# Extracts of the rosary pea, *Abrus precatorius,* are toxic to the invasive termite, *Coptotermes gestroi*

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## Abstract

In 2009 an outbreak of the termite *Coptotermes gestroi* caused major damage to buildings and vegetation in Fiji. Termite control is most commonly performed using synthetic insecticides, although many botanically-derived toxins are also being evaluated. The woody legume, *Abrus precatorius*, contains the toxic protein abrin, and has been investigated previously for insecticidal properties. The current study used laboratory experiments to evaluate extracts of *A. precatorius* as potential control agents for *C. gestroi*. In Petri dish assays, exposure to water- and methanol-based extracts of leaf and seed material significantly increased mortality and shortened time to death of *C. gestroi*. The results suggest that extracts of *A. precatorius* have potential for use as control agents of *C. gestroi* and should be evaluated further to determine their mode of action and efficacy under field conditions.

## Introduction

In 2009 an outbreak of the Asian subterranean termite, *Coptotermes gestroi* (Wasmann 1896) (Isoptera: Rhinotermitidae), occurred in the Lautoka district of Viti Levu in Fiji causing major damage to homes, schools and vegetation. According to the Biosecurity Authority of Fiji (*pers. comm*.) the Fijian government spent more than FJ$3 million between 2009 and 2011 on the problem of *C. gestroi* infestation, which involved application of chemical treatments, repair of damaged structures and education of local residents. Control of *C. gestroi* in the Lautoka area was primarily based on the commercially-available product 'Termidor' (which contains the active ingredient fipronil) imported from Australia, with prices to treat a typical Fijian house ranging between FJ$170 to FJ$250. The Fiji Government indicated that new termite infestations would only be treated on a 'user pays' basis, and home owners that could not afford to pay for the chemical treatments would also be liable to pay for their own house repairs, estimated in the region of FJ$8000, close to the estimated per capita gross national income of FJ$8300 in 2011 (Burese, 2011; Malo, 2011; UNdata Fiji, 2013).

*Coptotermes gestroi* originates from South East Asia and is considered one of the most destructive, economically important, termite species worldwide. Control of *C. gestroi* is still commonly performed using synthetic insecticides, such as bistrifluron, hexaflumuron and imidacloprid (Lee *et al.,*2004; 2009; Hafiz and Hassan, 2008). However, although these insecticides can be highly effective, the adverse effects of these chemicals on the wider environment and human health are of increasing concern and so alternative termite control methods continue to be investigated (Verma *et al.*, 2009).

A number of natural products and plant extracts have been screened for inhibitory activities against invasive or pest social insects such as ants, wasps and termites, with a view to controlling these species in a more 'environmentally friendly' manner. For example, extracts of boxthorn (*Lycium shawii*) and cassava (*Manihot esculenta*) have recently been shown to have strong toxic effects against samsam and leaf cutter ants respectively (de Cavalho Alves et al., 2013; Mashaly et al., 2014), whereas the argentine ant, now a major invasive species in New Zealand, was repelled from herbage treated with by essential oils of wintergreen, cinnamon, cloves, peppermint and (especially) spearmint (Scocco et al., 2012). With regard to termiticides, extracts from white cedar *Melia azedarach,* aroeira *Myracrodruon urundeuva,* teak *Tectona grandis,* stilt-mangrove *Rhizophora apiculata* and the 'suicide tree' *Cerbera odollum* have all been shown to have toxic effects against *C. gestroi* (Hashim *et al.*, 2009; Khalil *et al.*, 2009; Nascimento *et al.* 2009).

The leguminous plant *Abrus precatorius* L. (Fabaceae) ('rosary pea', 'jequirity') is a high-climbing or trailing woody vine that grows in tropical climates (Anand *et al.*, 2010). The plant is native (or an ancient introduction) to Fiji but, because of its tendency to smother or over-grow other native vegetation, is often considered as invasive or a nuisance weed. *Abrus precatorius* has numerous and wide-ranging bioactive properties and the insecticidal effects of this species have been investigated for over a hundred years (*e.g.* Stillmark, 1888; Bhatia et al., 2013). The aim of this study was to use laboratory assays to evaluate water- and methanol-based seed and leaf extracts of *A. precatorius* for toxicity towards *C. gestroi* by measuring both final mortality and time to death.

## Materials and Methods

Termites were collected from trees and posts in Tavakubu and Central College School, Lautoka, and housed in glass tanks (90% relative humidity; 28°C) with wood fragments until used in assays. *Abrus precatorius* leaves and seeds were collected from Malomalo Village and the leaves and seeds washed, frozen for 3 days, freeze dried for 3 days and then ground to a fine powder using an electric grinder. Methanol extracts were prepared by mixing 500g of leaf or seed powder with 1 L of methanol (99%) in a conical flask, shaking for 48 hours on an electric shaker and then repeatedly filtering until clear. The filtrate was dried using a rotary evaporator at 60˚C and a stock solution prepared by re-dissolving 10 g of the dried extract in 20 ml of methanol to obtain a 50% w/v solution, which was in turn serially diluted to give desired concentrations for bioassays (10% and 1% w/v). Water extracts were made by direct mixing of *A. precatorius* leaf or seed powder in distilled water at the required concentrations (10% and 1% w/v). The mixtures were placed on a shaker for 48 h and then filtered. All solutions were stored at 4°C until used.

Toxicity assays were performed using glass Petri dishes (9 cm diameter) as the experimental arenas. Filter papers were dampened with 2 ml of solution and excess fluid allowed to evaporate. There were three replicate Petri dishes for all test solutions. Distilled water or methanol (99%) were used as appropriate control treatments. Twenty five termites were added to each of the Petri dishes, and daily mortality of *C. gestroi* recorded in each dish for 10 days, with dead individuals being removed each day to prevent contamination. The Petri dishes were maintained in a hygrostatic tank at 28°C and 90±10% RH and the high humidity ensured the filter papers remained moist throughout the 10 d trial.

The mean time of death (MTD; d) for termites in each Petri dish was calculated by:

$$MTD = \frac{\sum d_i D_i}{\sum D_i}$$

where $d_i$ = the $i^{th}$ day since the assay started and $D_i$ = number of termites which were recorded newly dead on $d_i$.

## Results

There was a strong correlation between MTD and concentration for both the water and methanol extracts (Figure 1: $r_s > |0.75|$; P < 0. 0.005 for all extracts). Death was most rapid when termites were exposed to methanol extracts compared to water extracts (*cf.* Figure 1a & 1b), although mortality had still reached 100% in both of the 10% extracts by Day 10 (Table 1).

Termite mortality exhibited a clear positive relationship with extract concentration for all four types of extract after 1 d and 2 d exposure (Table 1). Mortality was more rapid when exposed to the methanol extracts compared to the water extract of comparable concentrations. For the highest (50%) concentration of methanol extracts, all termites were dead after 1 d exposure to the seed extract and after 2 d exposure to the 50% leaf extract.

There was no control mortality in the water and methanol extracts after 1 d and 2 d (Table 1). However mortality of termites in the control methanol treatment was high after 10 d (83%), suggesting exposure to methanol residues was having some chronic inhibitory effects

## Discussion

The results indicate that exposure to leaf and seed extracts of *A. precatorius* can cause mortality and shorten time to death in the termite *C. gestroi*. Previously, Setiawan *et al.* (2009) reported that methanol extracts of leaves and seeds of *A. precatorius* could produce 100% mortality of *C. gestroi* in 12 days using a 'force-feeding' method. Our study has reconfirmed the toxicity *A. precatorius* extracts against *C. gestroi*, and extended these results by demonstrating that water-based extracts are also toxic, and that mortality can be caused by contact with *A. precatorius* extracts or residues as well as by ingestion.

Bait toxicant systems, where active ingredients are incorporated into food baits which are then carried into the termite colony by foraging workers, can substantially reduce pesticide use (Su & Scheffrhan, 1998). In these bait systems, a slow mode of action is important as sufficient time is required for foraging termites to contact the toxin and then return to the colony. This type of action was apparent for some *A. precatorius* extracts: for example the 10% leaf extracts acted fairly slowly (MTD ≈ 4-6 d) but still caused 100% mortality after 10 days. A further desirable property of the *A. precatorius* extracts was that there were no obvious repellent effects towards *C. gestroi* and this may have prevented lethal doses being received.

Hoddle (2004) suggested there is scope to control invasive species in a sustainable fashion if one problem species is utilized in some manner that helps to control another. Many other important insect pests of South Pacific islands (*e.g.* mosquitoes and aphids) have also been shown to be susceptible to *A. precatorius* extracts. Thus the development of viable and effective insecticides from this plant may offer multiple advantages in a South Pacific setting, in terms of reduced synthetic pesticide use, more environmentally sound pest control, management of a problem weed, and, ultimately, some economic benefit to the local communities involved.

This study provides preliminary data describing the strong negative effects of *A. precatorius* on *C. gestroi*. However we have not as yet identified the active ingredient(s) and their concentrations in the extracts, and so could not estimate the actual doses of chemicals the termites received. Further investigation of *A. precatorius* extracts is required to ascertain their mode of action and their effectiveness when applied to natural substrates (e.g. soil; foliage; timber) or incorporated into bait toxicant formulations in a field setting.

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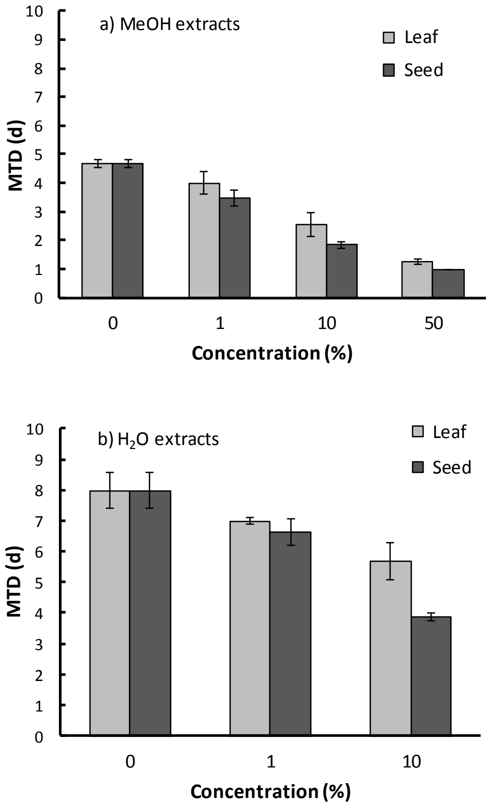


Figure 1. Mean time to death (MTD; days; mean ± SE; N = 3) of 25 *Coptotermes gestroi* exposed to filter papers treated with (*a*) methanol and (*b*) water extracts of *Abrus precatorius* leaves and seeds. The doses given are relative concentrations (%) of original leaf and seed extracts (see Methods for details).

| Solvent | Tissue | Time | Concentration | | | | $r_S$ | P |
|---|---|---|---|---|---|---|---|---|
| | | | 0% | 1% | 10% | 50% | | |
| Methanol | Leaf | 1 d | **0** | **1.3** | **20.0** | **74.7** | 0.931 | < 0.001 |
| | | 2 d | **0** | **8.0** | **45.3** | **100** | 0.987 | < 0.001 |
| | | 10 d | **82.7** | **100** | **100** | **100** | 0.598 | 0.011 |
| | Seed | 1 d | **0** | **10.7** | **41.3** | **100** | 0.986 | < 0.001 |
| | | 2 d | **0** | **28.0** | **81.3** | **100** | 0.941 | < 0.001 |
| | | 10 d | **82.7** | **100** | **100** | **100** | 0.598 | 0.011 |
| Water | Leaf | 1 d | **0** | **6.7** | **12.0** | - | 0.892 | < 0.001 |
| | | 2 d | **0** | **6.7** | **16.0** | - | 0.946 | < 0.001 |
| | | 10 d | **26.7** | **52.0** | **100** | - | 0.834 | 0.002 |
| | Seed | 1 d | **0** | **6.7** | **10.7** | - | 0.897 | < 0.001 |
| | | 2 d | **0** | **10.7** | **24.0** | - | 0.942 | < 0.001 |
| | | 10 d | **26.7** | **100** | **100** | - | 0.845 | 0.002 |

Table 1. Mortality (%; mean; N = 3) of 25 *Coptotermes gestroi* after 1, 2 and 10 days exposure to methanol and water extracts of *Abrus precatorius* leaves and seeds in Petri dish toxicity assays. The doses given are relative concentrations (%) of original leaf and seed extracts (see Methods for details). ($r_S$ - Spearman's rank correlation coefficient).