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Insecticide Activity of Lectins and Secondary Metabolites

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1. Introduction

Proteins are polymers of amino acids (molecules containing an amino group, a carboxylic group and a hydrophobic or hydrophilic side chain) present in all organisms. Apolar, polar uncharged and electrically charged amino acids are covalently linked through peptide bonds (amide bonds) and the sequence they form in the polypeptide chain (primary structure) determines the tertiary or quaternary structures ultimately presenting some biological activity. Proteins can be formed by one or multiple polypeptides (subunits) with or without a non-amino acid molecule (carbohydrate, ion, lipid, etc) linked to them.

Lectins comprise a heterogeneous group of non-immune proteins that interact with carbohydrates. This interaction is behind a number of biological properties, including antimicrobial, antitumoral, hemagglutinating, mitogenic and insecticide activities.

The specificity of the carbohydrate binding site is determined by the amino acids forming the lectin molecule, as well as shape and the spatial arrangement of neighboring amino acids; additionally, metal ions may contribute for correct positioning of the amino acid residues for binding to the carbohydrate (Sharon and Lis, 2001). Lectins can be divided into those that bind monosaccharides as well as oligosaccharides, and those that recognize only oligosaccharides (Sharon and Lis, 2007). Depending on carbohydrate specificity, they can be classified as: glucose/mannose, *N*-acetylglucosamine, galactose, *N*-acetylgalactosamine, fucose and sialic acid-binding lectins (Wu et al., 2001). The hemagglutinating activity assay (Figure 1A) in presence of free carbohydrates (Figure 1B) has been proved to be a useful tool to characterize lectin specificity.

Plant lectins have been isolated from bark, cladodes, flowers, leaves, rhizomes, roots and seeds. They differ from each other with respect to their molecular structures, carbohydrate-binding specificities, and biological activities. The compact globular structures, molecular aggregation and glycosylation of lectins in general result in high structural stability (Kawsar et al., 2008; Moreno et al., 2008).

In general, lectin isolation procedures include protein extraction steps with aqueous solvent, the production of a lectin-rich fraction, and separation of lectin from protein or non-protein

contaminants by chromatography. Lectin solubility and stability vary with the sequence of amino acids in the polypeptide chain, and such structural features can be exploited to provide concentrated lectin preparations. Lectins can be precipitated from extracts by adding ammonium sulfate at high concentration (salting out method) or organic solvents (Santana et al., 2008; Napoleão et al., 2011). Heat-stable lectins can be partially purified by submitting the extract to high temperature for removal of other proteins (Santana et al., 2008). Lectins are purified by ion exchange, molecular exclusion and/or affinity chromatography that rely on characteristics like charge, size and biological affinity of lectin for solid phases, respectively.

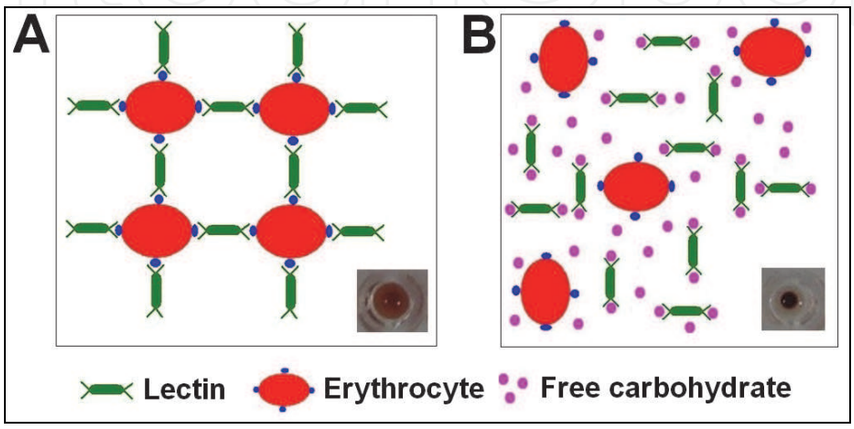

Fig. 1. Schematic representation of erythrocyte network promoted by lectin binding to surface carbohydrates (A) and inhibition of hemagglutinating activity by free carbohydrate (B). Aspects of assays in microtiter plates (insets).

Isolated lectin can be detected by polyacrylamide gel electrophoresis (PAGE) using dyes such as Coomassie Brilliant Blue or Amido black (Reisfeld et al., 1962; Laemmli, 1970). Specific staining techniques with Schiff's reagent (Pharmacia Fine Chemicals, 1980) or Concanavalin A-Peroxidase (Hinata and Nishio, 1981) can easily reveal the presence of glycan; carbohydrate moiety characterization can be performed after lectin tryptic digestion in gel followed by enzymatic deglycosylation and mass spectrometric analysis (Nasi et al., 2009).

1.1 Lectins from *Bauhinia monandra* leaf and secondary roots

Bauhinia monandra (Angiosperms, Eudicots, Rosids, Eurosids I/Fabidae, Order Fabales, Family Fabaceae) has the popular names "pata-de-vaca" in Portuguese, "orquidea del pobre" in Spanish, and pulse or Napoleon's plume in English (Judd et al., 2007; Souza et al., 2011b). *B. monandra* leaf infusions are used as medicine in the treatment of diabetes mellitus.

Two lectins were purified at milligram level from leaf and secondary roots of *B. monandra*, and were called BmoLL and BmoRoL, respectively. The isolation procedures included protein extraction with 0.15 M NaCl, ammonium sulphate (60%) fractionation and affinity chromatography on guar gel column (Coelho and Silva, 2000; Souza et al., 2011b).

BmoLL agglutinated rabbit and human (AB and B types) erythrocytes and this hemagglutinating activity was inhibited by D(+)galactose and D(+)rafinose. It was detected over a broad pH range, being heat stable up to 50 ^{o}C (Coelho and Silva, 2000).

Polyacrylamide gel electrophoresis for denatured proteins (SDS-PAGE) revealed that BmoLL is formed by two polypeptides (a 26-kDa subunit and a 33-kDa glycosylated subunit). This lectin did not induce genotoxic effects in a series of cell-free and bacterial assays (Sisenando et al., 2009).

BmoRoL showed hemagglutinating activity on human and rabbit erythrocytes at a pH range of 6.5 to 7.5, and was active up to 60 ºC, losing its activity above this temperature. SDS-PAGE revealed that the lectin was a 26-kDa glycoprotein. BmoRoL showed antifungal activity against *Fusarium solani* and *F. oxysporum* (Souza et al., 2011b).

1.2 Lectin from *Opuntia ficus indica* cladodes

Opuntia ficus indica Mill. (Angiosperms, Eudicots, Order Caryophyllales, Family Cactaceae) has the popular names "palma forrageira" or "figo-da-Índia" in Portuguese, "nopal" or "tuna" in Spanish, and Indian fig opuntia or barbary fig in English (Judd et al., 2007). Cladodes are used in folk medicine and studies demonstrated their diuretic, antiulcer and wound-healing activities (Galati et al., 2001; Galati et al., 2002; Trombetta et al., 2006). *O. ficus indica* is grown in northeastern Brazil as an important feed source for animals, and cladodes have been reported to be a component in sheep feed (Tegegne et al., 2007).

The procedure for isolation of *O. ficus indica* lectin (OfiL) included protein extraction with 0.15 M NaCl and chromatography of extract on a chitin column. OfiL agglutinated rabbit, chicken or human erythrocytes. The hemagglutinating activity was inhibited by monosaccharides and glycoproteins, stimulated by Ca^{2+} or Mg^{2+}, remaining stable across wide pH and temperature ranges. SDS-PAGE revealed that lectin is a single 8.4-kDa polypeptide. OfiL showed antifungal activity against *Colletotrichum gloeosporioides*, *Candida albicans*, *Fusarium decemcellulare*, *Fusarium lateritium*, *Fusarium moniliforme*, *Fusarium oxysporum* and *Fusarium solani*. This lectin was mainly active on *C. albicans* (Santana et al., 2009).

1.3 Lectins from *Moringa oleifera* seeds

Moringa oleifera (Angiosperms, Eudicots, Rosids, Eurosids II/Malvidae, Order Brassicales, Family Moringaceae) has the popular names ''moringa" in Portuguese, ''árbol del ben" in Spanish, and horseradish tree in English (Judd et al., 2007). The seeds are widely used in developing countries as a natural coagulant to treat water for human consumption. It has been demonstrated that a 3-kDa organic polyelectrolyte and proteins with molecular mass of 6.5 to 13 kDa and isoelectric points between 9.6 and 11.0 have coagulant properties (Gassenschmidt et al., 1995; Ndabigengesere et al., 1995; Okuda et al., 2001; Ghebremichael et al., 2005).

Santos et al. (2005) revealed the presence of water-soluble *M. oleifera* lectin (WSMoL) in *M. oleifera* seed extracts by detection of hemmaglutinating activity. The procedure for WSMoL isolation was defined by Coelho et al. (2009) and included the steps of protein extraction with water, precipitation of lectin with ammonium sulfate (60% saturation) and chromatography of precipitated fraction on a chitin column. WSMoL agglutinated human and rabbit erythrocytes in a broad pH range of 4.5 to 9.5 and when kept at 100 ºC for 5 h plus incubation overnight at 37 ºC. The carbohydrate binding site of lectin recognized D(+)-fructose and *N*-acetylglucosamine, since these monosaccharides inhibited the hemagglutinating activity (Rolim et al., 2011). MALDI-TOF/TOF analysis revealed that WSMoL showed similarity with *M. oleifera* protein (Coelho et al., 2009). Genotoxicity assessment of WSMoL using the cell-free plasmid DNA as well as the Ames and Kado

assays showed that this lectin was nonmutagenic (Rolim et al., 2011). WSMoL showed coagulant and antibacterial activities against *Escherichia coli*, *Staphylococcus aureus* and natural lake water bacteria (Ferreira et al., 2011).
The procedure for isolation of coagulant *M. oleifera* lectin (cMoL) was defined by Santos et al. (2009) and included the steps of protein extraction with 0.15 M NaCl, precipitation of lectin with 60% ammonium sulfate and chromatography of precipitated fraction on guar gel column. cMoL agglutinated human and rabbit erythrocytes in a broad pH range of 4.0 to 9.0 and when kept at 100 ºC for 7 h. The hemagglutinating activity of cMoL was inhibited by several carbohydrates, but not by D(+)-fructose. SDS-PAGE revealed that cMoL had a main 26.5-kDa polypeptide band. cMoL showed coagulant property and the ability to bind humic acid, which is interesting when the aim is to remove humic acids from water (Santos et al., 2009; Santos et al., 2011a; Santos et al., 2011b).

1.4 Lectins from *Myracrodruon urundeuva* bark, heartwood and leaf

Myracrodruon urundeuva (Angiosperms, Eudicots, Rosids, Eurosids II/Malvidae, Order Sapindales, Family Anacardiaceae) has the popular names "urundel" in Spanish, pepper tree in English and "aroeira do sertão" in portuguese (Leite, 2002; Judd et al., 2007). The plant has great importance in traditional medicine. Aqueous extracts of the bark showed anti-ulcer and anticholinergic, inflammatory, antidiarrhoeal and analgesic activities (Rao et al., 1987; Almeida-Cortez et al., 2007). *M. urundeuva* heartwood is excellent for poles, fences, pillars, beams, frames, bridges, mills, rafters, parquet, flooring, roofing and turned parts (Mainieri and Chimelo, 1989). Paes et al. (2002) showed that *M. urundeuva* heartwood was resistant to fungi (*Postia placenta* and *Neolentinus lepideus*) and termite (*Nasutitermes corniger*).
M. urundeuva bark, heartwood and leaf are sources of lectins called MuBL, MuHL and MuLL, respectively. The procedures for isolation of these lectins included protein extraction by 0.15 M NaCl, precipitation of lectins with ammonium sulphate (at different saturations for each lectin) and chromatography on a chitin column. The three lectins agglutinated human and rabbit erythrocytes in a broad pH range, and the hemagglutinating activities were inhibited by *N*-acetylglucosamine. SDS-PAGE revealed that MuBL, MuHL and MuLL are polypeptides of 14, 14.4 and 14.2 kDa, respectively (Sá et al., 2009c; Napoleão et al., 2011). MuHL showed antimicrobial activity inhibiting the growth of numerous bacteria (*Bacillus subtilis*, *Corynebacterium callunae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus faecalis*) and fungi *(Fusarium oxysporum, F. decemcellulare*, *F. fusarioides*, *F. solani* and *F. verticilioides* (Sá et al., 2009b).

2. Insecticidal activity of lectins

Lectins have deleterious effects against larvae, developing stages and mature forms of insects from orders Coleoptera, Diptera, Hemiptera, Homoptera, Hymenoptera, Isoptera, Lepidoptera and Neuroptera (Murdock et al., 1990; Eisemann et al., 1994; Powell et al., 1995; Zhu-Salzman et al., 1998; Bandyopadhyay et al., 2001; Isidro et al., 2001; Hogervorst et al., 2006; Kaur et al., 2006; Coelho et al., 2007; Macedo et al., 2007; Fitches et al., 2008; Sá et al., 2008; Coelho et al., 2009; Sá et al., 2009c; Silva et al., 2009; Napoleão et al., 2011; Oliveira et al., 2011; Souza et al., 2011b). Insecticide activity of lectin is generally evaluated by bioassays that incorporate the lectin into artificial diets offered to insects, with insects dying from nutritional deprivation. It has been shown that lectins are resistant to proteases present in the insect gut, a property responsible for their active presence in the digestive tract,

eventually with insecticide effects (Macedo et al., 2007; Napoleão et al., 2011; Oliveira et al., 2011).

The precise mechanisms of insecticidal action of lectins remain unknown, though it has been suggested that this entomotoxic activity seems to depend upon the carbohydrate recognition property they exhibit. Plant lectins with affinity for *N*-acetylglucosamine and chitin-binding property are able to bind chitin and glycosylated proteins of the peritrophic matrix, interfering in the digestion and absorption of nutrients (Tellam et al., 1999; Peumans and Van Damme, 1995; Zhu-Salzman et al., 1998; Zhu-Salzman and Salzman, 2001; Carlini and Grossi-de-Sá, 2002; Macedo et al., 2004; Macedo et al., 2007). The peritrophic matrix constitutes a membrane found in the midgut that separates the contents of the gut lumen from the digestive epithelial cells. The matrix contains a network composed by chitin (polymer of *N*-acetylglucosamine) and glycoproteins such as peritrophins. The importance of the integrity of the peritrophic matrix lies in the protection it offers to midgut epithelial cells against microorganism infection and mechanical damage by abrasive food particles, as apart from the compartmentalization of digestive processes (Hegedus et al., 2009).

Ultrastructural studies have shown abnormalities caused by *Triticum vulgaris* lectin in midgut of *Ostrinia nubilalis* and *Drosophila* such as hypersecretion of many disorganized layers of peritrophic matrix and morphological changes of microvilli (Harper et al., 1998; Li et al., 2009; Vandenborre et al., 2011). Lectin may also cross the midgut epithelial barrier by transcytosis, entering the insect circulatory system and resulting in a toxic action against endogenous lectins involved in haemolymph self-defense mechanisms (Fitches et al., 2001). Lectin may be internalized by endocytotic vesicles into the epithelial cells, blocking nuclear localization and nuclear sequence-dependent protein import, thus inhibiting cell proliferation (Yu et al., 1999).

2.1 Larvicidal activity of lectins against *Callosobruchus maculatus* and *Zabrotes subfasciatus*

Bruchid beetles (Family Chrysomelidae, Subfamily Bruchinae) are small insects under 1 cm in size mainly known for the damage they cause to leguminous seeds. *Callosobruchus* is a cosmopolitan genus behind lowered seed weight, germination viability and marketability, since eggs are laid attached to beans; larvae and pupae develop inside the seeds, which can be attacked both in the field and in storage (Edvardsson and Tregenza, 2005; Souza et al., 2011a). *C. maculatus* (cowpea weevil) is among the main pests of stored cowpea, *Vigna unguiculata* (Angus et al., 2011). Synthetic chemicals, grain protectants and fumigants are extensively used to control insect pests in stored grains; however, the usage of chemical insecticides leads to insecticide residues in grains, and has promoted the emergence of selected resistant populations (Loganathan et al., 2011).

Another important species of bruchid beetles is *Zabrotes subfasciatus* (Mexican bean weevil), which is native to Central and South America. It is one of the main pests of stored beans (*Phaseolus vulgaris*) in Brazil. The females of *Z. subfasciatus* are able to oviposit on the seeds after dehiscence or even when they are already inside the pods, which they enter through perforations (Credland and Dendy, 1992; Sari et al., 2003).

BmoLL showed deleterious effects against *C. maculatus* and *Z. subfasciatus* larvae (Table 1). An artificial seed containing 0.32% BmoLL promoted 50% mortality of *C. maculatus* larvae, while a 50% mass decrease was detected in larvae reared on a diet with seeds containing 0.4% BmoLL. Considering *Z. subfasciatus*, 50% mortality and 20% mass decrease were detected when larvae fed on artificial seeds containing 0.5% BmoLL (Macedo et al., 2007).

Additional assays revealed the ability of BmoLL to bind to a chitin column, the resistance of lectin to digestion by enzymes from *C. maculatus* and *Z. subfasciatus* larvae, the ability of BmoLL-Sepharose column to bind to proteins from midgut homogenates, and the inhibition of α-amylase activity from midgut by BmoLL. Based on these data, it was suggested that the larvicidal activity may be due to BmoLL binding to chitin from gut structures, cell surface glycosylated receptor or sugar moiety of glycoproteins, resistance of lectin to proteolysis by midgut enzymes, and a damaging effect on the digestive enzyme activity (Macedo et al., 2007).

Lectin source and abbreviation	Insect	Damage
Bauhinia monandra leaf (BmoLL)	*Callosobruchus maculatus*	Mortality of larvae; decreased larval weight; decreased α-amylase activity.
	Zabrotes subfasciatus	Mortality of larvae; decreased larval weight.
	Ephestia kuehniella	Decreased larval weight.
B. monandra secondary roots (BmoRoL)	*Nasutitermes corniger*	Mortality of workers and soldiers after ingestion.
Opuntia ficus indica cladodes (OfiL)	*N. corniger*	Mortality of workers and soldiers after ingestion.
Moringa oleifera seeds WSMoL	*Aedes aegypti*	Mortality of fourth-stage larvae (L_4); increased gut volume; disruption of gut underlying epithelium
	N. corniger	Mortality of workers and soldiers after ingestion.
cMoL	*E. kuehniella*	Decreased larval weight; delayed development; mortality of pupae; decreased adult emergence.
	N. corniger	Mortality of workers after ingestion.
Myracrodruon urundeuva Bark and heartwood (MuBL and MuHL)	*A. aegypti*	Mortality of L_4.
MuBL, MuHL and leaf lectin (MuLL)	*N. corniger*	Mortality of workers and soldiers after ingestion; bacteriostatic and bactericide effect against gut symbionts.

References: Macedo et al. (2007); Sá et al. (2008); Sá et al. (2009c); Coelho et al. (2009); Napoleão et al. (2011); Oliveira et al. (2011); Paiva et al. (2011); Souza et al. (2011b).

Table 1. Insecticidal activity of lectins.

2.2 Larvicidal and pupicidal activities of lectins against *Ephestia* (*Anagasta*) *kuehniella*

Ephestia kuehniella (many times referred to as *Anagasta kuehniella*, currently being *Anagasta*, ranked as a subgenus of *Euphestia*) is a moth belonging to the Pyralidae family, and today is a worldwide pest of stored grains, nuts, and legumes. It is commonly found in flour mills. Popularly known as flour moth, *E. kuehniella* also feeds on wheat flour, corn meal, seeds, dried fruits, pasta, baked goods, cocoa, and other stored foods (Gallo et al., 2002; Macedo et al., 2003; Tounsi et al., 2005). Its life cycle lasts 3-4 months and comprises egg, larvae, pupa and adult stages. The larvae (caterpillars) infest the stored product and are the most damaging stage. They produce silk building webs and cocoons in which they complete their development. Next, pupation occurs in the same site. The adults live approximately 14 days and do not feed (Bennett, 2003).

The effect of BmoLL against *E. kuehniella* was determined in a study using an artificial diet containing lectin concentrations of 0.25%, 0.5% or 1.0%. The moths were fed and the mass and number of neonate larvae (fourth instar) were determined (Macedo et al., 2007). The data showed that BmoLL up to 1% did not decrease the survival of larvae, though it produced a 40 % weight decrease (Table 1). The authors reckoned that for every 1% point increase in BmoLL dose, mass decreased by 0.61 mg. BmoLL was resistant to hydrolysis by *E. kuehniella* midgut extracts for 48 h.

The evaluation of insecticidal action of cMoL against *E. kuehniella* used neonate first instar larvae and artificial diet containing 0.5%, 1.0% or 2.0% cMoL. The effect of lectin was determined based on the parameters: weight and number of fourth instar larvae, weight of pupae, time at which the adults emerged and number of adults that emerged (Oliveira et al., 2011). cMoL reduced larval weight, delayed the larval development time by 15 days, promoted pupal mortality and produced low rates of adult emergence, though it did not interfere in larval survival (Table 1). The same study also reported the resistance of cMoL to proteolysis by *E. kuehniella* midgut enzymes.

2.3 Termiticidal activity of lectins against *Nasutitermes corniger*

The tropicopolitan genus *Nasutitermes* (Termitidae family) includes arboreal wood-feeding termites that build their nests in roofs, linings, and structural spans as well as on the soil or above its level (Edwards and Mill, 1986; Scheffrahn et al., 2002). Soldiers of all *Nasutitermes* are easily identified by the dark-brown color of their heads and the characteristically conical nasus that emits a defensive secretion, as well as the presence of six erect setae projecting from the vertex (Scheffrahn et al., 2002). One of the most dominant and broadly distributed species is *N. corniger*. These termites are able to invade the urban environment, attacking wood in the structures of buildings (Scheffrahn et al., 2005; Paes et al., 2007).

Termiticidal activity of lectins (Table 1) has been evaluated by a no-choice bioassay (Figure 2). Briefly, a filter paper disk impregnated with lectin solution or 0.15 M NaCl (negative control) is placed in petri plates. Workers and soldiers are transferred to each plate and the rate of insect survival is determined daily, upon the death of all insects.

The first report of toxic effect of a lectin against termites was the insecticidal activity of MuHL against *N. corniger*. When concentrations of 0.1, 0.2, 0.4 and 0.8 mg ml^{-1} of this lectin were used, it promoted mortality of termites with LC_{50} values of 0.248 mg ml^{-1} for workers and 0.199 mg ml^{-1} for soldiers (Sá et al., 2008). That study suggested that resistance of the heartwood to termite attack may be linked to termiticidal activity of MuHL. The termiticidal effect of lectins isolated from *M. urundeuva* bark and leaf (Table 1) was determined by

Napoleão et al. (2011). Both MuBL and MuLL killed workers (LC_{50} of 0.974 and 0.374 mg ml^{-1}, respectively) and soldiers (LC_{50} of 0.787 and 0.432 mg ml^{-1}, respectively).
Despite having the common property of binding to chitin, the lectins of *M. urundeuva* differently affected the survival of *N. corniger.* MuBL was less active against both castes than the other two lectins, and MuHL was more termiticidal against workers than. It has been reported that insecticidal activity of MuBL and MuLL can be due to the resistance of *M. urundeuva* lectins to proteolysis by enzymes from *N. corniger* gut, and to antibacterial action against symbiotic gut bacteria that is essential for termite survival (Napoleão et al., 2011).

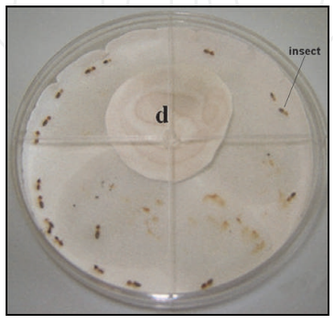

Fig. 2. Aspects of no-choice termiticidal assay used to evaluate insecticidal activity of lectins. The disk (d) of filter paper is impregnated with lectin solution.

The lectin from *B. monandra* secondary roots (BmoRoL) at concentrations of 0.025, 0.05, 0.1, 0.2 and 0.4 mg ml^{-1} also induced the mortality of *N. corniger* (Souza et al., 2011b). This lectin was more efficient against soldiers (LC_{50}: 0.014 mg ml^{-1}) than workers (LC_{50}: 0.09 mg ml^{-1}).
Termiticidal activity from *O. ficus indica* cladodes was determined using preparations (extract and OfiL) at concentrations of 0.25, 0.5, 1.0 and 1.5 mg ml^{-1} of protein (Paiva et al., 2011). The extract was termiticidal against workers at 1.5 mg ml^{-1}, though it did not interfere in survival of soldiers. OfiL was more active than cladode extracts, showing a stronger termiticidal activity against workers (LC_{50} of 0.116 mg ml^{-1}). The lectin was active against soldiers only at 1.5 mg ml^{-1}.
M. oleifera seeds were also sources of termiticidal preparations (Table 1). Bioassays used crude preparations (extracts and protein fractions) as well as purified lectins (cMoL and WSMoL) at concentrations of 0.125, 0.25, 0.5, 1.0 and 1.5 mg ml^{-1} of protein (Paiva et al., 2011). Both extracts containing cMoL and WSMoL were termiticidal on soldiers at 1.5 mg ml^{-1}, but only the protein fraction rich in WSMoL and pure WSMoL at 1.5 mg ml^{-1} interfered in the survival rate of soldiers. cMoL and WSMoL extracts (1.0 and 1.5 mg ml^{-1}), protein fraction from the WSMoL extract (1.5 mg ml^{-1}), as well the isolated cMoL and WSMoL (1.5 mg ml^{-1}) were all able to workers.
The repellent activity of MuBL, MuHL, MuLL, BmoRoL, OfiL, WSMoL and cMoL has also been investigated. Bioassays were performed in petri plates filled up with agar containing one central well at which termites were placed, and peripheral wells at which filter papers soaked with lectin were put. None of the lectins showed repellent activity *N. corniger*, since it was observed that the termites did not avoid contact with lectin-treated wells (Figure 3A).

2.4 Larvicidal activity of lectins against *Aedes aegypti*

The mosquito *A. aegypti* is native to North Africa, but it is a cosmopolitan species widely spread in tropical and subtropical regions (Forattini and Brito, 2003). Females feed more

frequently on blood than on plant sap, and have high affinity for human blood. Insect development occurs through the egg, larvae (four instars: L1, L2, L3 and L4), pupa and adult stages. Under favorable conditions of temperature, humidity and food availability, the period between the egg stage and adult emergence varies from 10 to 13 days (Forattini, 1965). *A. aegypti* is vector of human diseases of low (classic dengue) and high mortality (yellow fever and hemorrhagic dengue fever).

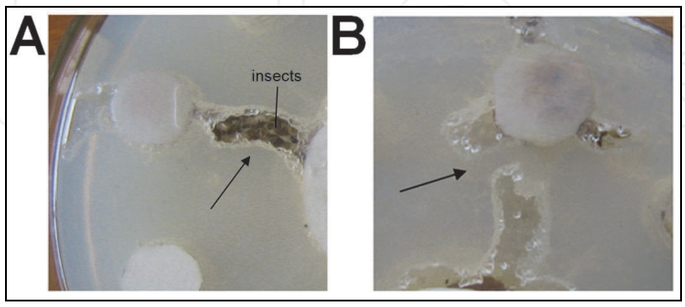

Fig. 3. Aspect of repellent activity assays. (A) non-repellent effect of lectin demonstrated by construction of tunnels in agar near a well containing lectin, and the galleries constructed in agar that remained open (arrow). (B) repellent action of methanolic extract from *M. urundeuva* heartwood detected by presence of closed gallery (arrow) constructed in agar next to peripheral wells containing the extract.

Strategies for control of *A. aegypti* immature forms includes: elimination of reproduction sites, biological control by *Bacillus thuringiensis* serovar *israelensis*, and *c*hemical control by larvicidal oils, repellents, organophosphorous, organophosphates and pyrethroids (Luna et al., 2004; Araújo et al., 2007). The control of mosquitoes using the insecticides Temephos, Malathion and Fenitrothion is the main measure adopted by public health programs; however, *A. aegypti* larvae have developed tolerance to these compounds, and this is one of the main problems in vector control programs (Poupardin et al., 2008; Melo-Santos et al., 2010).

M. oleifera seed extracts containing WSMoL interfered in the *A. aegypti* larval development (Figure 4). First larval instar (L1) incubated with extracts prepared with one, six and fifteen seeds (SE_1, SE_6 and SE_{15}) reached the last instar (L4) after longer development times than those recorded for the negative control (distilled water). The delay in larvae development promoted by SE_6 and SE_{15} was greater than that caused by SE_1, revealing a higher concentration of active principle (Coelho et al., 2009).

WSMoL and the lectins from *M. urundeuva* bark and heartwood (MuBL and MuHL) showed larvicidal activity against fourth-stage larvae in a concentration-dependent manner (Coelho et al., 2009; Sá et al., 2009c). Figure 5 shows that these lectins promoted larvae mortality with different efficiency; the values of lectin concentration (mg ml^{-1}) required to kill 50% (LC_{50}) of larvae in 24 h were 0.125 (MuBL), 0.04 (MuHL) and 0.197 (WSMoL). The hemagglutinating activities of MuBL and MuHL were not affected by exposure to sunlight, indicating that these lectins were resistant to environmental conditions of radiation and temperature, an important characteristic to be used in *A. aegypti* control (Sá et al., 2009c).

WSMoL heated at 100 ^{o}C for 5 h did not show hemagglutinating and larvicidal activities; these data reveal that the native protein structure is a requirement for these biological properties to remain in place. The larvae treated with WSMoL showed morphological

changes like hypertrophy of segments, increased gut volume and absence of epithelial layer that delimits the gut (Coelho et al., 2009).

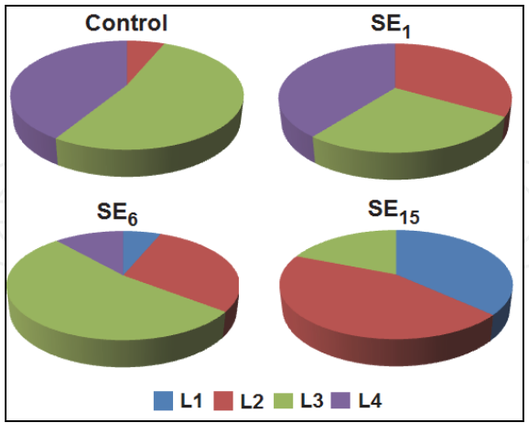

Fig. 4. Larval instar (%) of *A. aegypti* after incubation with *M. oleifera* seed extracts prepared with one, six and fifteen seeds (SE_1, SE_6 and SE_{15}, respectively) for 72 h.

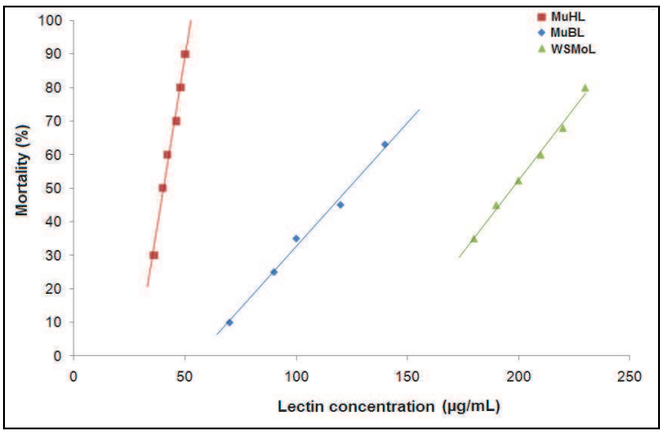

Fig. 5. Insecticidal activity of lectins from *Moringa oleifera* seeds (WSMoL) and *Myracrodruon urundeuva* bark (MuBL) and heartwood (MuHL) against *A. aegypti* fourth instar larvae (L4).

3. Secondary metabolites

Organic compounds produced by plants constitute a large and heterogeneous group known as secondary metabolites, characterized by a variety of structures and functions. They can be classified on the basis of chemical structure and composition, as nitrogen compounds (alkaloids, non-protein amino acids, amines, alcamides, cyanogenic glycosides and glucosinolates) and non-nitrogen compounds (monoterpenes, diterpenes, triterpenes, tetraterpenes, sesquiterpenes, saponins, flavonoids, steroids, coumarins).

Secondary metabolites may be found in several plant tissues. Differences in chemical properties and polarity of these molecules afford the use of different solvents for their extraction. Although aqueous extracts are usually rich in proteins, secondary metabolites can also be extracted by aqueous solutions. On the other hand, methanolic extracts generally contain large amounts of secondary metabolites with no protein content.

Natural functions and applications of secondary metabolites have been investigated employing separation techniques to isolate them from plant extracts or synthetic methods to obtaining equivalent compounds. However, many of the *in vivo* functions of secondary metabolites remain unknown. These substances do not appear to participate directly in the growth and development of plant, but many of them can be associated with survival and adaptation, including metal transporters, symbiotic agents, hormones, differential effectors and defense molecules (Demain and Fang, 2000).

The synthesis of secondary metabolites with defense role can be induced by water stress as well as seasonal variations in temperature and luminosity or infection by pathogens (Bray et al., 2000; Bulbovas et al., 2005). Experiments that simulated mechanical stimulation and damage promoted by phytopatogenous insects in *Glycine max* leaves demonstrated that secondary metabolites (mainly y-aminobutyric acid) can be accumulated in this tissue, when submitted to injuries (Ramputh and Bown, 1996).

After ingestion by herbivores, secondary metabolites can induce damage through several and different mechanisms. Alkaloids act as agonists or antagonists of neurotransmitters, and neuroreceptors or can insert themselves into DNA or induce DNA alkylation. In this way, the ingestion of alkaloids may disrupt the replication and transcription in phytophagous organisms. Non-nitrogen secondary metabolites, such as phenols, terpenoids and saponins affect herbivores through less specific mechanisms. Tannins and phenols can interact with several proteins through hydrogen bonds or ionic interactions inducing conformational changes that can lead to loss of protein activity and function. Lipophilic terpenes can affect the integrity of biomembranes. Finally, saponins have cytotoxic and antimicrobial effects by interacting with cellular membranes, inducing pore formation and causing disturbances in cell permeability (Wink, 2003).

3.1 Repellent activity of secondary metabolites from *Myracrodruon urundeuva* heartwood against *Nasutitermes corniger*

Methanolic extract from *M. urundeuva* heartwood contained secondary metabolites cinamic derivatives, flavonoids, gallic acid, luteolin, proanthocyanidins, hydrolysable tannins, and leucoanthocyanidins (Figure 6). Termiticidal and repellence bioassays revealed that the extract showed no termiticidal activity, though it induced repellent effect against *N. corniger* (Sá et al., 2009a). Insects closed the galleries constructed in agar next to peripheral wells containing the extract (Figure 3B). The presence of harmful compounds can be detected by insects through chemical receptors like olfactory or gustatory sensilla that detect chemicals with high or low-volatility, respectively (Bohbot and Vogt, 2005). Thus, termites can be repelled or attracted by a substance, depending on its chemical composition.

The studies on *M. urundeuva* heartwood indicate that two mechanisms seem to be involved in the resistance of this tissue against *N. corniger*: prevention of the arrival and attack of *N. corniger* by repellent action of secondary metabolites, and death of termites induced by MuHL, the heartwood lectin (Sá et al., 2009a).

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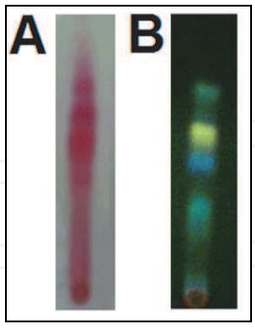

Fig. 6. Secondary metabolites from methanolic extract revealed by thin layer chromatography (TLC). (A) proanthocyanidins, hydrolysable tannins and leucoanthocyanidins. (B) kaempferol (green), quercetin (yellow) and gallic acid (blue).

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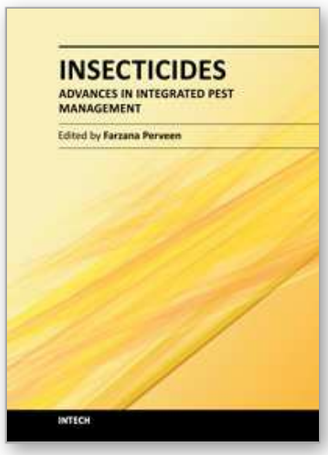

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This book contains 30 Chapters divided into 5 Sections. Section A covers integrated pest management, alternative insect control strategies, ecological impact of insecticides as well as pesticides and drugs of forensic interest. Section B is dedicated to chemical control and health risks, applications for insecticides, metabolism of pesticides by human cytochrome p450, etc. Section C provides biochemical analyses of action of chlorfluazuron, pest control effects on seed yield, chemical ecology, quality control, development of ideal insecticide, insecticide resistance, etc. Section D reviews current analytical methods, electroanalysis of insecticides, insecticide activity and secondary metabolites. Section E provides data contributing to better understanding of biological control through Bacillus sphaericus and B. thuringiensis, entomopathogenic nematodes insecticides, vector-borne disease, etc. The subject matter in this book should attract the reader's concern to support rational decisions regarding the use of pesticides.

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