TOXICITY OF *MURRAYA PANICULATA* (L.) JACK LEAF-DERIVED MATERIALS AGAINST *CALLOSOBRUCHUS MACULATUS* (F.) (COLEOPTERA: BRUCHIDAE)

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ABSTRACT

The toxicity of *Murraya paniculata* (L.) leaf-derived petroleum ether fractions (P_{1-} P_{13}) and ethyl acetate fractions ($E_{1-}E_{27}$) were evaluated by a residual film bioassay against adult male and females of *Callosobruchus maculatus* (F.). Petroleum ether fraction P_1 was more toxic than ethyl acetate fraction E_1. The 24-, 48- and 72-h LD_{50} values for P_1 and E_1 were: 11537.78, 1890.05, 347.02 and 26698.99, 5026.88, 411.14 ng/cm^2 (male) and 28227.67, 5809.14, 431.81 and 44838.78, 6691.01, 522.51 ng/cm^2 (female) respectively. Males were more susceptible than females. *M. paniculata* leaf-derived materials merit further study as potential insect-control agents for *C. maculatus*.

Key words: natural insecticide, *Murraya paniculata*, *Callosobruchus maculatus*, toxicity, susceptibility

INTRODUCTION

Callosobruchus maculatus F. is one of the most important and destructive pests of almost all kinds of pulses both in the field and storage. The damage in storage is more crucial than damage in the field. Control of this pest is primarily dependent upon continued applications of organophosphorus and pyrethroid insecticides and the fumigants, methyl bromide and phosphine (Kim *et al.*, 2003a, Park *et al.*, 2003b). Although effective, their repeated use for decades has disrupted biological control by natural enemies and led to outbreaks of other insect species and often resulted in the development of resistance. It has had undesirable effects on non-target organisms and fosters environmental and human health concerns (Kim *et al.*, 2003b, Park *et al.*, 2003a). To combat these problems, need for the development of safe and selective insect-control alternatives has been emphasized.

Many plant extracts and essential oils may be alternative sources for stored-products protection because they constitute a rich source of bioactive chemicals and many of them are largely free from adverse effect, are selective to certain insects, often biodegrade to nontoxic products (Isman, 1995) and are potentially suitable for use in integrated pest management (Kim *et al.*, 2003b). They can be applied to stored products in the same manner as the insecticides currently used. They also provide useful information on resistance management because certain plant extracts or phytochemicals can be highly effective against insecticide-resistant insects (Lindquist *et al.*, 1990; Ahn *et al.*, 1997).

Orange Jasmine, *Murraya paniculata* (L.) Jack (Rutaceae), known as Kamini in Bengali is an evergreen shrub or small tree. It is distributed to China, India, Australia, South and East Asia including Bangladesh (Anon, 1950; Ghani, 1998). In Bangladesh, it is widely grown in gardens and roadside as ornamental plant or for fencing the gardens in many areas of the country (Ghani, 1998). *M. paniculata* leaf essential oil is rich in mono- and sesquiterpenes (Li *et al.*, 1988), which showed growth disrupting activity against some insects (Slama *et al.*, 1974; Ghani, 1998). *M. paniculata* essential oil plays good role in the reduction of *C. maculatus* populations (Huixin *et al.*, 1998).

This paper describes a laboratory study to assess the potential of the fractions of *M. paniculata* leaf extracts in different solvents against adult male and females of *C. maculatus*.

MATERIALS AND METHODS

The study was carried out at the Integrated Pest Management (IPM) Laboratory, Institute of Biological Sciences, University of Rajshahi, Rajshahi, Bangladesh during April 2000 to October 2004.

Black gram, *Phaseolus mungo* L. seeds were used as food and breeding media. The Black gram seeds were purchased from local market at Shaheb Bazar, Rajshahi.

Cultures of *C. maculatus* was maintained in the laboratory for ten years without exposure to any known insecticide in petri dish (15 cm diam.) under controlled temperature and relative humidity, so that insects of uniform size and age were available.

Extraction and Fractionation
Fresh leaves *M. paniculata* were collected from Dinajpur district in the month of April-June/2004. They were dried in an oven (40°C). Dried leaves (500g) were pulverized and extracted in **Soxhlet's apparatus** with petroleum ether (pet. ether, 3 liter), ethyl acetate (EtOAc, 3 liter), acetone (3 liter) and methanol (MeOH, 3 liter) serially. The solvent portions were concentrated by vacuum rotary evaporator at 40°C to yield about 7.8, 12.5, 5.2 and 25.4 gm of an extract, respectively.

VLC procedure
Fractionation procedures were done by vacuum liquid chromatography. Silica gel (GF_{254}, 60-120 mesh, 2g/plate) was poured into the glass column (4.5 i.d × 2.5 cm in diameter and 18 cm in length) allowed to settle down by vacuum pressure. The crude extract (6.5 gm) was mixed with a small amount of pet. ether to form solution and a small portion of silica gel was added and well mixed. After drying in the air, the residue was loaded on the column carefully. Similar procedures were likewise applied to EtOAc extracts. A mobile phase of the solvent system Pet. ether-$CHCl_3$ (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 6:4, 5:5, 4:6, 3:7, 2:8) and finally the column was washed with 100% Pet. ether in case of petroleum spirit extract and EtOAc-$CHCl_3$ (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 6:4, 5:5, 4:6, 3:7, 2:8) and washed with 100% $CHCl_3$ and again $CHCl_3$-EtOAc (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9) and washed with 100% EtOAc in case of EtOAc extract were sued. At each step 40 ml of mixed different ratio of solvents were used. Thirteen (P_1 to P_{13}) and 27 sub-fractions (E_1 to E_{27}) from pet. ether and EtOAc extracts, respectively, were obtained. These sub-fractions were air-dried for subsequent bioassay.

Bioassay
A residual film bioassay was used to evaluate the toxicity of the tested materials to adult male and females of *C. maculatus*. Four concentrations (1.99, 0.398, 0.796 and 0.1592 ng) of each material used in 1ml of respective solvent of extraction (Pet. ether and EtOAc) were evenly applied to the bottom of petri dish (8 cm diam.). After drying for half an hour in the air, groups of 30 male and females (0-24h old) were individually placed on the bottom of the petri- dishes. Subsequently, a control batch was maintained with respective solvents. Treated and control (solvent only) insects were held at 30±1°C and 70% rh. All bioassays were replicate three times.

Data analysis
Mortalities were recorded 24, 48 and 72 hours of exposure and corrected according to Abbott's (1925) formula:

$$Pt = \frac{P_0 - P_c}{100 - P_c} \times 100$$

Where, Pt = corrected mortality
P_0 = observed mortality and
P_c = control mortality

The LD_{50} values were calculated by probit analysis according to Finney (1947) and Busvine (1971) using a Software developed for probit analysis (with the GW BASIC program language) in the Department of Agricultural and Environmental Science, University of Newcastle Upon Tyne, UK.

RESULTS AND DISCUSSION

The crude extracts of pet. ether and EtOAc were more toxic than those of acetone and methanol against *C. maculatus* adults. The LD_{50} values, 95% confidence limits, regression equations (Y) and χ^2-values for adult mortality of *C. maculatus* at different exposure times of the tested materials are shown in Tables 1 and 2. As judged by the LD_{50} values, P_1 fraction was more toxic than E_1 one. Males were more susceptible than the females. The correlation co-efficient (r) values showed significant positive correlation (P<0.01) among doses, exposure time and mortality in all the cases.
Our present study revealed that *M. paniculata* leaf extract was toxic against *C. maculatus*. This is apparently first report on insecticidal activity of *M. paniculata* leaf against *C. maculatus*. However, Li *et al.* (1988) isolated monoterpene and sesquiterpene rich oils from the leaf of *Murraya* spp., which showed growth disrupting activity

against insects (Slama *et al.* 1974, Ghani, 1998). Ito and Furukawa (1987a, b, 1989, 1990) and Ito (2000) isolated various compounds from *Murraya* spp. Flavone, flavonol, flavonoid, stigmasterol, alkaloid isolated from leaf, flower and brand cortex of *M. paniculata* (Kinoshita and Firman, 1995, 1997, Kinoshita *et al.*, 1996, Ferracin *et al.*, 1998, Riyanto *et al.*, 1999; Wu *et al.*, 1994; Barik and Kundu, 1987).

Table 1. LD_{50}, 95% confidence limits, regression equation, χ^2 and r-values of *M. paniculata* leaf-derived petroleum ether fraction P_1 tested against adult male and females of *C. maculatus* in different exposure time

Sex	Exposure Time (h)	LD_{50}-values ($\mu g/cm^2$)	95% confidence limits		Regression equation	χ^2-values in 2df	r-values
			Lower	Upper			
Male	24	11.54	2.118	62.853	Y=3.104+0.92X	0.855	0.8692
	48	1.89	1.156	3.088	Y=3.510+1.166X	0.363	0.9783
	72	0.347	0.257	0.468	Y=3.724+2.36X	7.318	0.9996
Female	24	28.229	1.952	408.064	Y=2.948+0.837X	1.753	0.7803
	48	5.8096	1.669	20.2118	Y=3.490+0.855X	0.787	0.9244
	72	0.4318	0.278	0.6691	Y=3.857+1.798X	11.132	0.9853

*Correlation coefficient = r < 0.01

Table 2. LD_{50}, 95% confidence limits, regression equation, χ^2 and r-values of *M. paniculata* leaf-derived ethyl acetate fraction E_1 tested against adult male and females of *C. maculatus* in different exposure time

Sex	Exposure Time (h)	LD_{50}-values ($\mu g/cm^2$)	95% confidence limits		Regression equation	χ^2-values in 2df	r-values
			Lower	Upper			
Male	24	26.701	1.93	369.45	Y=2.97+0.84X	2.24	0.7817
	48	5.03	1.57	16.098	Y=3.54+0.86X	4.21	0.9573
	72	0.41	0.27	0.64	Y=3.89+1.81X	11.02	0.9851
Female	24	44.84	1.42	1414.52	Y=2.90+0.79X	1.15	0.8033
	48	6.69	1.65	27.21	Y=3.51+0.81X	0.93	0.90
	72	0.52	0.35	0.77	Y=3.81+1.65X	8.04	0.9903

*Correlation coefficient = r < 0.01

CONCLUSIONS

This might be interesting to explore or use and to use *M. paniculata* leaf-derived materials or their constituents as insect-control agents. Further research is necessary on the structural elucidation of active compounds and their insecticide mode of action. Other areas requiring attention are changes in the quality of agricultural products treated with them (e. g. colour, flavour, and odor) and formulations for improving the insecticidal potency and stability and for reducing the cost.

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