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Artigo

Melia azedarach L. extracts and their activity on *Musca domestica* L. (Diptera: Muscidae)

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RESUMO: "Extratos de *Melia azedarach* L. e sua atividade sobre *Musca domestica* L. (Diptera: Muscidae)". Os extratos brutos e as frações obtidas das sementes de *Melia azedarach* L. (Meliaceae) foram testados em *Musca domestica* Linnaeus, 1758 (Diptera: Muscidae). Os bioensaios mostraram inibição no desenvolvimento pós-embrionário das moscas e um significativo aumento do período larva recém eclodida- adulto. Além disso, o peso pupal foi reduzido e a proporção sexual alterada. Foi observada toxicidade para os ovos das moscas.

Unitermos: Extratos de planta, Diptera, *Melia azedarach*, regulação do desenvolvimento, atividade biológica.

ABSTRACT: Crudes extracts and fractions from seeds of *Melia azedarach* L. (Meliaceae) have been assayed on *Musca domestica* Linnaeus, 1758 (Diptera: Muscidae). Thus, the post-embryonic development of the flies was reduced and the delay from newly hatched larvae to adults had significant increase. In addition, the pupal weights were reduced and the sexual ratio altered. Toxicity to fly eggs was also observed.

Keywords: Plant extract, Diptera, *Melia azedarach*, growth development, biological activity.

INTRODUCTION

The Meliaceae *Melia azedarach* L. is a huge tree, natural from South Asia and very common in Brazil, where it is known as "Cinamomo". A number of biological activities have already been described for crude extracts, fractions and metabolites isolated from this plant such as giardicial activity (Amaral et al., 2006), inhibitors of the enzyme acetylcholinesterase (Barbosa-Filho et al., 2006a), inhibitors of the angiotensin converting enzyme (Barbosa-Filho et al., 2006b) and antileprotic activity (Barbosa-Filho et al., 2007). Antitumor (Hartwell, 1971), anti-rheumatic, sedative and antiulcer properties (Duke & Wain, 1981; List & Horhammer, 1979) and used against flu and hypertension (Agra et al., 2008) are reported in traditional medicine. The plant also possesses insecticide (Cabral et al., 1995; Lepage et al., 1946) and vermicide activities (Zhao, 1984). Among secondary metabolites found in *M. azedarach*, one should mention triterpenes (Cabral et al., 1996; Mulholland et al., 2000), steroids, aromatic compounds (Mulholland et al., 2000) and the interesting limonoid meliaternin (Carpinella et al., 2003). In previous studies, we described a new euphane triterpene (Kelecom et al., 1996) and four lignans (Cabral et al., 1995) from the methanol extract of the seeds of *M. azedarach* collected in the city of Niterói, RJ. This was the first report of lignans in the family Meliaceae. We also reported anti-moulting activity of this crude extract against the bloodsucking bug *Rhodnius prolixus* (Cabral et al., 1996). The study described the biological activity of extracts and fractions of *M. azedarach* L. on the post-embryonic development of *Musca domestica* Linnaeus, 1758 (Diptera: Muscidae).

MATERIAL AND METHODS

Seeds of *M. azedarach* were dried, powdered

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and successively extracted with n-hexane, AcOEt (A) and MeOH (B). The oily hexane crude extract was then partitioned between cold hexane and 5% aqueous methanol to yield a hexane fraction (C) and a methanol fraction (D). Aliquots of the extracts A and B, and of the fractions C and D were carefully dried, traces of organic solvents eliminated under vacuum, and the organic residues bioassayed on *M. domestica*, a cosmopolitan diptera with high degree of synanthropy that acts as a mechanical and biological vector for enteroviruses, enterobacteria, protozoan cysts, helminth eggs and larvae, and fungi (Cox et al., 1912, Coutinho et al., 1957, Greenberg, 1971, 1973, Harwood & James, 1979; Oliveira, 1999; Oliveira et al., 2002). Insects used in this study were brought up in the colony maintained in laboratory (Queiroz et al., 1991). Samples were tested in triplicate by topic application at the concentration of 100 μg/μL on groups of 50 larvae (L1) of *M. domestica* and on egg masses (5 mg) at the concentration of 200 μg/mg (1 μL/mg). Insect viability, duration periods of larval, pupal and newly hatched larvae to the adult stage, were observed as well as sexual ratio and pupal weight.

Table 1. Viability (%) and sex ratio of post-embryonic development of *M. domestica* treated with extracts from *M. azedarach*, topic application on L1 larvae.

Treatment	Larval stage viability (%)	Pupal stage viability (%)	Larvae to adult viability (%)	Sex ratio
Control	85 ^{a}	85 ^{a} wt (mg) = 22.10 ±3.90 a	73 ^{a}	0.44
A	79 ^{c} ***	68 ^{b} ** wt (mg) = 20.17 ±3.28 b***	53 ^{b} **	0.50
B	77 ^{c} ***	65 ^{b} * wt (mg) =20.88 ±3.31 a	50 ^{b} ***	0.55
C	73 ^{c} ***	68 ^{b} *** wt (mg) =21.52 ±3.47X a, c*	50 ^{b} ***	0.49
D	54 ^{b} ***	69 ^{b} ** wt (mg) = 20.41 ±3.69 b, c**	37 ^{c} ***	0.38

Triplicate experiments with groups of 50 larvae of *M. domestica*, each. Numbers followed by the same letter did not differ among themselves and those followed by different letters have a significant difference (* P < 0.05, ** P < 0.01, ***P < 0.001) when the Tukey test was used; wt = pupal weight (mg), values are mean ± standard deviation (X ± SD); sex ratio refer to ratio number of females *versus* number of males.

Table 2. Duration in days of post-embryonic development of *M. domestica* treated with extracts from *M. azedarach*, topic application on L1 larvae.

Treatment	Larval stage (days)		Pupal stage (days)		Larvae to adult (days)	
	X ± SD	VI	X ± SD	VI	X ± SD	VI
Control	6.58 ± 0.77 a	5-10	5.56 ± 0.69 a	2-7	12.08 ± 0.62 a	11-14
A	6.45 ± 0.66 a	5 -8	5.89 ± 0.52 b**	4-8	12.25 ± 0.68 a	10-14
B	6.43 ± 0.76 a*	5-10	5.96 ± 0.52 b***	5-7	12.35 ± 0.86 a	11-14
C	6.72 ± 0.81 b*	5-10	5.96 ± 0.64 b***	5-8	12.63 ± 0.83 b***	11-14
D	6.65 ± 0.74 a	5-10	5.98 ± 0.65 b***	2-7	12.59 ± 0.65 b***	11-14

Triplicate experiments with groups of 50 larvae of *M. domestica*, each. VI= variation interval. Means followed by the same letter did not differ among themselves and those followed by different letters have a significant difference (* P < 0.05, ** P < 0.01, ***P < 0.001), when the Tukey test was used. Values are mean ± standard deviation (X ± SD).

Table 3. Viability of eggs (%) of *M. domestica* treated with fraction D from *M. azedarach*, topic application on eggs mass at the concentration of 200 μg/mg.

Treatment	Number of eggs	Number of larvae	Egg viability (%)
Control	167	152	91 a
Acetone control	266	233	88 b *
Fraction D	245	54	22 c ***

Triplicate experiments with groups of eggs mass (5mg) of *M. domestica*, each. Numbers followed by the same letter did not differ among themselves and those followed by different letters have a significant difference (* P < 0.05, ***P < 0.001) when the test χ^2 was used.

The results were expressed by their means and standard deviations, and the significance established statistically using the χ^2 and the Tukey tests.

RESULTS

The results are summarized in Tables 1-3. Thus, the post-embryonic development of the flies appeared to be drastically reduced on treatment with samples B, C and D, showing a viabilility of 50, 50 and 37% for each sample, respectively (Table 1). The viability of the larval stage was reduced by all tested samples of *M. azedarach*, mainly by fraction D that induced a reduction of 31% when compared with those of the control groups. The pupal weight was reduced by all the samples as compared to the control group (22 mg) with concomitant reduction of the flies size; thus, mean weights of 20 mg (spreading: 10-28 mg) were observed when larvae were treated with A ($P < 0.001$) and D ($P < 0.01$) respectively (Table 1). In addition, the sexual ratio (number females/number males) showed a reduction of the number of females (ratio = 0.38) in the group treated with fraction D (Table 1).

The duration of the pupal stage showed a significant increase when *M. domestica* were treated with samples A ($P < 0.01$), B ($P < 0.001$), C ($P < 0.001$) and D ($P < 0.001$). The duration of the newly hatched larvae to adults also had significant increase when treated with samples C ($P < 0.001$) and D ($P < 0.001$) (Table 2).

Treatment of egg mass with D, at the concentration of 200 μg/mg egg mass, resulted in only 22% of hatched larvae (Table 3) thus proving the high toxicity of this fraction from *M. azedarach* seeds on *M. domestica* eggs.

DISCUSSION AND CONCLUSION

This study describes preliminary results of the analysis of the biological activity of *M. azedarach* extracts focusing toxicity, pupation inhibition and outbreak of *M. domestica*, looking for fractions and substances that might be efficient to control dipterans vectors of pathogens. Inhibition of the post-embryonic development of *M. domestica* was observed in extract D that induced 46% and 63% larval and outbreak inhibitions respectively. Some authors described that an extract of the seeds of this tree is able to exert a repellent activity on *Locusta migratoria* (Pradhan et al., 1962), and, that the acetone extract interferes on the pupal development of *Culex pipiens* (Al Sharook et al., 1991). Similarly, anti-molting activity was found in *M. azedarach* against the triatomine insect *R. prolixus*, vector of Chagas's disease (Cabral et al., 1995, 1996).

High toxicity against larvae and pupae of *M. domestica* was evidenced in fractions C and D, both obtained by partition of the hexane crude extract from the Meliaceae. Moderate to high mortality of *M. domestica* was observed when these flies were exposed to extracts from Gymnospermae from New Zeeland (Singh & Upadhyay, 1993). A similar result was found for the AcOEt (80%) and hexane (85%) crude extracts on *R. prolixus* (Cabral et al., 1996). The same authors assayed extracts of *M. azedarach* and demonstrated molting inhibition and toxicity on the Hemiptera *O. fasciatus* and on the hematophagous triatomine *R. prolixus* (Cabral et al., 1999). Purifications of the crude extracts increased the activities. On fractionation, the anti-molting activity was found in fraction B, that inhibited 80% of the ecdysis of *R. prolixus* (Cabral et al., 1996), the active principle being identified as pinoresinol, that showed 90% (25 μg/μL) and 65% (25 μg/μL) molting inhibition on *O. fasciatus* and *R. prolixus*, respectively (Cabral et al., 1999).

Studies on species of Meliaceae of the genus *Trichilia* also appeared promising for insecticide use on soil plagues and on the armyworm *Spodoptera frugiperda* (Hernández et al., 1983). Similarly in the health area, several vegetal extracts are under study aiming at the control of insects, vectors of illnesses to man, such as hematophagous mosquitoes, domestic flies, cockroaches and earwigs (Lagunes et al., 1984, Simas et al., 2004).

In conclusion, the seed extracts of *M. azedarach* showed potent bioactivity against *M. domestica*, which is an important urban pest all over the world. Sample D demonstrated the highest activity. This seems to be the first report of biological activities of *M. azedarach* extracts against Muscidae insects.

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