damage caused by the Saharan termite *Anacanthotermes ochraceus* to house hold materials and agriculture in oasis and in continuation of our research programme in the valorisation of Saharan plants. The objective of this study was to evaluate Saharan plants extracts for their termiticidal activities against *Anacanthotermes ochraceus* for the research of environment and eco-friendly termite control agents. Thus, this study focused on the anti-termitic effect of aqueous extract from different part of four Algerian Sahara plants (*Calotropis procera*, *Hyoscyamus muticus*, *Pergularia tomentosa* and *Datura stramonium*) against *Anacanthotermes ochracus* workers.

MATERIALS AND METHODS

Collection of insects, experimental and biological specimens: The termite species, *Anacanthotermes ochraceus* (F/Hodotermitidae, sub.F/Hodotermitinae: Isoptera) (Logan *et al.*, 1999; Logan and El-Bakri, 1990); were collected from infected logs found at the arid area of LAHMAR district, Bechar (31°.56'.0,01"N and 2°.15'.34,95"W) (Fig. 1). Using a trapping technic (Salihah *et al.*, 1993). The colony was reared in an incubator at 26±2°C, 75±5% Relative Humidity (RH). Water and newspaper were used as food source. After separating from soil and debris, they were identified with the help of the taxonomic keys used by the Research Center in Arid Areas Beni abbes, Bechar (Algeria).

Plant materials and extraction: Plants were collected in October 2010 from an experimental station of plant and zoological experiments near LAHMAR district (north of Bechar). The botanical

Fig. 1(a-f): Experimental station (natural colony of termite) identification and voucher specimens were conserved at Phytochemistry and Organic Synthesis Laboratory (POSL) herbarium, University of Bechar, Algeria under to accession No. CA04/02 *Calotropis procera*, Asclépiadaceae, CA00/43 *Hyoscyamus muticus*, Solanaceae, CA00/44 *Pergularia tomentosa*, Asclépiadaceae and CA00/50 *Datura stramonium*, Solanaceae (Cheriti, 2000, 2002).

The leaves, stems, fruits and flowers were separated and oven air-dried (overnight) the plants were grounded into powder using a grinder. Extraction was done using a reflux apparatus. several aqueous solutions were prepared from different parts of plants at variable dilutions at 1, 2, 3, 4 and 5%. Then the extracts were filtered and stored at 5°C.

Anti-termitic bioassay: Force-feeding tests were conducted in the Petri dishes for both termite spp., Petri dishes were sterilized in an oven at 200°C for 2 h. The no-choice bio-essay method (Kang *et al.*, 1990; Cheng *et al.*, 2007) was employed to evaluate the anti-termitic activity of the four plant extracts during preliminary screening.

A piece of filter papers samples (Whatman No. 3, 8.5 cm in diameter) treated with bi-distilled water was used as a control. After the bi-distilled water was removed from the treated filter papers by air-drying at ambient temperature, 10 active termites (workers) above the third instars were put on each piece of filter paper in Petri dishes (9 cm in diameter, 1.5 cm in height). The dishes with covers were then placed in an incubator at 28±2°C, 75±5% RH. A few drops of water were periodically dripped on the bottom edge of each Petri dish. Four replications were made for each test sample and the mortality percentage of the termites was counted for 1 min and each 30-360 min. However, at the end of each time span the selected test samples turned out to be equal in their toxic potential.

Statistical analysis: The average termite mortality data were subjected to probits analysis for calculating LT_{50} (Ould El-Hadj *et al.*, 2006), by using the Schneider formula (Abbott, 1925; Finney, 1971):

$$M_C = \frac{M_2 - M_1}{100 - M_1} \times 100$$

Where:

M_C = Corrected mortality (%)

M_2 = Mortality in the treated population (%)

M_1 = Mortality in the control population (%)

Standard deviations chi-square, t-significance, correlation and ANOVA were calculated and analyzed using the software (Graph pad prism version 5.01); Results with $p<0.05$ were considered statistically significant.

RESULTS

Toxic and repellent responses of various aqueous extracts from *Calotropis procera, Hyoscyamus muticus, Datura stramonium, Pergularia tomentosa* were evaluated against Algerian saharan termite *Anacanthotermes ochraceus*. For this purpose, insects were treated with increasing doses of both extracts separately. The mortality rate was found dose-and time-dependent as it was found to be increasing with the increase in dose and exposure duration. The TL_{50} values for different extracts of 360 min are given in Table 1.

Table 1: Effects of different concentrations of plants extract activities on the mortality lethal time of 50% of the population

Plant extracts	Part used	Regression	Regression coefficient (R^2)	Lethal time 50 (TL_{50}) min
Evaluation of TL_{50} plant extracts at (1%)				
Calotropis procera CA04/02	Fruits	y = 2.6563x-2.3108	R^2 = 0.4904	565.26
Datura stramonium CA00/50	Fruits	y = 2.175x-2.0682	R^2 = 0.4181	1777.24
Hyoscyamus muticus CA00/43	Leaves	y = 2.4535x-1.4676	R^2 = 0.6708	432.58
Evaluation of TL_{50} plant extracts (2%)				
Calotropis procera CA04/02	Stems	y = 2.5781x-1.1114	R^2 = 0.8027	234.69
	Fruits	y = 2.9041x-2.2848	R^2 = 0.5756	322.44
Datura stramonium CA00/50	Stems	y = 2.6252x-1.6187	R^2 = 0.6686	332.06
	Fruits	y = 2.5358x-1.5879	R^2 = 0.6778	396.23
Hyoscyamus muticus CA00/43	Leaves	y = 2.4989x-0.9411	R^2 = 0.7975	238.49
Pergularia tomentosa CA00/44	Leaves	y = 2.8065x-1.7116	R^2 = 0.6759	246.29
	Stems	y = 2.864x-2.5435	R^2 = 0.4918	430.43
Evaluation of TL_{50} plant extracts (3%)				
Calotropis procera CA04/02	Stems	y = 2.5793x-0.2698	R^2 = 0.9690	110.43
	Fruits	y = 2.9829x-2.0045	R^2 = 0.6715	222.95
Datura stramonium CA00/50	Leaves	y = 2.655x-2.0965	R^2 = 0.5755	470.84
	Stems	y = 2.6403x-1.9883	R^2 = 0.5768	443.38
	Fruits	y = 2.8979x-1.4013	R^2 = 0.7926	161.78
Hyoscyamus muticus CA00/43	Leaves	y = 2.7329x-1.266	R^2 = 0.8008	196.24
	Flowers	y = 2.43x-1.8763	R^2 = 0.5808	675.69
Pergularia tomentosa CA00/44	Leaves	y = 2.87x-1.7687	R^2 = 0.6751	228.26
	Stems	y = 2.8286x-1.8888	R^2 = 0.6681	272.57
Evaluation of TL_{50} plant extracts (4%)				
Calotropis procera CA04/02	Leaves	y = 2.2711x + 0.0237	R^2 = 0.9941	155.28
	Stems	y = 2.6044x-0.2388	R^2 = 0.9729	102.68
	Fruits	y = 3.0033x-2.0033	R^2 = 0.6734	214.71
Datura stramonium CA00/50	Leaves	y = 2.9019x-2.1121	R^2 = 0.6266	282.38
	Stems	y = 2.829x-1.7876	R^2 = 0.6760	250.78
	Fruits	y = 2.8359x-1.3824	R^2 = 0.7940	178.06
Hyoscyamus muticus CA00/43	Leaves	y = 2.7482x-0.9425	R^2 = 0.7907	145.31
	Stems	y = 2.8386x-1.8371	R^2 = 0.6771	256.22
	Flowers	y = 2.8339x-2.1897	R^2 = 0.5803	344.37
Pergularia tomentosa CA00/44	Leaves	y = 2.6734x-0.287	R^2 = 0.9845	94.97
	Stems	y = 2.9521x-2.0266	R^2 = 0.6643	239.99
Evaluation of TL_{50} plant extracts (5%)				
Calotropis procera CA04/02	Leaves	y = 3.2358x-0.612	R^2 = 0.9414	54.24
	Stems	y = 3.2639x-0.7689	R^2 = 0.9040	58.54
	Fruits	y = 3.7863x-2.5805	R^2 = 0.6481	100.48
Datura stramonium CA00/50	Leaves	y = 3.1808x-1.7158	R^2 = 0.7735	129.22
	Stems	y = 3.2658x-2.5959	R^2 = 0.5729	211.78
	Fruits	y = 3.4664x-2.3847	R^2 = 0.6684	135.01
Hyoscyamus muticus CA00/43	Leaves	y = 3.2135x-1.4389	R^2 = 0.7845	100.85
	Stems	y = 3.1462x-2.1515	R^2 = 0.6691	187.52
	Flowers	y = 2.838x-1.4187	R^2 = 0.7650	182.68
Pergularia tomentosa CA00/44	Leaves	y = 3.215x-0.6838	R^2 = 0.9334	58.60
	Stems	y = 3.3459x-2.2394	R^2 = 0.6701	145.76

The results revealed two important aspects of toxicological inference TL_{50} irrespective of medium for feeding, movement was almost equal and non-significant depending upon fiducial limits. TL_{50} using aqueous extract was shorter than that of other extracts. Many studies have shown activity of plant extracts when applied on filter paper and/or mixed in soil to determine mortality (Jembere *et al.*, 2005) and concluded that plant extracts have the potential for under- and above-ground application for termite control.

DISCUSSION

In this study, the effect of four toxic plants extract have evaluated on the causal agent of degradation of wood and cellulose part of plants, this insect is considered as the second telluric ravager after fusarium fungi of the date palm tree "*Phoenix dactylifera* L." in sahara (Boulenouar *et al.*, 2012).

The used toxic plants *Calotropis procera*, *Hyoscyamus muticus*, *Datura stramonium* and *Perlgularia tomentosa* extracts have an anti-termitic activity. Three different concentration extracts have a similar TL_{50} (Table 1), respectively *Calotropis procera* leave (5%) TL_{50} = 54, 24 min, *Calotropis procera* Stem (5%) TL_{50} = 58,54 min and *Pergularia tomentosa* leave (4%) TL_{50} = 94,97 min while *Calotropis procera* Stem (3%) TL_{50} = 110,43 min (Table 1), *Datura stramonium* fruit (3%) TL_{50} = 161, 78 min, *Calotropis procera* Stem (2%) TL_{50} = 234, 69 min and *Hyoscyamus muticus* leave (2%) TL_{50} = 238, 49 min.

The traditional use of toxic plants as a pesticide is greatly justified. The termicide activity of the extract increase with increasing of concentration of the extract, except in the most toxic extract which is more effective in lower concentrations (Giridhar *et al.*, 1988).

Badshah *et al.* (2004) observed toxic effects of *C. procera* extracts against *H. indicola* and *C. heimi* and Singh *et al.* (2002) reported a great termicidal activity against *O. obesus*.

Concerning the action mode of the toxic plant extract, contact and inhalation are the main factors of effectiveness. They can be also transmitted in the colony during social tasks however, they do not, act when ingested and are appetite inhibiting for termites.

Nevertheless when developing a new bio-insecticide to meet regulatory requirements, it is necessary to have an understanding the degree of the toxicity of the bio-insecticides to the target insect pests (Lee *et al.*, 2003).

The aqueous plant extracts can be a good termicide, but field trials with suitable formulations need to be carried out to further assess the efficacies of these plant extracts. It would be interesting to determine the lowest concentration of these plant extract at which they are still effective as fumigants or through solution contact and to study the effects of combining components to identify potential synergisms and antagonisms (Gillij *et al.*, 2008).

It was reported that termite are strongly repelled by the toxic material to the extent that they will starve rather than consume cross treated simples and when kept close to the extract, they become disoriented and eventually die (Osipitan and Oseyemi, 2012).

Three species of flagellated protists (*Spirotrichonympha leidyi*, *Holomastigotoides hartmanni* and *Pseudotrichonympha grassii*) are found in the hindgut of *Formosan subterranean* termites. Ohmura *et al.* (2000) and Doolittle *et al.* (2007) investigated a the ability of three natural products (*neem* extract, *capsaicin* and *gleditschia*) to reduce the number of microbes (*S. leidyi*, *H. hartmanni*, *P. grassii* and *P. spirochaetes*) present in the hindgut of the *Formosan subterranean* termite. Neem extract significantly reduced the population of *P. grassi* and *P. spirochaetes* and was found to be most potent at (1 ppm) concentration, causing 100% termite mortality. Anthracenes, anthrones, anthraquinones and xanthones (Rudman and Gay, 1963) act as deterrents, monoterpenoids, alkaloids and toxic hydrocarbons (Cornelius *et al.*, 1997) and plant flavonoids and related compounds have both toxic and anti-feedant effects against termites (Ohmura *et al.*, 2000; Boue and Raina, 2003).

CONCLUSION

The screening results suggest that *Calotropis procera, Pergularia tomentosa* are promising in termite control. It may be used as environment friendly and sustainable insecticides to combat termites take protection from them. Plant extracts should be exploited to develop new wood preservatives to protect wooden structures, agricultural crops, plants and trees, as these are less harmful to the environment and humans. Further, short and long-term field studies are required to use them as commercial termiticides. Finally, laboratory bioassays with a range of plant extracts in particular, indicated the potential use of some of them as termiticides.

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