Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

Journal of Entomology and Zoology Studies

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2017; 5(4): 11-15

Received: 02-05-2017
Accepted: 03-06-2017

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Control potentials of *Saraca indica* L. extracts against the adults of stored product pests *Callosobruchus chinensis* L., *Sitophilus oryzae* L. and *Tribolium castaneum* (Hbst.)

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Abstract
The aim of the current research work was to analyze the control potentials of plant extracts against stored product pests. The research work was carried out in the Crop Protection & Toxicology Lab, University of Rajshahi, Bangladesh, during April 2016 to November 2016. Pet. ether, $CHCl_3$ and CH_3OH extracts of *Saraca indica* L. were subjected to repellent activity and dose-mortality tests against *Callosobruchus chinensis* (L.), *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Hbst.). Pet. ether extracts of root and stem bark; $CHCl_3$ extracts of leaves, root and stem bark didn't show mortality at all. However, other parts of the test plant extractives provided mortality to the test insects by yielding different LD_{50} values in different time exposure. In repellency test the extracts were found moderately repellent ($P<0.01$) and mild repellent ($P<0.05$). However, CH_3OH extracts of leaves, $CHCl_3$ and CH_3OH extracts of stem bark didn't show repellent activity at all.

Keywords: Dose-mortality, Repellency, *Saraca indica*, *Callosobruchus chinensis*, *Sitophilus oryzae*, *Tribolium castaneum*

1. Introduction
Saraca indica L. is a small evergreen tree, 7 to 10m in height. Leaves are narrow, cork like at the base and with short intrapetiolar and completely united [1]. The stem bark is rough and uneven due to the presence of rounded or projecting lenticels. Flowers are polygamous, yellowish orange turning to scarlet, in short laterally placed corymbose, axillary panicles, bract small, deciduous, calyx petaloid [2]. It is extensively found in Malayan Peninsula, Myanmar, Srilanka and Bangladesh [3]. It is widely distributed throughout Indian Subcontinent in evergreen forests up to an elevation of about 750meters and propagation is done by the seeds [4]. It is commonly called Asok tree in English; Ashoka and Ashok in Bengali [5]. *S. indica* is a religious and the most ancient tree of India. It has a number of medicinal properties hence used by physicians since centuries in Unani system of medicine along with Ayurveda [3]. Married women in India are known to eat Ashoka flower buds as a ritual to invoke deities for child protection as well as gynecological problems. Women suffering from menorrhagia drink a decoction on an empty stomach in the morning, which is prepared from the bark of Ashoka in water in combination with other herbs such as *Terminalia chebula* and *Coriandrum sativum* [6]. In leucorrhoea, the decoction of Ashoka bark in water and milk after evaporation of water is consumed by women. In India, Srilanka, Bangladesh and Pakistan Ashoka bark is used by womenfolk in treating menorrhagia, menstrual and uterine disorders [7-8].
Test insect *C. chinensis* (Family: Bruchidae) is a common species of beetle found in stored legumes [9]. The eggs are cemented to the surface of pulses and are smooth, domed structures with oval, flat bases. The larvae and pupae are normally found only in cells bored within the seeds of pulses [10]. The developmental period from egg to adult takes 20-25 days [11-12].
S. oryzae (rice weevil) (Family: Curculionidae) is a serious stored product pest which attacks several crops and worldwide in distribution. The adult rice weevil is a dull reddish-brown to black in color. The larval rice weevil must complete its development inside the seed kernel. The larva develops within the seed, hollowing it out while feeding. Total life cycle from egg to adult took 34 to 49 days with an average of 42 days at 15 to 34°C temperature and 58 to 89 per cent relative humidity [13].

T. castaneum (Family: Tenebrionidae) is a worldwide pest of stored products and of Indo-Australian in origin [14]. These beetles have chewing mouthparts, but do not bite or sting. The red flour beetle may elicit an allergic response [15]. The eggs are microscopic and the slender larvae are creamy yellow to light brown in colour. The adult is a small reddish-brown beetle. Total life cycle contain subsequently for egg incubation 8.8 days, larval development 22-100 days depending on temperature, pupal development 4.5 days, and for reproductive maturation 4-5 days [16]. The strategy for the present investigation was designed to carry on screening of the crude extracts of the test plant species on three test organisms [*Callosobruchus chinensis* (L.), *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Hbst.)] for the detection of biological activity and keeping an option to show the extent of activity by analyzing the data statistically that read on various parameters during the course of the investigation.

2. Materials and Methods

2.1 Collection and preparation of test materials

S. indica plant leaves, root and stem bark were collected from the Botanical Garden belongs to the Department of Botany, University of Rajshahi, Bangladesh in the month of April, 2016. Firstly, the plant was identified by the Department of Botany, University of Rajshahi where voucher specimens are kept in the herbarium. The leaves, roots and stem bark were separated while excess soil was removed from the roots without washing. Leaves, roots and stem bark of the plant were then sliced and chopped into small pieces, dried under shade and powdered with the help of a hand grinder, weighed and placed in separate conical flasks to add solvents. Petroleum ether, $CHCl_3$ and CH_3OH (Merck, Germany) were used (200g × 600ml × 2 times) successively each of which took for 48h on a shaker. For each of the extract filtration was done by Whatman filter paper (made in USA) at 24h interval in the same flask followed by evaporation until the extract was left as a scum. The extracts was then removed to glass vials and preserved in a refrigerator at 4 °C with proper labeling.

2.2 Collection and culture of test insects

The test insects *C. chinensis*, *S. oryzae* and *T. castaneum* were used in repellency activity and insecticidal activity tests in the crude extracts from the different parts of *S. indica*. These test insects were selected because they are easy cultivable and noble laboratory animals. Moreover, they are important stored grain pests in a wide variety of cereal products. All the test insects of same age used in this investigation were collected from the stock cultures of the Crop Protection and Toxicology Laboratory, Department of Zoology, University of Rajshahi, Bangladesh.

2.3 Repellent activity

The repellency test was adopted from the method of McDonald *et al*. (1970) with some modifications [17]. A general concentration for each of the extracts (Pet. ether, $CHCl_3$ and CH_3OH) was selected as stock dose for repellency applied against the adults of *C. chinensis* and *S. oryzae* to make other successive doses by serial dilution to give 1.42, 0.71, 0.35, 0.18 and 0.088mg/cm^2 and for *T. castaneum* the doses were established as 629, 314, 157, 80 and 39µg/cm^2.

For the application of the extracts on *C. chinensis* and *S. oryzae* Petri dish (of 9cm in diam.) was divided into three parts and marked with two narrow stick through adhesive tape. Then the both side filled with food where in one side treated food and other side with non-treated food followed by the concentration except the middle one. Then ten adult insects were released into the middle of the petri-dish.

Whereas, in case of *T. castaneum* half filter paper discs (Whatman No. 40, 9cm diam.) were prepared and selected doses of all the extracts separately applied onto each of the half-disc and allowed to dry out as exposed in the air for 20 minutes. Each treated half-disc was then attached lengthwise, edge-to-edge, to a control half-disc with adhesive tape and placed in a Petri dish (9cm diam.). For each of the test samples three replicates were maintained. Being volatile the solvent was evaporated out within a few minutes. Then ten insects were released in the middle of each filter paper circles. Repellency was observed for one-hour interval and up to five successive hours of exposure for the all three insect species population. In case of *C. chinensis* and *S. oryzae* just by counting the number of insects from the non-treated part and the middle part of the 90mm Petri dish floor. While, for *T. castaneum* just by counting the number of insects from the non-treated part of the filter paper spread on the floor of the 90mm Petri dish. The values in the recorded data were then calculated for percent repellency, which was again developed by arcsine transformation for the calculation of analysis of variance (ANOVA). The average of the counts was converted to percentage repellency (PR) using the formula of Talukder and Howse (1993, 1995) [18, 19]: PR = (Nc-5) × 20; where, Nc is the average hourly observation of insects on the untreated half of the disc.

2.4 Dose-mortality test

2.4.1 Dose-mortality test on *C. chinensis* and *S. oryzae*

For insecticidal activity test each of the three extracts were dissolved in its solvent of extraction at different concentrations to go through *Ad Hoc* experiments to set considerable mortality and that were considered as doses. The concentrations of Pet. ether leaves extract used against *C. chinensis* in this experiment were 13, 10, 8, 7 and 5µg/kg; for *S. oryzae* the concentrations of CH_3OH extracts were 14.5, 13.5, 13, 12.5 and 11µg/kg for leaves; 17.5, 16.5, 15, 14 and 12.5µg/kg for root and 14.5, 13.5, 12.5, 11.5 and 10µg/kg for stem bark. For each dose 1ml of prepared dose was mixed with the prepared food and being volatile the solvent was evaporated out shortly. The actual extract present in 1ml mixture was calculated just dividing the value by the amount of used calculated food. After drying 10 insects of the same age were released on the food in 3 replications. A control batch was also maintained with the same number of insects after preparing the food by applying and evaporating the solvent only. The treated insects were placed in an incubator at the same temperature as reared in stock cultures and the mortality of the insects were counted after 0.5h, 6h, 12h and more 5 times with 12h intervals.

2.4.2 Dose-mortality test on *T. castaneum*

The experiment for insecticidal test on *T. castaneum* is not the same as on *C. chinensis* or *S. oryzae* since the feeding is different. Here also the *Ad Hoc* experiments were set to find out the final concentrations for dose selection. The concentrations for Pet. ether leaves extract used against *T. castaneum* in this experiment were 2.291, 2.037, 1.935, 1.783 and 1.528mg/cm^2. For each application 1ml of the dose was dropped on a petri-dish (50mm) in such a way that makes a uniform film over the Petri dish. Then the Petri dishes were air dried leaving the extraction on it. The actual extract present in 1ml mixture was calculated and divided the value

by the area of the Petri dish to find the dose per square centimeter was calculated. After drying 10 insects were released (3-5 days old) in each of the Petri dishes and the whole experiment was set in three replicates. A control was also maintained with the same number of insects. The treated beetles were then placed in the incubator at the same temperature as reared in stock cultures and the mortality was counted as like as *C. chinensis* and *S. oryzae* were counted.

2.4.3 Statistical analysis

The mortality (%) was corrected using Abbott's formula (1925) [20]:

$P_r = \frac{P_o - P_c}{100 - P_c} \times 100$; Where, P_r = Corrected mortality (%), P_o = Observed mortality (%), P_c = Mortality in the control (%). The data were then subjected to Probit analysis [21-22].

3. Results

3.1 Repellent effects on *C. chinensis*, *S. oryzae* and *T. castaneum*

The Pet. ether, $CHCl_3$ and CH_3OH extracts of *S. indica* leaves, root and stem bark showed repellency to *C. chinensis* and *S. oryzae*. The $CHCl_3$ extracts of leaves and stem bark against *S. oryzae*; root against *C. chinensis* showed repellency. The CH_3OH extracts of root against *C. chinensis* and stem bark against *S. oryzae* showed repellency. The Pet. ether extracts of leaves against *C. chinensis*; the Pet. ether extracts of root and stem bark and the $CHCl_3$ and CH_3OH extracts of stem bark offered moderate repellency at 1% level of significance *(P<0.01)*; the leaves extracts of Pet. ether and $CHCl_3$ against *S. oryzae* and the root extracts of Pet. ether, $CHCl_3$ and CH_3OH against *C. chinensis* gave mild repellency at 5% level of significance *(P<0.05)*. However, the Pet. ether extracts of stem bark, the $CHCl_3$ extracts of leaves and stem bark, the CH_3OH extracts of leaves and stem bark against *C. chinensis* did not show repellency. On the other hand, the CH_3OH extracts of leaves and root and the $CHCl_3$ extracts of root against *S. oryzae* did not offer repellency. Moreover, none of the extracts showed repellent activity against *T. castaneum*. According to intensity of repellency *S. indica* extracts to *C. chinensis* the result could be arranged in a descending order: leaves (Pet. ether) > root ($CHCl_3$, CH_3OH and Pet. ether) extracts. On the other hand, the intensity of repellency against *S. oryzae* the result could be arranged in a descending order: stem bark ($CHCl_3$, Pet. ether and CH_3OH) > root (Pet. ether) > leaves ($CHCl_3$ and Pet. ether) extracts.

3.2 Dose mortality effects on *C. chinensis*, *S. oryzae* and *T. castaneum*

The results of the dose mortality assays Pet. ether extracts of *S. indica* leaves against beetles of *C. chinensis* are represented in Table 2. The LD_{50} values were 15.3, 14.7. 14, 9.25, 6.93, 6.43 and 5.47µg/kg after 6h, 12h, 24h, 36h, 48h, 60h and 72h of exposures respectively. The lethal activity of CH_3OH extracts of *S. indica* leaves, root and stem bark against the weevils of *S. oryzae* are represented in Table 3. The LD_{50} values were 13.6, 13.5, 12.8, 12.1, 11.7, 11.1 and 11µg/kg for leaves; 15.7, 15, 14.4, 13.9, 13.4, 12.9 and 12.7µg/kg for root and 15.9, 15.7, 13.6, 12.2, 11.4, 11.1 and 11µg/kg for stem bark after 6h, 12h, 24h, 36h, 48h, 60h and 72h of exposures respectively. The insecticidal activity against *T. castaneum* for the Pet. ether extract of leaves of *S. indica* is represented in Table 4. The LD_{50} values were 3.21, 2.51, 2.15, 1.76, 1.64, 1.51 and 1.52mg/cm^2 after 6h, 12h, 24h, 36h, 48h, 60h and 72h of exposures respectively. According to intensity of activity the extracts of *S. indica* could be arranged in the following descending order: leaves (Pet. ether) > stem bark (CH_3OH) > root (CH_3OH) > leaves (CH_3OH) extracts.

Table 1: ANOVA results of the repellency against *C. chinensis*, *S. oryzae* and *T. castaneum* by the Pet. ether, $CHCl_3$ and CH_3OH extracts of *S. indica* L. (leaves, root and stem bark).

Selected parts of *S. indica*	Name of the solvents used for extraction	Name of the used stored product pests	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
Leaves	Pet. ether	*C. chinensis*	4	4	16	40.603**	0.551	0.701	0.348
	$CHCl_3$		4	4	16	1.203	0.754	0.348	0.570
	CH_3OH		4	4	16	6.791	0.608	0.002	0.151
	Pet. ether	*S. oryzae*	4	4	16	10.023*	0.162	0.001	0.954
	$CHCl_3$		4	4	16	19.835*	0.819	4.82E-06	0.532
	CH_3OH		4	4	16	6.435	0.753	0.003	0.570
	Pet. ether	*T. castaneum*	4	4	16	1.084	0.109	0.397	0.978
	$CHCl_3$		4	4	16	1.502	0.751	0.248	0.572
	CH_3OH		4	4	16	8.547	3.682	0.001	0.026
Root	Pet. ether	*C. chinensis*	4	4	16	9.763*	2.370	0.003	0.096
	$CHCl_3$		4	4	16	14.224*	1.160	3.9E-05	0.365
	CH_3OH		4	4	16	11.807*	0.130	0.001	0.096
	Pet. ether	*S. oryzae*	4	4	16	37.712**	1.995	5.89E-08	0.144
	$CHCl_3$		4	4	16	3.466	0.196	0.032	0.937
	CH_3OH		4	4	16	7.078	1.239	0.002	0.334
	Pet. ether	*T. castaneum*	4	4	16	2.552	1.585	0.079	0.226
	$CHCl_3$		4	4	16	6.494	1.284	0.003	0.318
	CH_3OH		4	4	16	3.371	1.586	0.035	0.226
Stem bark	Pet. ether	*C. chinensis*	4	4	16	4.769	0.619	0.010	0.656
	$CHCl_3$		4	4	16	7.737	2.034	0.001	0.138
	CH_3OH		4	4	16	8.320	4.244	0.001	0.016
	Pet. ether	*S. oryzae*	4	4	16	32.836**	0.405	1.95E-07	0.803
	$CHCl_3$		4	4	16	39.343**	0.604	4.35E-08	0.665
	CH_3OH		4	4	16	24.302**	1.223	1.25E-06	0.340
	Pet. ether	*T. castaneum*	4	4	16	5.209	1.293	0.007	0.314
	$CHCl_3$		4	4	16	3.709	0.433	0.026	0.783
	CH_3OH		4	4	16	2.432	1.032	0.090	0.421

** = Significant at 1% level (*P<0.01*) * = Significant at 5% level (*P<0.05*)

Table 2: LD_{50} values of Pet. ether extracts of *S. indica* leaves extracts against *C. chinensis.*

Solvent	Extract	LD_{50} µg/kg at different exposures (in hours)						
		6h	12h	24h	36h	48h	60h	72h
Pet. ether	Leaves	15.3	14.7	14	9.25	6.93	6.43	5.47

Table 3: LD_{50} values of CH_3OH extracts of *S. indica* leaves, root and stem bark against *S. oryzae.*

Solvent	Extract	LD_{50} µg/kg at different exposures (in hours)						
		6h	12h	24h	36h	48h	60h	72h
CH_3OH	Leaves	13.6	13.5	12.8	12.1	11.7	11.1	11
	Root	15.7	15	14.4	13.9	13.4	12.9	12.7
	Stem bark	15.9	15.7	13.6	12.2	11.4	11.1	11

Table 4: LD_{50} values of Pet. ether extracts of *S. indica* leaves extracts against *T. castaneum.*

Solvent	Extract	LD_{50} mg/cm^2 at different exposures (in hours)						
		6h	12h	24h	36h	48h	60h	72h
Pet. ether	Leaves	3.21	2.51	2.15	1.76	1.64	1.51	1.52

4. Discussion

The findings of the present investigation receive supports from works done by previous researchers. Works on *S. indica* extracts for insects mortality and repellency is scanty, however a lots of work have been done on larvicidal activity. The findings on the test insects mortality through this investigation are supported by Mathew *et al.* (2019) that revealed the Pet. ether extract of *S. indica* leaves and the $CHCl_3$ extract of the bark were effective against the larvae of *Culex quinquefasciatus* with respective LC_{50} values, 228.9 and 291.5ppm, which follows the WHO standard protocols [23]. The results are also supported by Jinu and Jayabaskaran (2015), which yielded that the Pet. ether extract of *S. indica* leaves and $CHCl_3$ extract of bark exhibited more than 50% larval mortality against *C. quinquefasciatus* larvae at an exposure period of 48h [24]. No such reports have been reported so far on insect repellent activity of *S. indica* extract especially against the adults of the test insects. However, the findings by Singh *et al.* (2009) showed that *S. indica* leaves were effective against antibacterial activity where ethanol (95%) and water extracts on agar plate *E. coli*, *S. aureus* by inhibitory effects on their growth which is similar to our findings in case of repellency test [25]. However, another finding in the same experiments by Singh *et al.* (2009) showed that *E. coli* were found active whereas tested against *S. aureus* gave negative results [25]. The findings of the present investigation also gets support from the findings of Sarojini *et al.* (2011) which revealed that the methanolic extracts were found relatively more potent as an anthelmintic agent due to presence of alkaloids [26]. The mortality results also gets support from the findings of Verma *et al.* (2010) where *S. indica* methanolic leaves extracts showed that the central nervous system (CNS) of albino mice was depressant [27]. The findings of the inhibitory or mortality results gets support from the findings by Dabur *et al.* (2007) that the methanolic extracts of *Saraca indica* exhibited good inhibitory activity against *A. canjani* while it is effective at lower concentrations against other fungi also [28]. The findings of Dubey *et al.* (2008) revealed that food grain losses due to insect infestation during storage are serious problem, particularly in the developing countries [29]. It is estimated by Ahmed and Grainge, (1986) that more than 20,000 species of field and storage pests destroy approximately one-third of the world's food production, valued annually at more than $100 billion among which the highest losses (43%) occurring in the developing world [30]. The present investigation was carried out against *C. chinensis*, *S. oryzae* and *T. castaneum* to yield promising repellency and insecticidal activity as all the three insects are stored product pests and they cause a huge damage in stored products and ultimately cause economic damage. Moreno & Racelis, (2015) finds out that repellency is the system tends to dissuade pests away from a susceptible crop (repellent) what can be called a push approach and our findings in controlling these pests gets support from it [31]. Thus, plants are natural source of these repellent agents, reported in numerous ethnobotanical information. Ali *et al.* (2017) concluded that plant-derived repellents or insecticides do not pose hazards of toxicity to humans and domestic animals, and are easily biodegraded compared to synthetic compounds, natural products are presumed to be safer for humans [32]. The extracts of *S. indica* leaves can be used in the control of these stored product pests as the results of the investigation showed both repellency and mortality against the test insect pests. This study was attempted to highlight *S. indica* claimed to be used or associated with insect repellent and mortality activity, and it was found considerable. However, test result on other attributes also support the present finding, such as mortality and repellency for the extracts of *S. indica* against stored product pests.

5. Conclusion

Stored products cover a major portion of agricultural produces but several species of insects infest these in storage and causing a huge damage. Using plants with insecticidal properties is therefore an attractive alternative to save them in comparison to the more expensive synthetic pesticides. Various plants by-products have been dried recently with a good degree of success as protectants against a number of stored grain insect pests. The findings of the present study indicate the repellent and mortality effects of some extracts of *S. indica* on *C. chinensis*, *S. oryzae* and *T. castaneum.*

6. Acknowledgements

The authors are grateful to the University Grants Commission (UGC) of Bangladesh. They would like to extend thanks to the Chairman, Department of Zoology, University of Rajshahi, for providing laboratory facilities.

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