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Screening of Repellent, Termiticidal and Preventive activities on Wood, of *Azadirachta indica* and *Carapa procera* (Meliaceae) seeds oils

Djenontin Tindo S.[1,2], Amusant N.[3,4], Dangou J.[1], Wotto D.V.[1], Avlessi F.[1], Dahouénon-Ahoussi E.[1], Lozano P.[2], Pioch D.[2,4] and Sohounhloué K.C.D.[1*]

[1]Laboratoire d'Etude et de Recherche en Chimie Appliquée (LERCA), Ecole Polytechnique d'Abomey – Calavi (EPAC), Cotonou, BENIN
[2]CIRAD, UMR 016 GPEB, Montpellier, FRANCE
[3]CIRAD, UMR ECOFOG, Equipe Matériaux et Molécules, Kourou, FRANCE
[4]CIRAD, UR 40 Tropical Wood Products, Biorefinery team, Montpellier, FRANCE

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Abstract

To avoid environmental pollution and health problems caused by traditional wood preservatives or synthetic pesticides which are becoming more restricted to use, there is an increasing search of naturally occurring toxicants from plants. Extractives of phytobiomass having termiticidal activity can be used as low hazard termite control agents. Meliaceae species are well represented in Africa and cover a size range from magnificent forest trees to small shrubs. They have a wide range of uses in ethno medicine, prompting further investigations for biological activity due to the variety of isolated compounds. In the present article the termiticidal activities of Azadirachta indica and Carapa procera (Meliaceae) seeds oils (AISO and CPSO) are screening by no-choice test and standard test EN 118 (2005). Only AISO shows a promising termiticidal activity and both oils have shown a promising repellent activity. AISO and CPSO have the same preventive activity by standard test EN 118 (3: moderate attacks). Additional work will be undertaken to check whether some seeds extracts (non timber products from sustainable management of native forest) could show even higher termiticidal activity under more adverse conditions (polar solvents, cold temperature extraction under pressure) and could be alternative active natural products to synthetic compounds.

Keywords: *Azadiracta indica*, *carapa procera*, repellent activity, termiticidal activity, wood preservation, Benin.

Introduction

Termites are global problem all around the world and especially in tropical areas where relative humidity is high. They have enormous potential value to man, in the degradation and bioconversion of lignocellulosic wastes[1]. Developing methods that prolong the service life of wood have always been the interest of wood researchers[2,3].

Phytochemicals possess a wide spectrum of biological properties against insects. They may act as antifeedants, repellents, growth inhibitors, attractants, chemosterilants or as insecticides[4]. Since these naturally occurring phytochemicals are usually biodegradable and non-toxic to plants, warm-blooded animals and the environment[5-9]. Meliaceae species are well represented in Africa and cover a size range from magnificent forest trees to small shrubs. Certain species are important as timber trees and they have a wide range of uses in ethno medicine, prompting further investigations because relatively few extracts were screened for biological activity in spite of the variety of isolated compounds like limonoids, mono-, di-, sesqui-, and triterpenoids, coumarins, chromones, lignans, flavonoids and other phenolics[10,11].

Azadirachta indica (Neem) is an evergreen tree growing in almost every state of India where it has been used for centuries for medicinal purpose and pest management[12]. Neem kernels contain 30-50 % of oil mainly used by the soap, pesticide and pharmaceutical industries and contain many active ingredients having antifeedant, growth-inhibiting, anti-oviposition and insecticidal activities[13]. *Carapa procera*, "African crabwood", species are distributed all over Africa, as well as in the Amazon basin. The fruit is edible and oil can be extracted from the seeds. The oil is well known for its anti-inflammatory, insect repellent activity[12,13]. It is believed to have potential as a multipurpose (oilwood) crop[12].

Sharma et al. have evaluated termiticidal potential of non-edible oil seeedcakes (Jatropha, Karanja, Neem and Mahua) and their crude active components (phorbol esters, karanjin, saponins and azadirachtin) *in vitro* and *in vivo*[14]. Several authors have worked on termiticidal activities but the diversity of structures and selectivity of the biological actions in Meliaceae family have stimulated a continuing search for new members of this class of potent botanical products[2-5,12,13].

Collected *A. indica* seeds have an oil content of 44.0 wt% dry nut which falls within the range of the data reported by Kaura et al., Sidhu et al. and Kaushik and Vir, and is comparable to groundnut for example 45–53 %[15-17], while *C. procera* seeds have a rather exceptional oil content (61.5 wt% dry nut), higher than the reported data (48–52%)[18,19], even exceeding most known nuts including palm kernel. Owing to the high content, these oils could be extracted simply by pressure, without solvent, bringing several advantages for small scale exploitation (low capital cost, simple technique, safety and environmental impact). Sustainable wood protection methods can also be developed using these seeds oils due to their active components largely discussed in the literature[1-12].

Under the course our exploratory investigations of non food seed oils from underused species[20,21], we explore here by no-choice test and standard European test EN 118[22], the termiticidal activity of *Azadirachta indica* and *Carapa procera* seeds oils. The previous work was dealed with chemical composition of seeds oil and defatted cake[21].

Material and Methods

Collection and preparation of samples: *A. indica* and *C. procera* seeds were collected respectively from Abomey-Calavi, Atlantic Department, and Sakete, Plateau Department (BENIN). They were identified at the National Herbarium of Abomey-Calavi University (BENIN).

Extraction of the seeds oils: The seeds were separated manually, cleaned for any adhering flesh and dried at 50 °C for 48 hours. The dried seeds were ground in a mill and screened through a mesh of 0.6 mm. The oils were extracted from powdered whole seeds in a Soxhlet extractor with hexane at 69 °C and named respectively AISO and CPSO.

Biological assays: Termite species: Assays were conducted with a European species of termites, *Reticulitermes flavipes.* Termites were collected from Oleron Island, FRANCE. The colony was reared in a climatic room at 27 °C and 70 % relative humidity (RH).

Repellent activity: The repellent activities assays were carried out in 9 cm diameter plastic Petri dishes, containing 30 g of damp Fontainebleau sand. A central hole, dug in the sand, contained 2 g of sawdust or a 1 cm^2 filter paper square (Whatman N°42) impregnated with the seeds oils. As a control, termites without paper and filter paper impregnated with extraction solvent were used. 50 active termites (45 workers and 5 soldiers) were placed in a Petri dish (9 cm diam × 1.5 cm height). Repartition of termite galleries crossing concentric circles around the central sawdust were an indicator of repellent activity[23-26].

No-choice test: The method of Kang et al. was used to evaluate the termiticidal activity of the seeds oils: 20 µl aliquots extracts were applied to 1 cm² filter paper discs (Whatman N°42)[26]. After the solvent was removed from the treated papers by air drying at room temperature (65 % and RH – 20 °C), 50 active termites (45 workers and 5 soldiers) were placed on each filter paper impregnated with the test material housed in a Petri dish (9 cm diam x 1.5 cm height). Wet sand of Fontainebleau (20 g) was used as substrate to maintain the humidity. The test dishes with covers were then placed in an incubator maintained at 27 °C – 70 % RH. A few drops of water were periodically dripped on the bottom of each Petri dish. Filter paper samples treated with solvent alone (20 µl) were used to control the no toxicity (Solvent) and untreated paper samples were used as termite vitality controls (Diet). Also termites without paper sample were used as control. Each treatment was tested in triplicate and the termite mortality was scored.

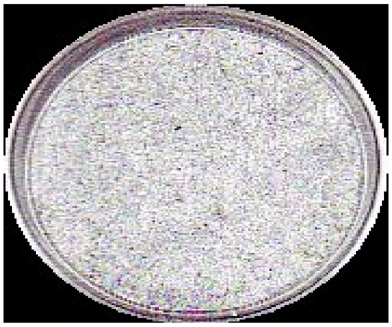
Standard test EN 118[22]: Test wood blocks were cut in pine sapwood (25 × 100 × 10 mm R x T x L). The wood samples were treated with 20 mg of seed oil per cm².

Three wood samples were treated with the solvent to control the no toxicity and no treated sapwood wood blocks was use to control the virulence of the termites. All samples were dried in a climatic room (20°C – 65 % RH) for 72 hours. Surfaces of the treated wood samples and controls were exposed to 250 workers, 5 nymphs and 5 soldiers for 8 weeks in a climatic room maintained at 27 °C and 80 % RH. Three replicates were conducted per concentration. At the end of the exposure period, the worker mortality rate was calculated and a score related to the extent of degradation was allocated to samples according to the standard: 0 = no attacks, 1 = attempted attacks, 2 = light attacks, 3 = moderate attacks and 4 = heavy attacks.

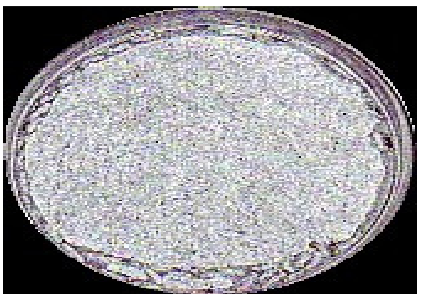
Results and Discussion

Validation of no-choice test and EN 118: The termiticidal activities of AISO and CPSO are carried out by two tests: i. no-choice test can evaluate the toxicity of the seed oils by allowing to compute the mortality rate; ii. standard test EN 118[22] can confirm the termiticidal activity revealed by screening, and evaluates the preventive activities against wood degradation. These tests on living organisms (termites) with seed oils obtained with an organic solvent are validated by the following observations: The termites survive during 24 days without feeding (diet). This period is quite longer than the 13 days assay required by the no-choice test, The rate of survival for the control (termites normally supplied with cellulose) is equal to 65 %, thus higher than the 50 % necessary for the validation, The effect of solvents shows only 4-10 % of mortality rate, The control of virulence presents the visual quotation 4 (strong wood attacks) by standard test EN 118[22], A ratio of 57 % of survivors -higher than 50 % necessary for the validation- is observed at the end of the test. These observations can also validate the assays.

Termiticidal activity of the seeds oils: Repellent activity: When exposed to a "repellent substance"[23-26], the termites organize themselves in order to limit as much as possible their contact with the active substrate. They make then in tunnels in the lower part of the sand of Fontainebleau (used as model medium in the petri dish). The scan of the lower face of the Petri dish a few time (2 hours) after the beginning of the test, have revealed the repellent activity. According to the organization of the termites observed in figure 1, we note the average repellent activity for both seeds oils (AISO and CPSO) obtained in the hot temperature (69 °C) and denote the highest repellent activity can be achieve with polar solvents or with oils obtained under cold temperature conditions.



(a) Repellent activity of *A. indica* seed oil (AISO)



(b) Repellent activity of *C. procera* seed oil (CPSO)

(c) Diet

(d) Solvent control
Figure -1
Repellent activity of seeds oils (AISO, CPSO) by no-choice test (Dose: 20 mg/cm²)

CPSO would be most repellent than Neem oil (AISO). These repellent activities of AISO and CPSO were previously reported respectively by Schmutterer and Konan et al. against mosquito and other insects[13,27].

No-choice test: The mortality rates during the assay are presented in figure 2. The Neem oil (AISO) is the most active with a TM 65 % after 13 days exposure. This activity is slightly higher than the one observed for *Carapa procera* seed-oil (CPSO), only TM 21%. Insecticidal activity of Neem oil is largely discussed in the literature by Schmutterer[13] for general insecticidal activity, and specially by Sharma et al. who have evaluated termiticidal potential of non-edible oils (Jatropha, Karanja, Neem and Mahua) and their crude active components (phorbol esters, karanjin, saponins and azadirachtin) *in vitro* and *in vivo,* but *Azadirachta indica* was not found to be the most potent[14]. They also suggested that it would be beneficial to further purify and characterize active components and develop commercial formulations for termite management. The literature provides the data on repellent activity of solvents extracts of *C. procera* reported by Konan et al.[27]. Several authors have worked on termiticidal activities[1-9,28] but the diversity of structures and selectivity of the biological actions in *Meliaceae* family will stimulate further biological investigations for new potent botanical products[29,30].

Standard test EN118[22]: In order to confirm the activity of oils shown by no-choice test, standard test EN 118 was carried out (figure 3). Types of attack of wood observed are of moderate intensity for both species. According to this standard test, none of these oils should be used as wood preservatives because the type of attack is higher than 2. Although the mortality rate of CPSO is weaker than the one of AISO by no-choice test, both oils show the same preventive activity on wood (moderate attacks). It is thus preferable to kip *C. procera* for complementary tests with extracts obtained with solvent covering a larger range of polarity. Indeed, these oils obtained under a relatively high temperature (69°C) are not relevant. It will be wise to test oils obtained by simply pressing under cold temperature, without solvent, these conditions bringing several advantages for small scale exploitation (low capital cost, simple technique, safety and environmental impact).

This simple technology can be developed because our team has recently reported that collected *A. indica* seeds have an oil content of 44.0 wt% dry nut, which is comparable to groundnut for example (45–53%), and that *C. procera* seeds have a rather exceptional oil content (61.5wt% dry nut), even exceeding most known nuts, including palm kernel[21]. Sustainable wood protection methods could be developed using these seeds oils due to their active components largely discussed in the literature[1-12].

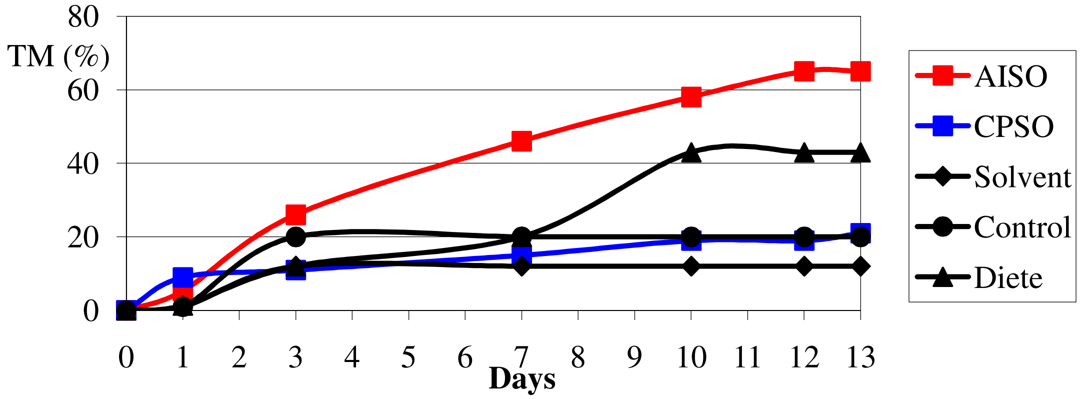
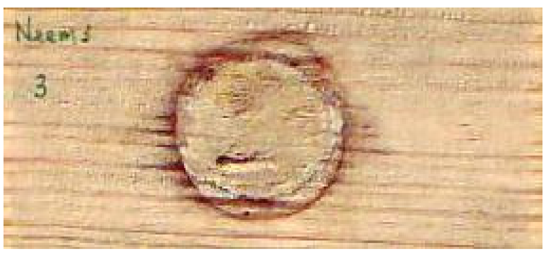
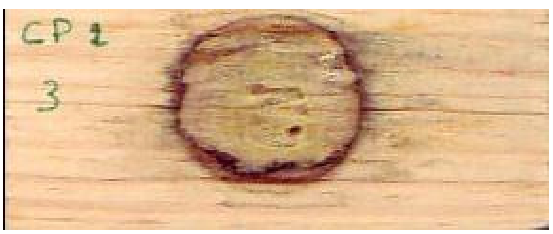


Figure -2
Termiticidal activity of *Azadirachta indica* and *Carapa procera* seeds oils (AISO and CPSO)



(a) Type of attack for the wood treated by AISO



(b) Type of attack for the wood treated by CPSO

(c) Control

Figure -3
Attacked area of the treated wood (*Pinus sylvestris*) exposed to termites according to EN118 (2005)
(Dose applied: 20 mg/cm²)

Conclusion

The repellent activity by no-choice test of *Carapa procera* seed oil is higher than that observed for *Azadirachta indica*, but both oils showed the same preventive activity on wood based on the standard procedure EN 118 (3: moderate attacks).

Azadirachta indica was also promised for a good termiticidal activity after further purification of extract or extraction with solvent covered a large polarity and/or in cold conditions. Both oils were promised for further high repellent activity. Additional work will be undertaken to check whether some seeds extracts (non timber products of sustainable management of native forest) obtained under a broader range of conditions (polar solvents, cold temperature extraction by pressure) could show an even higher termiticidal activity, and be alternative active natural products to replace synthetic compounds.

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