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Toxicity of the Essential Oil of *Lippia alba* against *Tribolium castaneum* and *Sitophilus oryzae*

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ABSTRACT: *Lippia alba* is a flowering shrub in the verbena family and its essential oil (EO) is known for its sedative, antidepressant and analgesic properties. In the Amazon region of Brazil, it is used in aquaculture to anesthetize fish during transport. Many of the specialized metabolites found in EOs presumably evolved to protect plants from herbivores, especially insects. Gas chromatography–mass spectrometry identified the major components of the EO as the monoterpenes citral (59%), carvone (7%) and limonene (7%). The essential oil of *Lippia alba* is reported as an antifungal against human pathogenic microorganisms but few articles report its use as an alternative to synthetic fungicides on green mould control. Several papers have presented ethnopharmacological studies dealing with *L. alba* as sedative, antidepressant and analgesic properties. The essential oil of *L. alba* also has many applications such as stomachic, anti-spasmodic, digestive, anti-hemorrhoidal and anti-asthmatic. Different biological activities such as cytotoxic, antifungal, antibacterial, antiviral and anti-inflammatory, have been identified in essential oils or extracts of *L. alba*. The antifungal activity of the essential oils of *L. alba* against human pathogenic fungi such as *Candida albicans*, *Candida guilliermondii*, *Candida parapsilosis*, *Candida neoformans*, *Trichophyton rubrum* and *Fonsecaea pedrosoi* has been previously demonstrated for the citral and myrcene-citral chemotypes. *L. alba* essential oil and two of its major components were evaluated for fungitoxicity and anti-aflatoxigenicity against *Aspergillus flavus*. The antifungal activity was evaluated against *C. parapsilosis*, *Candida krusei*, *A. flavus* and *Aspergillus fumigatus* strains. Antifungal screening was carried out also against *Saccharomyces cerevisiae*, *A. flavus*, *Aspergillus niger* and *C. albicans*.

KEYWORDS: *Lippia alba*, *Tribolium castaneum*, *Sitophilus oryzae*, antifungal, antibacterial essential oil, medicinal

I. INTRODUCTION

Lippia alba is a species of flowering plant in the verbena family, Verbenaceae, that is native to southern Texas in the United States,^[3] Mexico, the Caribbean, Central America, and South America.

In Ethiopia the plant is also known as Coseret and classified under herbal group. They use the plant leaf for preparing butter.

The species is also present in Australia and India, where it is probably a human introduction.^[4] Common names include bushy matgrass,^[1] bushy lippia, hierba negra,^[3] juanilama, pamporegano, poleo and pitona.^[5] It is a multi-branched shrub, reaching a height of 1.5 m (4.9 ft). Leaves measure 1 to 3 cm (0.39 to 1.18 in) in length and 0.9 to 2 cm (0.35 to 0.79 in) in width and are opposite or in threes. Flowers with white, pink, or light blue-purple corollas form on spikes 2 cm (0.79 in) long.^[6] Bushy lippia is widely cultivated as an ornamental for its aromatic foliage and beautiful flowers.^[3] The essential oil composition is unique to each plant, but may include piperitone, geranial, neral, caryophyllene, camphor, eucalyptol, limonene, carvone, germacrene, α -guaiene, β -ocimene, linalool, or myrcene.^[6] The leaves are used for flavoring foods,^[7] such as mole sauces from Oaxaca, Mexico.^[8] The plant is used medicinally for its somatic, sedative, antidepressant, and analgesic properties.^[9]



Lippia alba (Mill.) N.E.Br. - VERBENACEAE - Jardim Botânico de Brasília - Distrito Federal - Brasil.

The insects *Sitophilus zeamais* and *Tribolium castaneum* cause losses in stored grains and are considered pests of wide distribution and global significance. The toxicity and repellency of essential oils of different *Lippia alba* genotypes (carvone chemotypes LA-13 and LA-57 and citral chemotypes LA-10 and LA-44) and their major monoterpenes, carvone and citral, on *S. zeamais* and *T. castaneum*. For *S. zeamais*, the monoterpene citral had the lowest lethal time ($LT_{50} = 6$ h), whereas for *T. castaneum*, the monoterpenes carvone and citral showed a more rapid toxicity ($LT_{50} = 7.3$ h). The compounds tested were highly repellent to *T. castaneum*; however, no repellency's effect was observed against *S. zeamais*, except for LA-13 chemotype. The essential oils from the carvone chemotype and the monoterpene carvone have potential for the development of natural insecticides^[10] against stored grain insects *S. zeamais* and *T. castaneum*.^[11]

II. DISCUSSION

The red flour beetle (*Tribolium castaneum*) is a species of beetle in the family Tenebrionidae, the darkling beetles. It is a worldwide pest of stored products, particularly food grains, and a model organism for ethological and food safety^[1] research. Adult beetles are small, around 3-4mm long (1/8 inches), of a uniform rust, brown or black color.^[2] Head and pronotum are sometimes darker than rest of body. The red flour beetle attacks stored grain and other food products including flour, cereals, pasta, biscuits,^[12] beans, and nuts, causing loss and damage. The United Nations, in a recent post-harvest compendium, estimated that *Tribolium castaneum* and *Tribolium confusum*, the confused flour beetle, are "the two most common secondary pests of all plant commodities in store throughout the world."^[3] The red flour beetle is of Indo-Australian origin and less able to survive outdoors than the closely related species *Tribolium confusum*. It has, as a consequence, a more southern distribution, though both species are worldwide in heated environments. The adult is long-lived, sometimes living more than three years. Although previously regarded as a relatively sedentary insect, it has been shown in molecular and ecological research to disperse considerable distances by flight.^[4] Female red flour beetles exhibit polyandrous mating behavior^[13] Within a single copulation period, a female will mate with multiple males. Any one male may have a low sperm count, having depleted its supply through prior matings, so mating with several increases the female's likelihood of obtaining an adequate amount of sperm.^[5] Although multiple mating events may also increase the likelihood of finding genetically compatible sperm, genetic compatibility is not always a major factor driving polyandrous behavior.^[5] Increased embryo viability due to increased genetic compatibility did not significantly increase the number of adult beetles over time, which indicates that it did not play a significant role in the fitness of the overall population.^[5] However, increased genetic compatibility^[14] could increase the genetic diversity of a population, which can be advantageous in situations of rapid environmental change.^[6]

The availability of resources and population size can greatly affect how many matings each individual participates in. Increased population size within a given area with fixed resources can limit how many offspring can survive.^[6] This can result in heavy competition between males to be a female's last mate, which carries an advantage as ejaculate from

one mating can be removed during subsequent matings.^[7] When resources are limited, increased cannibalism among competing males can ultimately decrease fitness for the population^[15] as a whole, as it decreases both offspring production and survival.^[6] In red flour beetles, a male's ability to attract females (via pheromones) is genetically based. Higher attractiveness does not, however, correlate with higher fitness of offspring. Genes for more attractive pheromones are not a reliable indicator that the male also has genes which will improve offspring survival.^[8]



Tribolium castaneum

The rice weevil (*Sitophilus oryzae*) is a stored product pest which attacks seeds of several crops, including wheat, rice, and maize. The adults are usually between 3 and 4.6 mm long, with a long snout. The body color appears to be brown/black, but on close examination, four orange/red spots are arranged in a cross on the wing covers. It is easily confused with the similar looking maize weevil. The maize weevil is typically somewhat larger than the rice weevil, but rice weevils as large as the largest maize weevils and maize weevils nearly as small as the smallest rice weevils have been found^[16] Some external features can be used to differentiate the vast majority of adults, but the only reliable features are on the genitalia (see table below). Both species can hybridize. The genitalic structure of hybrids is unknown.^{[2][3][4][5]} Adult rice weevils are able to fly,^[6] and can live for up to two years. Females lay 2-6 eggs per day and up to 300 over their lifetime. The female uses strong mandibles to chew a hole into a grain kernel after which she deposits a single egg within the hole, sealing it with secretions from her ovipositor. The larva develops within the grain, hollowing it out while feeding. It then pupates within the grain kernel and emerges 2-4 days after eclosion.

Male *S. oryzae* produce an aggregation pheromone called sitophilure ((4S,5R)-5-Hydroxy-4-methylheptan-3-one) to which males and females are drawn. A synthetic version is available which attracts rice weevils, maize weevils and grain weevils. Females produce a pheromone which attracts only males.^[17]

Its gammaproteobacterial symbiont *Candidatus Sodalis pierantonius* str. SOPE is able to supply rice weevil with essential vitamins like pantothenic acid, riboflavin, and biotin.^[7] During larvae development, bacteria rely on up-regulation of type three secretion system genes and genes for flagellum so they can infect insect stem cells.^[8]

Control of weevils involves locating and removing all potentially infested food sources. Rice weevils in all stages of development can be killed by freezing infested food below -18 °C (0 °F) for a period of three days, or heating to 60 °C (140 °F) for a period of 15 minutes.^[9]

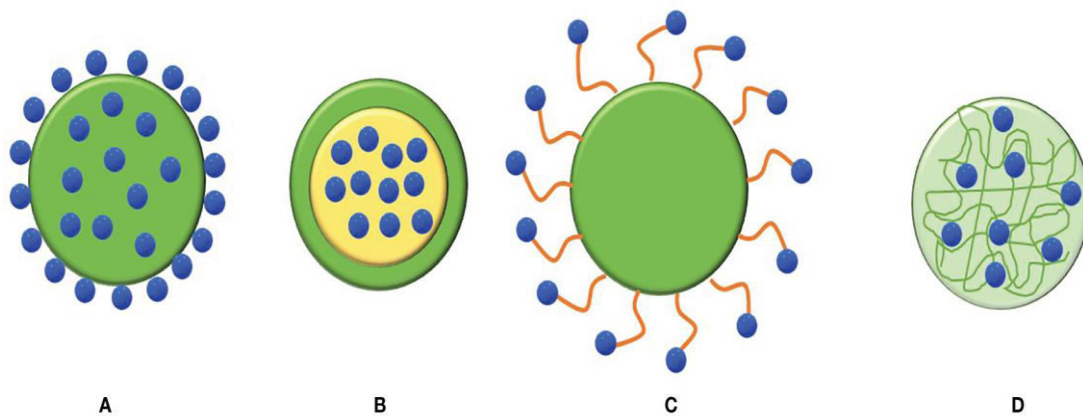


Sitophilus oryzae

III. RESULTS

A tool for integrated pest management is the use of essential oils (EOs) and plant extracts. In this study, EOs from *Lippia alba*, grown in Colombia, were analyzed by gas chromatography–mass spectrometry. These oils as well as several of their constituents were tested for repellent activity against *Tribolium castaneum*, & *Sitophilus oryzae*^[18]

using the area preference method. The main components (>10%) found in EOs were methylchavicol, limonene/ α -pinene, carvone/limonene, benzyl acetate/linalool/benzyl benzoate, and α -pinene, *L. alba*, basically. All EOs were repellent, followed a dose–response relationship, and had bioactivity similar to or better than that of commercial compound IR3535. EOs from *L. alba* were the most active. Compounds from EOs, such benzyl benzoate, β -myrcene, and carvone, showed good repellent properties. In short, EOs from plants cultivated in Colombia are sources of repellents against *T. castaneum* & *Sitophilus oryzae*^[19]



Schematic forms of nanomaterials with essential oils, (A) adsorbed on the nanoparticle's surface, (B) encapsulated by a hydrophilic or hydrophobic polymer shell, (C) attachment to nanoparticle using different linkers, and (D) entrapment inside the polymer matrix.

IV. CONCLUSIONS

Essential oils have a great potential as natural insecticides against curculionids or other species. In fact, they can play an important role in pest management and organic farming because they do not generate toxic residues and are environmentally friendly. The disadvantages they present, which are related to their high volatility and degradation, can be minimized by using current encapsulation methods. Therefore, future research should focus on determining how encapsulation of EOs or mixtures of EOs can increase the effectiveness in controlling different life stages of pests under changing environmental conditions^[20]

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