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Potentiality of use extracts from *Tetraclinis articulata* like biocide against wood destroying organisms: *Reticulitermes santonensis*

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ABSTRACT
Screening trials were conducted to determine the antitermitic and antifungal properties of essential oil and methanolic extractives obtained from *Tetraclinis articulata* heartwood. Extract-treated cellulose pads were used to evaluate antitermitic activity and complete termite mortality was obtained with the essential oil: the threshold is situated under 5% (v/v). Standardised tests according European standard EN 118 allow validate the use of essential oil like biocide.

Keywords: *Tetraclinis articulata*, heartwood, wood extractives, essential oil, methanolic extracts, termites, Basidiomycetes fungi, antitermitic.

1 INTRODUCTION

The role of the extractives in the natural protection of the wood against fungi and insects is important for standing tree and wood products (Schultz and Nicholas, 2000). The secondary metabolites synthesis constitute actively or passively a chemical of physical barrier which can be inhibiting or toxic against pathogens. Generally, these substances have weak molecular weight (lignane, terpene, stilbene…) with large action spectrum (Hart, 1981, Regnault-Roger *and al*, 2003, Woodward and Pearce, 1988,). *Tetraclinis articulata* also called Thuya grows naturally, especially in Morocco regions: East of Morocco, Moyen Atlas, Haut Atlas, Anti Atlas and region of Essaouira (Benabid, 2000) and represents 10 % of the forested area. Its timber has been used for cabinet making, joinery. It is also a source of vegetable tar for traditional medicinal uses (Bellakhdar, 1997). This species belongs to the order of Cupressales which knew for the presence of chemical compounds with biocide properties like antitermitic or fungicidal (Haluk and Roussel, 2000). Termites and fungi cause destruction to wooden structures. Many commercial termicides and fungicides are potential environmental contaminants and even carcinogens. For example, the most common method of subterranean termite infestation prevention involves applying a chemical termicide to a structure's peripheral grounds. This often leads to soil and water contamination when applied improperly. Though past environmental contamination caused by synthetic biocides is irreversible, future damage is not inevitable. New source of biocides must be reconsidered. The use of natural antitermtic and/or fungicide is a potential alternative. This research examines one possible solution: extracts of *T. articulata* native to Mediterranean regions.

2 EXPERIMENTAL METHODS

2.1 EXPERIMENTAL PLANTS

The plant was collected from Moyen Atlas (Morocco) from a sawmill in March 2005. The timber material was dried by natural seasoning and the sawdust was imported and stored at room temperature until processing in our laboratory.

Essential oil distillation: the essential oil of wood of *T. articulata* was obtained by hydrodistillation for 6 hours in Clevenger device (Guenther, 1972). The yield obtained was 1.3 % of dried material.

Methanol extract: The sawdust of *T. articulata* (100g) was extracted in soxlhet apparatus with methanol for height hours. The crude extract was evaporated to dryness under vacuum to yield 5 g of solid.

Gas chromatography analysis: The usual analysis procedure was followed as described previously (Barrero *and al*, 2005).

Essential oil constituents: Forty components have been identified from the wood oil of *T. articulata*, which represents 85% of the total oil, thirty one of them being oxygenated terpenoids (80.8% of total oil). The major components were cedrol (30.7%), 1,7-di-epi-cedrol (18.4%), 1,7-di-epi-isocedrol (4.7%) and p-methoxy thymol (4.4%) (Barrero ,Herrador ,Arteaga *and al*, 2005).

Methanolic extract constituents: the diterpenoids were the main components: a pimarane derivative, a menthane dimer, an abietane, a cedrol, p-methoxythymol, ferruginol were isolated, the characterisation of these products was reported in an earlier paper (Barrero *and al*, 2003).

2.2 TERMITE ASSAYS

Termites: *Reticulitermes santonensis* was collected from Oleron Island in France. The colony was reared in a climatic room at 27°C and 80% relative humidity. Water and cellulose paper were used as food sources in the no- Choice Test. In order to maintain the humidity, we used for all the bioassays wet Fontainebleau sand. Only workers were used in the bioassays.

No-choice Test: a preliminary test was performed to detect the presence of natural antitermitic properties in the heartwood of the *T. articulata* tree. First series of tests were carried out with crude sawdust in order to evaluate the activity of the whole vegetal material. A disc of sawdust in the proportion of 33 % and 1 % (w/w of wet sand) was placed into the bottom of cylindrical plastic containers with wet sand. Fifty termites were then added to each container. In order to determine the biological activity of the extracts: 20 µl aliquots of essential oil and methanolic extracts were topically applied to 1 cm² cellulose pads. The essential oil dilutions of 5%, 25%, 100% (v/v) were made using diethyl ether. The methanolic extracts dilutions of 50% (weight/weight) were made using methanol. The solvent was allowed to evaporate, and the pads were placed into the bottom of cylindrical plastic containers. Cellulose pads that had been treated with solvent alone were also air-dried in order to control its harmless. Cellulose pad no treated (blank control) were used to control the vitality of the colony. Fifty termites were then added to each container. Termite mortality rates were monitored at 1, 12 and 30 days which correspond to the duration of the test. To identify the specific termite behaviour alterations caused by each extract, triplicate were performed for each concentration. The locations of the termites were noted for both tests and based on the termites which chose to stay on the extract-treated pad or on the sawdust and the extracts and the sawdust were designated to be an attractant, repellent or toxic. The test plastic container was placed in a climatic room maintained at 27 °C and 80 % HR (relative humidity) during all the period of the bioassay. A few drops of water were periodically dripped to the bottom edge of each of them to maintain the humidity of the sand.

Inhalation Test: for assayed termite inhalation, 1 cm² of cellulose pads were treated with 20 µl of crude essential oil. These treated pads were placed on the lid of the cylindrical plastic containers in order to be not in contact with the termites. Fifty termites were added and the test were performed in triplicate. Based on rate of mortality number of termites the extract was designated to act by inhalation if all termites die.

Standardised Tests: The tests were carried out according to the NF-EN 118 standard. Test wood blocks were cut from Pine sapwood (50 × 40 × 10 mm). One face of wood samples was treated with 0.3 g of a solution of treatment. Three concentrations were tested: 20%, 10% and 1% (v/v) of essential oil in diethyl ether. Treated and untreated wood blocks (controls) were dried in climatic room (20 °C, 65% HR) during 72 hours. Three replicates were made for each concentration and for the control. The surface of the wood samples treated and the controls were exposed to 250 termites for 8 weeks in a climatic room maintained at 27 °C and 80 % HR. At the end of the duration, survival of workers was calculated and a quotation related to the extend of the degradation was given to samples: 0 no attack, 1 tentative of attack, 2 light attack, 3 moderate attack, 4 heavy attack.
Statistical analysis was conducted with the analysis of variance with the computer software Statistica.

3 RESULTS AND DISCUSSIONS

The solvents (methanol and diethyl ether) are without impact on the termites. The termites can survive during 30 days without feeding. In the case of blank control (untreated paper), the mean number of alive termites is superior to 75%. After the no-choice test termite mortality responses (Table 1) were analysed, the strongest termite toxicant overall was found to be the sawdust and the essential oil. Total termite mortality occurred in all replications after only one day exposure. The activity of the sawdust is due to the presence of different compounds and we can suppose that the volatile compounds play an important role. The threshold of activity is under 5% (v/v) for the essential oil and is under 1% for the sawdust. At low concentration, the essential oil is repellent since most of termites tended to avoid the contact with the treated zone. They moved at the periphery of the container. Previous reports have revealed the sensibility of termites against some wood species due to the presence of extractives (Betty *and al*, 2001 , Bläske and Hertel, 2001 , Chang *and al*, 2000 , Yusiasih *and al*, 2003). Allelochemicals like terpenoids, are the chemical compounds that are responsible for the natural repellent and toxic properties of termite resistant wood species (Lajide *and al*, 1995a , Lajide *and al*, 1995b , Ohmura and Hoaraa kato, 1997 , Saeki *and al*, 1970 , Watanabe *and al*, 2005).

Inhalation test allows to show that the essential oil acts by inhalation and not by contact or stomodeal poisoning. While the pads of cellulose treated with the methanol extracts at 50 % (w/w) was not toxicant. The termites didn’t eat the treated support, so they are died because of the absence of food. During the first seven days, they hide the treated area with the sand in order to avoid contact. While the relative importance of the methanol soluble fraction, its activity is moderately efficient and characterised by antifeedent properties. The methanolic fraction contains molecules that induce an inhibition of the diet of the termites. Polyphenolic compounds are recognised by the receptor taste (Regnault-Roger ,Philogène and Vincent, 2003). These data suggest that the more volatile components of the wood may be more important for termite resistance of *T. articulata* than the higher molecular weight or more polar compounds that are soluble in methanol. This finding is in accordance with previous work with other species that found the volatile fraction to be specially important (Ohtani *and al*, 1996 , Smythe and Carter, 1970). For the EN 118 test, percentage of survival termites and visual attack ratings are shown in Table 2. Termites in control tests were healthy, more than 50% of individuals survived at the end of the test and the non treated wood blocks were heavily attacked (level 4). The results obtained with the wood samples treated with a solution of 1% of essential oil are similar to the controls; the treatment is without effect on the termites. We can observe that the treatments with 10 % and 20% of essential oil are efficient: no survival is observed and all blocks showed no signs of attack (rated 0). The strong termicidal activity is observed after six 6 weeks of exposition. The standard tests confirm the results obtained with the screening tests: the threshold is situated between 1 % and 10 %. From these presented results, it appears that it would be possible to use the essential oil extracts of *T. articulata* as active ingredient and the threshold of the activity is between 1% and 10 % (v/v). So, the activity against termites is due to the presence of some active molecules in the essential oil. These compounds have repellency and toxic effect. Cedrol is one of main compound present in the essential oil (30%). Several authors are showed the activity of this compound present in some species like *Taiwania cryptomerioides* or *Juniperus Virginia* (Chang *and al*, 2001 ; Mc Daniel, 1989) against fungi or termites. The abietane (diterpenes based on 20 carbons skeleton) are well known as antifeedants (Isman, 2002). But, these results may be due also to a synergistic effect of other constituents like cedrol derivatives: 1, 7-di-epi-cedrol (18.4%), 1, 7-di-epi-isocedrol (4.7%). It will be interesting to control the activity of the crude compounds in order to compare its activity with the activity of the essential oil and to determine a synergetic effect or not of all the constituents.

Table1: Percent of mortality of termites in no-choice tests according tested fractions

Tested fractions	dosage	Termite mortality (%)		
		1 day	12 days	30 days
Sawdust	33 % (w/w)[a]	100	/	/
Sawdust	1 % (w/w)	100	88	100
Essential oil	100 % (v/v)[b]	100	/	/
Essential oil	25% (v/v)	100	/	/
Essential oil	5% (v/v)	1	66	100
Essential oil (inhalation)	100 (v/v)	100	/	/
Methanolic fraction	50 % (w/w)	3	22	100
Diethyl ether	/	0	8	24
Methanol	/	2	2	20
ControlControl Control	/	1	2	25
			222	

[a]w/w: weight/weight; [b]v/v: volume/volume)

Table 2: Termites survival and visual rating according to standard EN 118

Tested fraction	Termite survival	Visual rating[a]
Control	53	4
Essential oil 1 %	50	4
Essential oil 10 %	0	0
Essential oil 20 %	0	0

[a] rating scale: 0 no attack; 4 heavy attack

4 CONCLUSIONS

T. articulata is known for cabinet making sector, its antitermitic potentials were largely unrealised before this research was conducted. The *T. articulata* tree's list of many uses could now be extended to include natural antitermitic biocide. In further studies, we will interest about practical applications of the extracts: termite repellents, toxicants, bait toxicant, soil barrier treatments, topical wood treatments treatment. Each of these termite elimination methods aims to eliminate current environmentally-deleterious termite suppression practices. A natural antitermitic treatments developed from the *T. ariculata* tree could prove to be not only environmentally benign, but economically valuable as well. The *T. articulata* industry is well developed in Mediterranean countries. The industry discharge waste that could be recycled and would be an incentive for our *T. articulata* tree growers to augment their orchards. Future plans of study include isolating specific antitermitic and compounds and further examining the extracts' effects of the physiology of termites.

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