Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2017; 5(6): 692-696

Received: 03-09-2017
Accepted: 07-10-2017

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Potentiation of *Mucuna pruriens* (L.) DC. for insecticidal activity, insect repellency and brine shrimp lethality tests under the laboratory conditions

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Abstract
Aerial part, fruit and root extracts of *Mucuna pruriens* (L.) DC. were obtained with petroleum ether, chloroform and methanol and evaluated for their insecticidal and insect repellent activity against the adult beetles of *Tribolium castaneum* (Hbst.) and cytotoxic activity against *Artemia salina* L. nauplii. The chloroform extract of fruit found most effective with LD_{50} 0.534mg cm^{-2} followed by the petroleum ether and methanol extracts of root with LD_{50} 0.814and 1.056mg cm^{-2} after 48 hours of exposure. Petroleum ether and chloroform extracts of the fruit and methanol extract of the aerial part showed repellent activity against the adult beetles at 5% level of significance ($P<0.05$). The same extracts responded through (Cytotoxicity) brine shrimp lethality assay while the methanol extract of aerial part found most effective (LC_{50} 12.793ppm)followed by the petroleum ether extract of root and chloroform extract of fruit with LC_{50} 24.414 and 25.208ppm after 30h of exposure.

Keywords: *Mucuna pruriens*, dose mortality, repellency, *Tribolium castaneum*, brine shrimp lethality, *Artemia salina*

Introduction
Mucuna pruriens (L.) DC. of the family Fabaceae is the native in the tropical Africa, Asia and West Indies. It is a climbing annual legume known as Cowhage, velvet-bean or Bengal velvet-bean that can be up to 6-18m long and contains a taproot with numerous 7-10m long, lateral roots. The stems are slender and slightly pubescent and the leaves are generally slightly pubescent, alternate, trifoliate with rhomboid ovate, 5-15cm long × 3-12cm broad leaflets [1-2].It can grow on a wide range of soils, from sands to clays but thrives on well-drained, light textured soils of appreciable acidity [3-4]. *M. pruriens* finds importance as food, feed, cover crop and fodder and is extensively cultivated worldwide [5-8]. It is considered a viable source of dietary proteins [9-10] due to its high protein concentration (23–35%) in addition to its digestibility, which is comparable to that of other pulses such as soybean, rice bean, and lima bean [11]. *M. pruriens* is a popular Indian medicinal plant, which has long been used in traditional Ayurvedic Indian medicine, for diseases including Parkinsonism [12].The present investigation was carried out to find out the potential of its insecticidal and insect repellent activity against the red flour beetle, *Tribolium castaneum* (Hbst.), and lethality against the brine shrimp, *Artemia salina* L. nauplii. The red flour beetle is reddish-brown in color and its antennae end in a three-segmented club [13]. Although small beetles, about ¼ of an inch long, the adults are long-lived and may live for more than three years [14], and thus became a suitable lab insect. The *A. salina* belongs to a genus of very primordial crustacean (crawfish-crayfish) the *Anostraca* (Fairy Shrimps). Crawfish of this genus just has a divided exoskeleton made of chitin enhanced protein, no usual crust of chitin (escutcheon) as the crawfish has. There are many species within the genus of *Anostraca*, but the *A. salina* is very nice to grow, and since the rate of successful hatches is very highthis species is used as a test agent in the toxicology laboratory.

Materials and Methods
Collection and preparation of test materials: *M. pruriens* was collected from the University of Rajshahi Campus and identified by the Department of Botany, University of Rajshahi,

where a voucher specimen is kept in the herbarium. The plants were chopped into small pieces, dried under shade and powdered using a hand grinder, weighed and placed in separate conical flasks to add Pet. ether, $CHCl_3$ and MeOH (Merck, Germany) (100gm × 300ml × 2times) for 48h. Filtration was done by Whatman filter paper (made in USA) at the 24h interval in the respective flasks followed by evaporation until the extracts were left. The extracts were then removed to glass vials and preserved in a refrigerator at 4°C with proper labeling.

Collection and culture of test insect: Adults of *T. castaneum* were reared in glass beakers (500ml) in a standard mixture of whole-wheat flour with powdered dry yeast (19:1) in an incubator at 30 ±0.5°C without light and humidity control for a continuous supply of adults during experimentation.

Dose-mortality test: The dose-mortality responses of *M. Pruriens* were observed by surface film method. The concentrations used were 2.038, 1.529, 1.019, 0.510 and 0.254mg cm^{-2} for Pet. ether extract of fruit followed by 2.548, 2.038, 1.529, 1.019, 0.510 and 0.254mg cm^{-2} and 3.567, 3.057, 2.548, 2.038, 1.529, and 1.019mg cm^{-2} for $CHCl_3$ and CH_3OH extracts; 4.080, 3.567, 3.057, 2.548, and 2.038mg cm^{-2} for Pet. ether extracts of aerial part as well as 2.548, 2.038, 1.529, 1.019, 0.510 and 0.250mg cm^{-2}; and 2.548, 2.038, 1.529, 1.019, 0.510 and 0.254mg cm^{-2} for Pet. ether and CH_3OH extracts of root against *T. castaneum* in the dose mortality experiments. Each of the doses were diluted in 1ml of solvent, poured into Petri dishes and allowed to dry out. Ten adult beetles were released in each Petri dish, and the experiment of all the doses for each of the extracts were replicated in a thrice. The mortality was assessed for 12h, 24h, 36h, and 48h of exposure.

Statistical Analysis: The mortality (%) was corrected using Abbott's formula [15]:

$P_r = Po - Pc /100 - Pc \times 100$

Where, P_r = Corrected mortality (%), P_o = Observed mortality (%), P_c = Control mortality (%). The data were then subjected to probit analysis according to Finney [16] and Busvine [17] using a software developed at the University of Newcastle upon Tyne, UK.

Repellent Activity: The repellent activity test was adopted from the method (No. 3) of McDonald *et al*. [18] with some modifications. Half filter paper discs (Whatman No. 40, 9cm diam.) were treated with the selected doses of 0.629, 0.314, 0.157, 0.0786, 0.0393mg $cm^{-2}$for all extracts and were then attached lengthwise, edge-to-edge, to a control half-disc with adhesive tape and placed in the Petri dishes. The orientation was changed in the two remaining replicates to avoid the effects of any external directional stimulus affecting the distribution of the test insects. Ten adult insects were released in the middle of each of the filter paper circles.

Each concentration against of the solvents was tested for five times. Insects that settled on each of the non-treated half of the filter paper discs were counted after 1h and then observed repeatedly at hourly intervals for five hours. The average of the counts was converted to percent repulsion (*PR*) using the formula of Talukder and Howse[19-20]: $PR = (Nc - 5) \times 20$, where, Nc is the percentage of insects on the untreated half of the disc.

Brine shrimp nauplii lethality test: Brine shrimp cycts were purchased from Kalabagan, Dhaka and kept in aerated seawater at room (25-30°C) temperature and took 30-48h to give nauplii. The series of concentration were for Pet. ether extracts of the fruit were: 400, 200, 100, 50 and 25ppm; followed by 100, 50, 25, 12.5 and 6.3ppm; and 100, 50, 25, 12.5 and 6.3ppm for CH_3OH and $CHCl_3$ extracts as well as 800,400, 200,100 and 50ppm; 400,100 and 25ppm; and 200, 100, 50, 25 and 12.5ppm for Pet. ether, $CHCl_3$ and CH_3OH extracts of aerial parts and for Pet. ether extracts of root were 50, 25, 12.5 and 6.3ppm; followed by 200, 100 and 50ppm; and 50, 25 and 12.5ppm for $CHCl_3$ and CH_3OH extracts were selected respectively. Ten freshly hatched nauplii were added to each of the test tubes with different concentrations mentioned earlier and observed mortality after 6, 12, 18, 24 and 30h of exposures. The data were then subjected to probit analysis.

Results and Discussion

Dose mortality effects: The dose mortality assay of Pet.E., $CHCl_3$ and CH_3OH extracts are represented in Table 1. All the extracts found effective against *T. Castaneum* adults. The $CHCl_3$ extracts of fruit found most effective showing LD_{50} 0.933,0.702,0.579 and 0.534 mg cm^{-2} followed by the Pet. ether extract of root with LD_{50} 2.845,1.820,1.048 and 0.814mg cm^{-2} and methanol extract of root with LD_{50} 3.275, 2.259, 1.844 and 1.056 mg cm^{-2} for 12, 24, 36 and 48h of exposures respectively.

Table 1: LD_{50} values of the test extracts of *M. pruriens* established through residual film assay against *T. castaneum* adults.

Test plant	Plant parts used	Solvents	LD_{50} value (mg cm^{-2}) Duration of exposures			
			12h	24h	36h	48h
M. pruriens	Fruit	Pet.E.	8.896	2.428	1.709	1.031
		$CHCl_3$	0.933*	0.702	0.579	0.534*
		CH_3OH	-	28.982	3.997	2.869
	Aerial part	Pet. E.	4.069	4.842*	3.436	3.210*
	Root	Pet.E.	2.845*	1.820	1.048	0.814
		CH_3OH	3.275	2.259	1.844	1.056*

* Variance has been adjusted for heterogeneity

Repellent effects: The extracts of fruit obtained in Pet. ether; and $CHCl_3$ and CH_3OH extract of aerial part offered promising repellent activity at 5% and 1% level of significance ($P<0.05$ and $P< 0.01$) respectively while the other extracts didn't show any significant repellency (Tables 2 and 3).

Table 2: ANOVA results of the repellency against *T. castaneum* by the Pet. ether, $CHCl_3$ and CH_3OH extracts of *M. pruriens* (fruit, aerial part and root).

M. pruriens plant parts	Solvents used for extraction	Sources of variation			F-ratio with level of significance		P- value	
		Between doses	Between time interval	Error	Between doses	Between time interval	Between doses	Between time interval
Fruit	Pet. ether	4	4	16	19.358*	1.477	5.6E-06	0.255
	$CHCl_3$	4	4	16	8.972*	0.160	0.0005	0.955
	CH_3OH	4	4	16	2.819	2.593	0.060	0.076
Aerial part	Pet.ether	4	4	16	1.107	1.303	0.387	0.311
	$CHCl_3$	4	4	16	6.201	2.340	0.003	0.099
	CH_3OH	4	4	16	10.751*	2.461	0.0002	0.087
Root	Pet.ether	4	4	16	3.281	3.075	0.038	0.047
	$CHCl_3$	4	4	16	1.839	1.429	0.1707	0.270
	CH_3OH	4	4	16	2.928	4.586	0.054	0.012
* = Significant at 5% level (P<0.05)								

Table 3: Repellent effect of Pet. ether and $CHCl_3$ extract of fruit and CH_3OH extract of aerial part of *M. Pruriens* against *T. castaneum.*

Solvents	Between doses (df=4)		Between time interval	
	F- values	Level of significance	F- values	Level of significance
Pet. ether (Fruit)	19.358*	P<0.05	0.865	-
$CHCl_3$ (Fruit)	8.972*	P<0.05	0.705	-
CH_3OH (Aerial part)	10.751*	P<0.05	2.093	-

** = Significant at 1% level (P<0.01) * = Significant at 5% level (P<0.05); (-) = Not significant at any level.

Brine shrimp lethality effect: The brine shrimp lethality for Pet.E., $CHCl_3$ and MeOH extracts of *M. pruriens* represented in Table 4. The highest lethality was observed in the CH_3OH extract of aerial part with LC_{50} value 12.793ppmfollowed by the Pet. ether extract of root with LC_{50}24.414ppmboth after 30h of exposure against brine shrimp nauplii.

Table 4: LC_{50} values of the test extracts of *M. pruriens* established through brine shrimp lethality assay against *A. salina* nauplii.

Test plant	Plant part Used	Solvents	LC_{50} value (ppm) Duration of exposure				
			6h	12h	18h	24h	30h
M. pruriens	Fruit	Pet. ether	217.120	120.414	69.093	56.085	44.139
		$CHCl_3$	80.511	59.947	40.234	31.767	25.208
		CH_3OH	-	17117.130	218.850	144.431	77.675
	Aerial Part	Pet. ether	3297.292	2110.736	2107.780	1355.386	321.291
		$CHCl_3$	-	247.889	165.053	58.738	34.273
		CH_3OH	60.486	33.064	25.936	17.256	12.793
	Root	Pet. ether	175.297	72.111	51.943	33.436	24.414
		$CHCl_3$	108.639	45.254	43.905	43.910	34.111
		CH_3OH	66.647	40.977	35.020	23.738	19.527

Discussion

These findings receive support from previous researchers' achievements. All parts of *Mucuna* plant are known to possess high medicinal value [21].Its extracts have been long used in tribal communities as a toxin antagonist for various snakebites. Aqueous extracts of *M. pruriens* seeds possess compounds, which inhibit the activity of cobra and krait venoms. About 0.16mg and 0.19mg of *M pruriens* seed extracts were able to completely neutralize the lethal activity of cobra and krait venom respectively [19]. *Mucuna* pod hairs are blended with honey and are used as vermifuge. The paste prepared from pod hairs are also used as a stimulant and mild [20]. *M. pruriens* has been found to contain L-DOPA, that converts into dopamine which is a potent neurotransmitter precursor that is believed, in part, to be responsible for the toxicity of the *Mucuna* seeds [22]. A study was conducted to develop suitable method(s) for extraction of L-DOPA from the powdered seeds of *M. pruriens* using different solvents and conditions [23]. Rajeshwar *et al.* [24] demonstrated that the methanol extract of *M. pruriens* seeds has significant *in vitro* anti-oxidant activity, and there are also indications that methanol extracts of *M. pruriens* may be a potential source of natural anti-oxidants and anti-microbial agents. Its anti-venom activities have been investigated [25] and its anti-helminthic activity has also been demonstrated by Jalalpure [26]. *M. pruriens* has also been shown to be neuroprotective [27] and has demonstrated as analgesic and anti-inflammatory agents [28]. The Antiparkinsonian and Anti dyskinetic mechanisms of *M. pruriens* have also been reported [29]. In the Ayurvedic system of medicine, *M. pruriens* was used for the management of male infertility, nervous disorders and also as an aphrodisiac [30-31]. Fruit, aerial part and root extracts of *M. pruriens* of Pet. ether, $CHCl_3$ and CH_3OH were found effective against the brine shrimp nauplii in different hour of exposure. Patoary *et al*. [32] conducted a research where he reported the $CHCl_3$ and CH_3OH extracts of the seed coat and seed kernel of *M. pruriens* were tested against the brine shrimp, *Artemia fransiscana* nauplii for mortality at 24h post exposure. All the test extracts were found to be effective. A review study conducted by Yadav *et al*. [33] established the plant *M. pruriens* as a herbal drug. It has been shown that its seeds are potentially of substantial medicinal importance. In the ancient Indian medical system, Ayurveda, *M. pruriens* used traditionally to treat Parkinson's disease. *M. pruriens* has also been shown to have antiparkinson and neuroprotective effects, which may be related to its antioxidant activity and used for the management of male infertility, nervous disorders, and also as an aphrodisiac. Some authors reported

the repellent effects of some well-known plants such as neem, turmeric, etc. Leaf of *Withania somnifera* repelled *Ryzopertha dominica* [34].

Conclusion

Dose-mortality, insect repellent activity and brine shrimp lethality tests revealed that this renowned medicinal plant *M. pruriens* has potentials to be a potent tool in the global pest control strategy. Thus, comprehensive phytochemical analyses of the test plant for its biologically active potentials, as well as the pharmacological studies of the active ingredients are very much to be solicited for their effective use in the future pest control and pharmaceutical endeavors.

Acknowledgements

The authors are grateful to the University Grants Commission (UGC) of Bangladesh. The Authors are also thankful to the Ministry of Science & Technology of the People's Republic of Bangladesh for the Bangabandhu Fellowship to S. B. Chhabi on Science & ICT Projects. They would like to extend thanks to the Chairman, Department of Genetic Engineering and Biotechnology and Department of Zoology, University of Rajshahi for providing laboratory facilities.

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