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Evaluation of polyphenols-rich natural compounds as treatments to prevent attacks by subterranean and drywood termites: preliminary results

Abstract - In the view to find sustainable methods to prevent termite attacks to wooden objects and structural timbers, this study represents a preliminary step in the evaluation of some natural substances considered as effective by some African popular traditions. Dark shea cake, obtained from the kernels of *Vitellaria paradoxa* (Sapotaceae), is the phase just before the extraction of shea (= karitè) butter. In some West African regions, by-products from this extraction are traditionally believed to protect houses from termites. To verify if this practice has scientific basis, shea cake was used in experiments with both subterranean and drywood termites, respectively *Reticulitermes lucifugus* (Rossi) (Rhinotermitidae) and *Kalotermes flavicollis* (Fabricius) (Kalotermitidae). As shea nuts are extremely rich in polyphenols, the trials included a comparison with tannins from chestnut (*Castanea sativa*). Short-term experiments to evaluate repellency and feeding deterrence of the two compounds were performed. Results showed differences in the behavior of the two termites species and that shea cake is significantly more repellent than chestnut tannins, especially for *K. flavicollis*. No feeding deterrence activity was detected for either substance for either termite species. Further investigation is currently being performed to better clarify how these compounds affect termite biology.

Key words: *Vitellaria paradoxa*, chestnut tannins, *Kalotermes flavicollis*, *Reticulitermes lucifugus*, repellence.

INTRODUCTION

Termites (Isoptera) are among the worse insect pests for wooden structures and objects and their presence may represent a serious threat for cultural heritage, especially in those areas where climate and environmental conditions are especially favourable to these insects. Prevention of their attacks instead of expensive and polluting a-posteriori control is especially desirable and there is a growing interest in the use of plant compounds as alternatives to synthetic insectides (Verma *et al*., 2009). In the view to find sustainable and valuable methods to protect wood and eventually whole buildings from termites, attention is focused on natural substances that popular traditions consider effective against these insects. *Vitellaria paradoxa* (= *Butyrospermum parkii*) C. F. Gaertn. (Sapotaceae), is a very common tree in West-Central Africa. Its kernels are used to produce shea (= karitè) butter, well known for its use in cosmetic and food industries. People use different parts of this plant against local insect pests (Lehman *et al.*, 2007) and employ the byproducts obtained from shea-butter extraction to protect their houses from termites (Dalziel, 1937), which are extremely abundant in these regions. Among the constituents of shea nuts and their derivatives there are alkaloids, saponins and especially poliphenols like tannins (Morgan & Trinder, 1980; Annongu *et al.*, 1996; Maranz *et al.*, 2003). Presence of tannins and flavonoids has been associated to termite resistance properties (Ohmura *et al.*, 2000; Yamaguchi *et al.*, 2002). The present study represents a first step in the evaluation of *V. paradoxa* kernels extracts in comparison with another polyphenols-rich source, chestnut (*Castanea sativa* Miller) tannins, using as target species the subterranean termite *Reticulitermes lucifugus* (Rossi) (Rhinotermitidae) and the drywood termite *Kalotermes flavicollis* (Fabricius) (Kalotermitidae).

MATERIALS AND METHODS

K. flavicollis and *R. lucifugus* were extracted from naturally infested wood logs collected in San Rossore forest (Pisa) in Tuscany (Italy). Shea cake (indicated as SHEA), is a dark solid waxy paste obtained from finely crushed kernels of *V. paradoxa* that represents the intermediate product in the traditional shea butter processing, just before butter extraction. The sample used for the experiments was produced in a factory located in the area of Sanankoroba, Mali (Africa). Chestnut tannins (indicated as TAN) were used as powder (SAVIOTAN FEED®, Nuova Rivart, Radicofani, Siena, Italy). SHEA and TAN solutions were prepared by dissolving respectively 1.5 g shea cake (melted using bain-marie at 35-90°C) or 1.5 g chestnut tannins in 10 ml ethanol and treatment was performed by placing filter paper disks inside the solutions for 10 seconds. All disks were left to dry in a hood at 60°C for one hour both before and after the treatment to determine retention (w/v), which was 2.1% for ethanol, 7.7% for TAN and 16.2% for SHEA. For *R. lucifugus* containers for the bioassays were maintained inside plastic boxes (30 x 19 x 7 cm) closed with a hermetic lid, whose bottom had layers of absorbing paper completely soaked in water, so that inside each box R.H. was 90%. For the whole duration of the trials, for both species, all the enclosures were kept in a thermostatic chamber in the dark at T 25 ± 2°C.

Termite bioassays

Repellency was tested by means of a choice bioassay system consisting of a Petri dish basement (90 mm diameter) and a plastic cylinder (60 mm diameter x 35 mm high) as cover. The basement was lined with 2 half disks where one was treated with the test substance and the other half was treated with the solvent (ethanol). Ten termites were initially placed in the middle of the dishes and their position was recorded after 10, 20, 30, 60 min. For each termite species 4 replicates were performed for each treatment (CONTROL, SHEA, TAN). For each substance and species a repellency index was calculated as: RI=[(T-C)/TOT] x 100, where T = number of insects on the treated side; C= number of insects on the untreated side; TOT= total number of insects. For this parameter, negative values indicate repellency and positive values attraction. RI were obtained for each observational time point, and an average repellency index over the whole hour of the test (Av 1 h) was also obtained. Repellency indexes (RI) were compared by means of one-way ANOVA and Tukey HSD test to separate the means. RI values, being in percentage form, were transformed in arcsin of the square root before analyses. Nevertheless, results are represented as untransformed data.

To test feeding deterrence a short term two-choice bioassay was set up, where groups of 30 termites were initially placed in the middle of plastic Petri dishes (90 mm diameter) with 15 g sterilized sand moistened with 500 µl micro-filtered water. A treated and a control (ethanol) filter paper disks (30 mm diameter), were diagonally placed in the same Petri dish. Distribution of the termites was checked after 1 h, 24 and 72 h. At the end of the experiment (72 h) mortality was recorded and the filter papers were removed, brushed clean of faeces and debris, oven-dried (60°C for 1 h), and weighed to determine consumption. The test was replicated three times for *R. lucifugus* and four times for *K. flavicollis*. The percent weight loss and the mean numbers of termites detected in each interval on the treated and control-paper were compared by a paired comparisons t-test ($P \leq 0.05$). For mortality statistical analyses (one-way ANOVA) were performed on angular transformations of the percentage data, although untransformed data are reported.

RESULTS

For *R. lucifugus* significant differences were detected in the comparison of repellency indexes (Tab. 1, Fig. 1) after 20 min, when the RI indicated a strong repellency (-87) for shea cake. Considering the average RI over one hour, both chestnut tannins and shea cake were significantly different from the control, but with an average RI indicating medium-low repellency (-42.5 and -52, respectively). For *K. flavicollis* sig-

	R. lucifugus		***K. flavicollis***	
Time (min)	**F**	**p**	**F**	**p**
10	4.21	> 0.05	5.32	0.03
20	5.25	0.03	3.55	> 0.05
30	1.72	> 0.05	4.95	0.04
60	1.38	> 0.05	4.92	0.04
Av 1 hr	7.40	0.01	14.29	0.001

Tab. 1 - Values of one-way ANOVA performed to compare the RI (repellency indexes) for the two termite species.

	R. lucifugus				*K. flavicollis*			
	Mean (±SE)				**Mean (±SE)**			
Experiment	**% weight loss**		**t value**	**p**	**% weight loss**		**t value**	**p**
Control	2.47	± 0.57	-0.58	> 0.05	2.76	± 0.81	0.2	> 0.05
Control	3.17	± 0.88			2.57	± 0.54		
Control	2.82	± 0.28	0.27	> 0.05	2.53	± 0.38	0.51	> 0.05
TAN	2.48	± 1.03			2.08	± 0.82		
Control	3.66	± 0.79	-0.8	> 0.05	2.45	± 0.68	0.46	> 0.05
SHEA	4.48	± 0.40			2.12	± 0.28		

Tab. 2 - Results of the feeding deterrence choice test, reporting the "t values" for paired comparisons t-test.

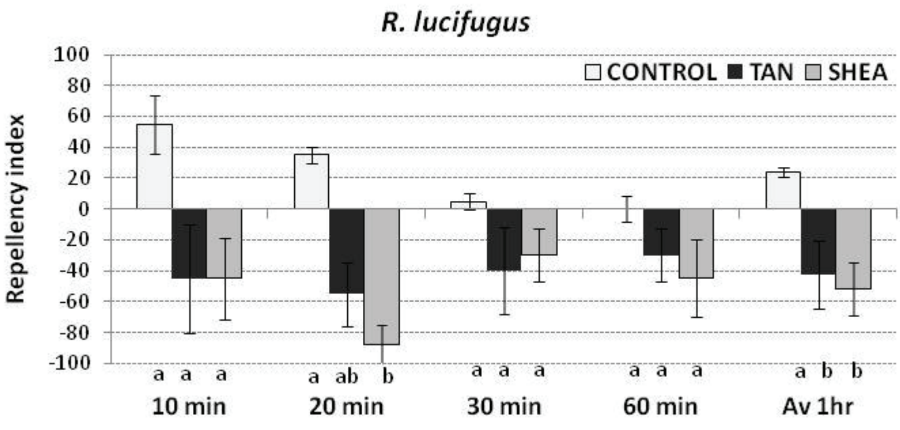

Fig. 1 - Mean (± SE) repellency indexes shown by *R. lucifugus* towards chestnut tannins (TAN) and shea cake (SHEA). Av 1 h shows average repellency index over the whole hour of the test. For each time point, columns indicated by different letters show significant differences at the Tukey HSD test ($p < 0.05$).

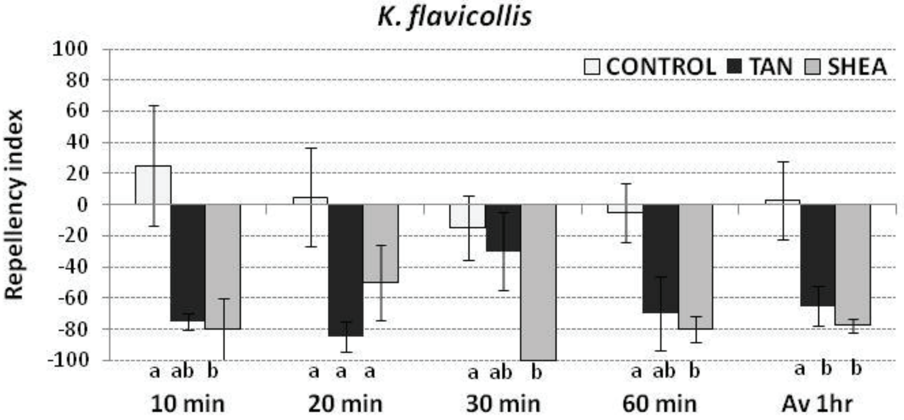

Fig. 2 - Mean (± SE) repellency indexes shown by *K. flavicollis* towards chestnut tannins (TAN) and shea cake (SHEA). Av 1 h shows average repellency index over the whole hour of the test. For each time point, columns indicated by different letters show significant differences at the Tukey HSD test ($p < 0.05$).

nificant differences were detected for almost all the time points (Tab. 1, Fig. 2), with the RI indicating a strong repellency (-80 to -100) for shea cake. Considering the average RI over one hour, both chestnut tannins and shea cake were significantly different from the control, with an average RI indicating medium repellency (-65 and -77.5, respectively).

In the two-choice feeding deterrence test, no significant differences were detected in the percent weight loss between the control and the treated disks for SHEA or TAN (Tab. 2). Similarly, no significant differences were found in the distribution of termites on the two paper disks at any considered time point. At the end of the experiment no significant differences were detected in the mortality of termites observed in the different treatment groups (average mortality 5-8%).

DISCUSSION AND CONCLUSIONS

The present work shows the results from the preliminary trials performed to evaluate the anti-termitic potential of shea cake from *V. paradoxa* kernels and chestnut tannins. Filter paper disks treated with ethanol solutions of these substances were used to test repellency and feeding deterrence on *R. lucifugus* and *K. flavicollis*. Shea cake was a repellent for both termite species, with a stronger effect on the drywood termite. At a lower degree, also chestnut tannins elicited a negative behavior on both species. Neither shea cake or chestnut tannins acted as feeding deterrents and no short-term toxic effects were observed during the experiments. Termite repellence is usually associated to the presence of volatile compounds, such as terpenoids (Maistrello *et al*., 2003; Nix *et al*., 2006), whereas feeding deterrence is often associated to the presence of alkaloids and flavonoids (Ohmura *et al*., 2000; Verma *et al*., 2009) and in some cases termite resistance is linked to condensed tannins (Yamaguchi *et al*., 2002). Chestnut tannins and shea kernels are extremely rich in polyphenols, being good sources of hydrolysable tannins, on the other hand the second contains also saponins, alkaloids and triterpenes (Marantz *et al*., 2003). It is also possible that lack of anti-feedant effects is due to the heat treatments sustained by the compounds during their processing (Ohmura *et al*., 1999). A further investigation is needed to characterize which constituents of shea cake are those responsible for its repellence. Currently, trials are being performed to test different extracts from shea kernels as wood and sand treatments to evaluate effects on termites in short and long range experiments.

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