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# Toxicity of Turmeric Extracts to the Termite *Reticulitermes flavipes* (Blattodea: Rhinotermitidae)

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**ABSTRACT** Turmeric is an important spice crop with documented human health benefits associated with chemicals called curcuminoids. In this study, the termite *Reticulitermes flavipes* (Kollar) was exposed to different solvent extracts of turmeric to investigate potential termiticidal properties. Treating termites with hexane extracts of purified lab-grade curcuminoids had no effect on termites. However, in continuous exposure assays, the LC<sub>50</sub> for hexane extracts of crude turmeric powder was 9.6 mg, or 1.0 mg starting material per square centimeter of filter paper substrate. These active components were soluble in a range of polar and apolar solvents, but only hexane could selectively fractionate active components away from the inactive curcuminoids. The active constituents of turmeric separated by thin layer chromatography (TLC) fluoresced in short-wave UV light but were not visible in long-wave UV light. By re-extracting TLC-separated bands in hexane and performing bioassays and gas chromatography–mass spectrometry, we demonstrated that termiticidal components of turmeric are extractable as a blend containing mainly ar-turmerone, turmerone, and curlone. This determination is consistent with findings of preceding work by other researchers that investigated insecticidal properties of turmeric in other pest insects.

**KEY WORDS** natural product, turmeric, curcumin, turmerone, solvent extract

Plant-based products have been considered as likely alternatives to synthetic insecticides due to their seemingly less harmful effects on the environment and human health compared with conventional pesticides, which can harm nontarget organisms and ecological communities (Isman et al. 2011). Major plant-based products used for controlling insects are pyrethrum, rotenone, neem, essential oils, ryania, nicotine, and sabadilla (Isman 2006), and there are many examples of their phytochemical effects on insects (reviewed by Isman 2008).

Turmeric (*Curcuma longa* L.) belongs to the family Zingiberaceae and is grown in the tropical region of southern Asia. *C. longa* rhizomes are ground to yield a yellow powder that is used as a food spice and also as a natural dye (Su et al. 1982). The characteristic yellow color of turmeric is attributed to polyphenols called curcuminoids present in the rhizomes (Revathy et al. 2011). In the Indian traditional system of medicine “Ayurveda,” turmeric has been used to treat various disorders (Sivananda 1958, Raghunath and Mitra 1982, Srimal 1997, Tripathi et al. 2002), and more recently western medicine has considered its pharmacological properties for a number of medicinal uses (e.g., Lee et al. 2011, Hucklenbroich et al. 2014).

Prior studies have investigated turmeric toxicity and repellency against insects. For example, turmeric is toxic to the maize weevil (*Sitophilus zeamais* (Motschulsky)) and the fall armyworm (*Spodoptera frugiperda* (J. E. Smith)) (Tavaresa et al. 2013), and essential oil extracts from turmeric leaf are toxic against *Sitophilus oryzae* L., *Rhyzopertha dominica* (F.), and *Tribolium castaneum* (Herbst) (Tripathi et al. 2002). Turmeric in powdered form or its raw extract strongly repels *Tribolium confusum* Jacquelin du Val, *R. dominica*, *Sitophilus granarius* L. adults and *Attagenus megatoma* (F.) larvae (Su et al. 1982).

Some prior studies have investigated using plant-derived chemicals to manage termites. Oil extracted from nuts of the *Aleurites moluccana* tree has been used to treat wood, protecting it from feeding by the Formosan termite (*Coptotermes formosanus* Shiraki; Nakayama and Osbrink 2010). Defatted neem oil along with other inert ingredients is more efficacious in protecting wood, as well as acting as a soil barrier against the subterranean termite, *Coptotermes gestroi* Wasmann compared with the azadirachtin-containing fraction (Himmi et al. 2013). Also, four plants viz. *Rhazya stricta* Decne, *Lantana camara* L., *Ruta chalepensis* L., and *Heliotropium bacciferum* Forssk displayed toxic effects on the subterranean termite *Pseudotermes hybostoma* (Desneux) (Alshehry et al. 2014). However, to our knowledge no prior studies have investigated direct lethal or toxic effects of turmeric on termites. In this study, we used the eastern subterranean termite,

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*Reticulitermes flavipes* (Kollar) to: 1) test the toxicity of different solvent extracts of turmeric, 2) determine the LC<sub>50</sub> of active extracts based on amount of starting material, and 3) identify the active components present in termitically active turmeric extracts.

### Materials and Methods

**Maintenance of Termite Colonies.** Five laboratory colonies of *R. flavipes* were initiated by collecting termites from five separate locations or colonies on the Purdue University campus (West Lafayette, Tippecanoe County, IN). Subterranean traps baited with corrugated cardboard were used for collecting termites. Wooden shims (Nelson Wood Shims, Cohasset, MN) covered in paper towels (Scott Brand, Kimberly-Clark Professional, Roswell, GA) moistened with deionized water were provided as a food source to termite colonies. Colonies were maintained in the laboratory for 1–12 mo in darkness at 22°C before their use in experiments. Ten or 20 termites from each colony were used per assay replicate for both treatments and solvent controls, with three to five biological replicates per treatment.

**Test Materials and Solvents.** Two sources of non-organic turmeric (Deep Foods, Inc., Union, NJ) and one source of organic turmeric (Frontier Natural Products, Norway, IA) were tested. Laboratory grade curcumin (>90% pure), containing a mixture of curcuminoids, was purchased from Sigma-Aldrich (St. Louis, MO). All analytical grade solvents (acetone, acetonitrile, chloroform, ethanol, and hexane) were either purchased from Fisher Scientific (Pittsburgh, PA) or Sigma-Aldrich (St. Louis, MO).

**Toxicity Bioassays with Different Solvent Extracts.** Initially, components of turmeric were extracted by passing 500 µl of solvent (i.e., water, ethanol, acetonitrile, acetone, chloroform, or hexane) through 0.1 g turmeric contained within a column composed of a 27.2-cm-long flint glass Pasteur pipette (Fisher Scientific, Pittsburg, PA) that was plugged with glass wool. Extracts were eluted directly onto 3.5-cm-diameter Whatman grade 4 filter papers (GE Healthcare UK Limited, Buckinghamshire, UK) contained within Petri dishes (TPP Techno Plastic Products AG, Trasadingen, Switzerland). After absorbing the turmeric extract, the filter papers were allowed to dry in a fume hood prior to use in bioassays. Lab grade curcumin (0.1 g) was extracted in an identical manner. Solvent-treated filter papers served as controls in all experiments. After the solvent evaporated, 100 µl of deionized water was added to keep the filter paper moist (Boucias et al. 2013). Each replicate dish then received 10 or 20 worker termites. After initiating treatments, observations were made once every 24 h for the first 2 d and then on the fifth day before assays were terminated. Termites that were immobile were considered intoxicated, whereas moving termites were considered healthy.

**Thin-Layer Chromatography (TLC).** All solvent extracts of turmeric were analyzed by TLC to separate and visualize chemical constituents of the extracts.

TLC plates with fluorescent indicator (Fluka 60763, Sigma-Aldrich) were also spotted with ethanol extracts of technical grade curcumin, organic turmeric, or conventional turmeric from two sources (see above). Plates were developed with a mobile phase consisting of chloroform: methanol (95:5) (Revathy et al. 2011, Kulkarni et al. 2012). TLC plates were observed under long-wave (366 nm) and short-wave (254 nm) UV light. The band of interest that appeared near the leading edge of the mobile front (active ingredient band) was scraped off and re-extracted in 500 µl ethanol for further testing in bioassays, high pressure liquid chromatography (HPLC), and gas chromatography–mass spectrometry (GC-MS).

**Vapor Toxicity Assay.** To determine the extent to which termites are affected by vapors of turmeric extracts, termites from three different colonies were tested as biological replicates. Ten termites from each of three colonies were held in separate 4-ml amber vials with vented caps (Wheaton, Millville, NJ; Scharf et al. 2006) placed on top of dried filter papers treated with ethanol extracts made from 0.2 g turmeric. The mesh was glued onto the septum caps and served to confine termites within the amber vials while allowing gasses to pass through. Ten termites from the same three replicate colonies were also placed directly on treated filter papers. The assay dishes, with and without mesh-covered vials, were held inside an airtight container. Ethanol extracts were tested because they contained the widest spectrum possible of candidate insecticidal compounds (see Results). Ethanol was allowed to evaporate before placing termites in assays. The termites were scored once every 24 h for 2 d.

**LC<sub>50</sub> Determination.** To estimate a median lethal concentration of raw turmeric, 1.0 ml hexane extracts were made from 40, 20, 18, 16, 14, 12, 10, 8, 6, and 4 mg turmeric starting material and applied to filter paper substrates. Controls received 1.0 ml hexane alone. After solvent evaporated, 0.1 ml of water was added. Termites were placed on treated filter papers in Petri plates and scored as described above. Five independent replicates using termites from five source colonies were tested for each concentration and solvent controls. Each replicate consisted of 20 worker termites per dish. SAS software was used to conduct probit analysis for calculating LC<sub>50</sub>, LC<sub>99</sub>, and associated parameters (SAS Institute Inc., Cary, NC).

**Analytical Chemistry Procedures.** The following samples were analyzed using HPLC: ethanol extracts of turmeric, ethanol extracts of the most mobile turmeric active ingredient band (scraped from TLC plates), ethanol extracts of lab grade curcumin, and ethanol alone. These samples were transferred to 2-ml autosampler vials (Fisher Scientific, Pittsburgh, PA) and analyzed using a Shimadzu series HPLC system (Shimadzu, Kyoto, Japan) consisting of a LC-20AB pump, a CBM-20A communications bus module, a SPD-M20A diode array detector, a DGU-20A3 degasser, and a SIL-20A HT autosampler. The autosampler was used to inject 10-µl aliquots of samples onto a reversed phase C18 column (5 µm, 25 cm × 4.6 mm; Phenomenex, Torrance, CA) after passing through a C18 guard

column. Synchronized control of the various HPLC components was achieved through Shimadzu EZstart 7.4 SP3 software. Analytes were eluted using a series of ACNl (acetonitrile)+water gradients: 1) 20% ACNl from 0 to 5 min, 2) linear gradient to 100% ACNl from 5 to 18 min, 3) 100% ACNl from 18 to 26 min, 4) linear gradient to 20% ACNl from 26 to 36 min and 5) 20% ACNl from 36 to 41 min. A constant flow rate of 1 ml per min was used throughout. A wavelength of 202 nm was used to detect peaks in all the samples.

For GC-MS analysis, the turmeric active ingredient band was purified by TLC and re-extracted in hexane. GC-MS analysis with electron impact ionization (EI, 70 eV) was performed using an Agilent 6890 N GC (Agilent Technologies, Santa Clara, CA) equipped with a DB-5MS capillary column (30 m  $\times$  0.25 mm  $\times$  0.25  $\mu$ m film; J&W Scientific, Folsom, CA) in splitless mode, and interfaced to a 5975B mass selective detector (Agilent Technologies) with helium carrier gas. The oven temperature was programmed from 100°C for 1 min, ramped to 150°C at 5°C/min, then to 200°C at 2°C/min, then to 300°C at 5°C/min, and held for 15 min. Inlet temperature was set to 250°C. Compounds were identified by comparing their mass spectra with those in the National Institute of Standards and Technology (NIST) mass spectral library (ca. 120,000 spectra) with a minimum match of 90% (Chemstation Version D.05.01; Hewlett Packard Corp., Palo Alto, CA).

## Results

**Toxicity Bioassays of Turmeric and Curcumin Extracts.** Water extracts caused no significant mortality above controls. The effects of ethanol, acetonitrile, acetone, chloroform, and hexane extracts of 0.1 g of turmeric on termites were visible within 24 h. Treated termites appeared sluggish and showed flaccid paralysis-like symptoms, while control termites were active. All extracts killed 100% of termites within 5 d, whereas control termites receiving only dried solvent residues showed no significant mortality above 10%. These results suggest that active components of turmeric have physical properties that allow them to be extracted into both polar and apolar solvents.

To evaluate the extent to which the curcumin and other curcuminoid components of turmeric were responsible for toxicity, a commercially available curcumin extract (see TLC and HPLC analyses below) was assayed. Curcumin-treated termites showed 0% mortality, as did control termites, indicating that curcumin and related curcuminoids are not the termiticidally active components of turmeric.

In vapor toxicity bioassays, termites exposed only to vapors were alive, while those exposed to the turmeric extract-treated filter paper died within 2 d. These results indicate that the active components of turmeric extract are active by contact and feeding and not volatility.

**LC<sub>50</sub> Determination.** Termites treated with hexane extracts prepared from a range of crude turmeric quantities (40, 20, 18, 16, 14, 12, 10, 8, 6, and 4 mg) revealed an approximate median lethal turmeric

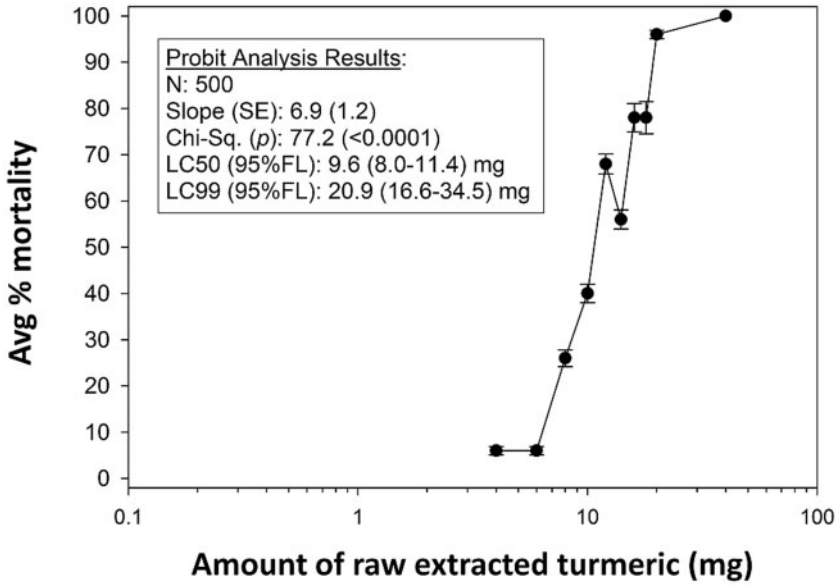
quantity (LC<sub>50</sub>), pre-extraction, of 9.6 mg (Fig. 1). These results indicate that the active components of turmeric can be selectively extracted from crude turmeric powder using hexane, with extracts of ca. 1 mg starting material per square centimeter of treated substrate causing median lethal mortality.

**Thin-Layer Chromatography.** Based on a TLC comparison of extracts using solvents across a continuum of polarities (acetonitrile, ethanol, acetone, chloroform, and hexane) only hexane effectively excluded some components present in all other extracts (results not shown). Specifically, a group of compounds with greatest TLC mobility (R<sub>f</sub> > 0.85) was selectively isolated by hexane extraction (Fig. 2, top three lanes). These hexane-extractable compounds retained toxicity in bioassays, causing 100% mortality when prepared from 0.1 g turmeric. Hexane extracts were also devoid of the yellow color present in all other solvent extracts, which is caused by curcuminoids. In a comparison of ethanol extracts of turmeric from different sources against a reagent-grade curcumin preparation (Fig. 2, top three lanes), all bands of R<sub>f</sub> < 0.5 present in purified (lab grade) curcumin were also present in ethanol extracts of organic and standard turmeric. However, the highly mobile bands of R<sub>f</sub> > 0.85 were present in turmeric extracts and not in the purified curcumin standard. These more mobile bands were only visible under short-wave UV light. The curcumin bands, alternatively, were visible under both long- and short-wave UV light, but fluoresced more intensely under short-wave UV. The more mobile bands were also those unique to hexane extracts as noted above. Termites exposed to filter papers treated with the upper-most mobile bands re-extracted in ethanol started showing the characteristic symptoms of turmeric toxicity within 24 h, and reached 100% mortality by day 5. No mortality occurred in solvent controls.

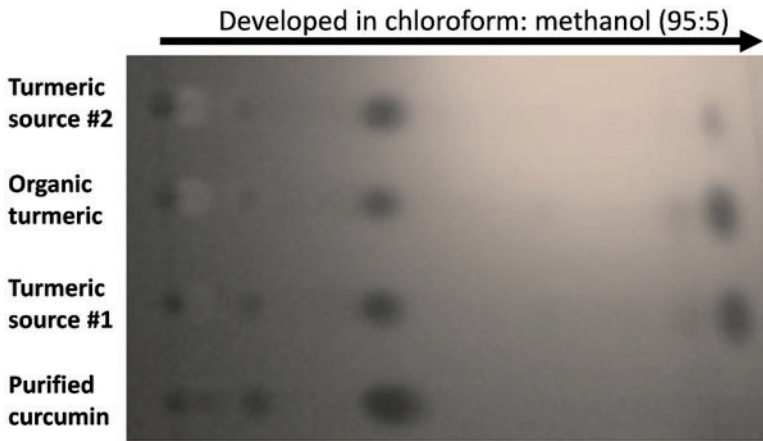
**Analytical Chemistry.** HPLC analysis was performed to further define characteristics of crude and purified active fractions of ethanol extracts (Fig. 3). Chromatograms of active termiticidal extracts contain several peaks eluting between 19 and 22 min that represent novel compounds (Fig. 3). Although ethanol was the extracting solvent used for HPLC studies, the active components eluting between 19 and 22 min can be selectively extracted from crude turmeric using hexane (results not shown). Lastly, GC-MS identified components of termiticidally active hexane extracts as aromatic (ar)-turmerone (peak 1), turmerone (peak 2), and curlone (peak 3), with turmerone analogs being the dominant components (Fig. 4).

## Discussion

This study revealed that a range of solvents with widely varying polarities can be used to extract active ingredient(s) from turmeric that are toxic to termites. Previous studies cite the use of petroleum ether and hexane extracts of turmeric on *T. castaneum* and *Plutella xylostella*, as well as hexane and acetone extracts on *T. castaneum* (Jilani and Su 1983, Jilani et al. 1988,



**Fig. 1.** Concentration–response data for hexane extracts of raw turmeric. The median lethal concentration estimate (LC<sub>50</sub>) for hexane extract of turmeric was 9.6 mg.

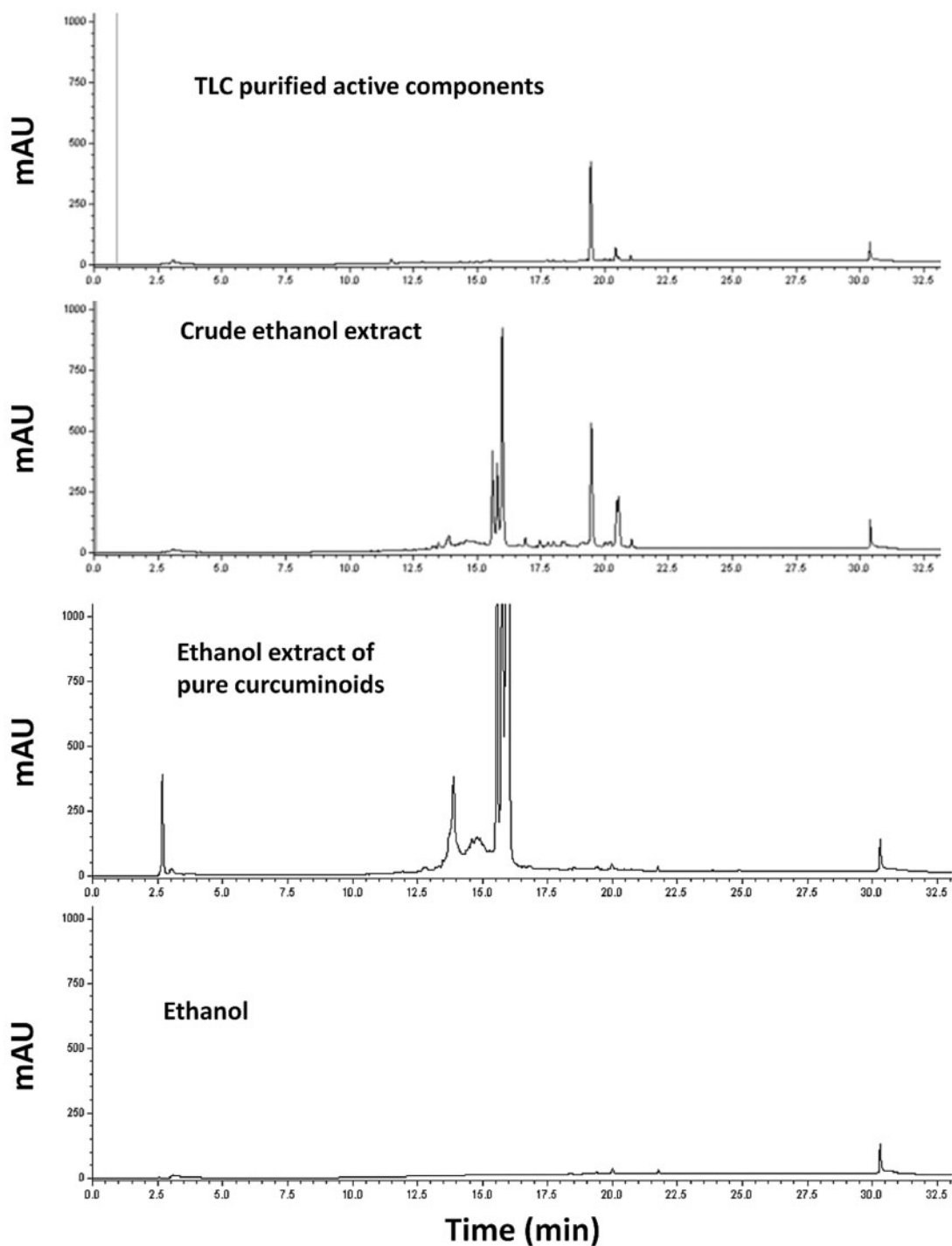


**Fig. 2.** Analysis of turmeric from three different sources and purified curcuminoids by thin-layer chromatography. Spots with greatest mobility at the far right of the plate were determined to be the active components of extracts, and are selectively extractable in hexane.

Morallo-Rejesus et al. 1992, Chander et al. 1994, Tripathi et al. 2002).

This study also revealed insecticidal activity of a purified blend of turmeric components containing turmerone, ar-turmerone, and curlone. For the LC<sub>50</sub> study we used hexane as the solvent mainly because it excludes most of the inactive curcuminoids and retains the toxic blend ingredients (turmerone, ar-turmerone, and curlone) as opposed to other solvents that retain the curcuminoids in addition to the toxic ingredients. We also determined that hexane extracts prepared from ca. 2.2 mg of starting material are capable of causing

99–100% lethal mortality (i.e., the LC<sub>99</sub> in our assays system was 2.2 mg of starting material per square centimeter of assay substrate). Sequential extracts of ethanol and hexane and vice-versa further verified this concept by demonstrating that the majority of the active ingredients are taken up by any solvent that first passes through the turmeric (results not shown). Thus, irrespective of polarity, all active components are extractable by a wide range of polar to apolar solvents that include ethanol and hexane, which are at each end of the polarity spectrum. However, hexane is unique because it offers the ability to separate turmerone and

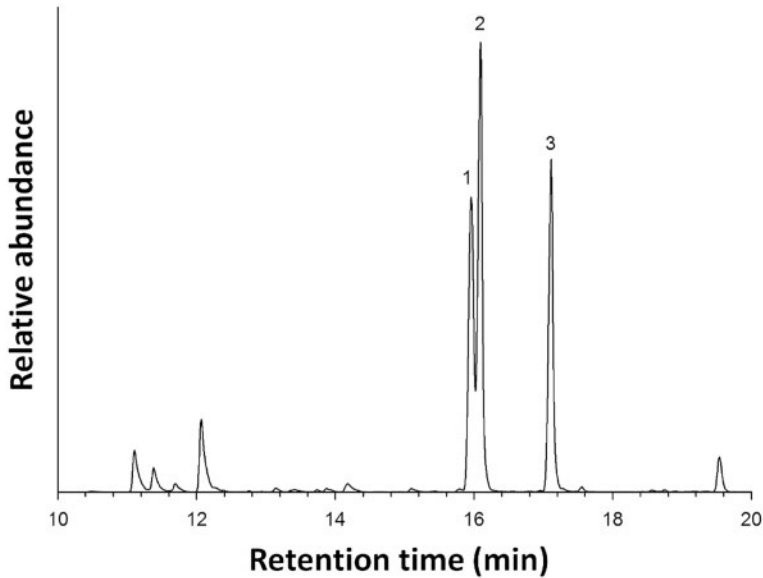


**Fig. 3.** HPLC chromatograms at 202nm showing (top to bottom) TLC-purified active components of ethanol extracts, crude ethanol extract, ethanol-extracted pure curcuminoids, and ethanol alone.

related active components from the curcuminoids that also possess a strong yellow color (Figs. 2–4 and Surwase et al. 2011). Subjecting turmeric to hexane and ethanol extraction would thus result in the isolation of tumerones and curcuminoids by the respective

solvents. This approach could be very helpful for both pest management and human health applications. The separation of these two important constituents could add value to each process and help in reducing the wasting of raw material. We also found that organically





**Fig. 4.** GC-MS analysis showing peaks identified by MS: (1) aromatic (ar)-turmerone, (2) turmerone, and (3) and curlone.

grown and conventional turmeric contain identical components, suggesting that the active ingredient is not a pesticidal contaminant, but rather is inherent in turmeric. Our findings that ar-turmerone and turmerone are present in the termite-active fraction are concordant with previous research showing that ar-turmerone and turmerone are repellent to *T. castaneum* (Su et al 1982). The effects of pure curlone remain unknown in this respect. We did not consider repellency here, as our focus was to identify chemistries that acutely impact survival, and not behavior. Finally, the fact that no vapor toxicity was observed is consistent with the physical properties of chemical components identified in active extracts (O'Neil et al. 2001, Surwase et al. 2011).

The mechanisms of termiticidal activity of the active blend identified here are presently unknown. However, multiple modes of action remain plausible, including symbiont mortality, neurological activity, and respiratory disruption. Symbiont mortality is an interesting possibility because the *R. flavipes* gut houses a rich complement of protist and bacterial symbionts (Lewis and Forschler 2004, Boucias et al. 2013). Acute symbiont mortality does not seem likely, as the onset of mortality happens so quickly. Also, defaunated *R. flavipes* termites typically live 2 wk or longer (Cleveland 1923, Wheeler et al. 2007). However, a blend of extracted turmeric essential oils containing mostly alpha-turmerone was found to inhibit bacterial biofilm formation (Lee et al. 2011). If biofilm disruption is occurring in the *R. flavipes* gut, which contains many bacterial species that form biofilms on the gut lining and potentially protect against water loss (Tamschick and Radek 2013), this could at least partially explain the symptoms observed. Another possible mode of action is neurotoxicity; for example, ar-turmerone has

been shown to affect nervous tissues of mammals (e.g., Lee et al. 2011, Hucklenbroich et al. 2014). Target sites that cause neuroexcitation do not seem to be affected because intoxicated termites only displayed paralytic symptoms and immobility (Scharf 2008). Finally, respiratory inhibition is another mode of action commonly observed for natural products and essential oils (Song and Scharf 2009). Respiratory disruption via mitochondrial impacts can be responsible for paralysis-like symptoms such as those that were observed (Scharf 2008). Thus, at the present time symbiont mortality, neurological activity, and respiratory disruption are all being considered as possible modes of action.

In summary, this study reveals that turmeric extracts prepared with a range of solvents are active against termites, with hexane being the only solvent of those tested that offers any degree of fractionation. Hexane extracts had an  $LC_{50}$  of 9.6 mg crude turmeric powder, or 1.0 mg per square centimeter of treated substrate. The chemical blend present in active hexane extracts contained three main components that included turmerone, ar-turmerone, and curlone. Further work will be required to test individual blend components for activity and to determine the mechanism by which turmeric is toxic to termites. Pinpointing the active blend components and determining their modes of action can reveal candidate natural product termiticides and potential new niches to explore for termiticide research and development.

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