

# Toxic and Growth Inhibitory Effects of *Sapium indicum* Willd Seed Extracts on the Rust Red Flour Beetle, *Tribolium Castaneum* (Herbst) and the Confused Flour Beetle, *Tribolium Confusum* (du Val.)

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**Abstract:** The study was conducted to find out the toxic effect of different solvent extracts of *Sapium indicum* seed against mature larvae and adult of *Tribolium castaneum* and *Tribolium confusum*. Among this Acetone (Ac) extract was found to be the most effective toxicant against the larvae and adult of both the insect species. The LD<sub>50</sub> values have been calculated at 24, 48 and 72 hours after treatment (HAT). The values of Ac extract at 72 hours were 173.98 for *T. castaneum* and 193.32 µg/cm<sup>2</sup> for *T. confusum* larvae. The LD<sub>50</sub> values of the same extract for adult beetle at 72 hours were 74.34 and 155.90 µg/cm<sup>2</sup> for *T. castaneum* and *T. confusum* respectively. Depending on the toxic nature, Ac extract was used to assess the growth and development of the insect spp. All the treated food of the extract significantly reduced the wt. of larvae, pupae and adult of both the insect spp. The treatment also lengthened the developmental period; reduced pupal and adult recovery in comparison with the control. Ac extract proves to have good insecticidal value for suppression of population of these insect pests.

**Keywords:** *Sapium indicum* seed, *T. castaneum*, *T. confusum*, Contact toxicity, Growth and development.

## 1. Introduction

The Red flour beetle, *Tribolium. castaneum* (Herbst) and the confused flour beetle, *T. confusum* (du Val.) are worldwide in distribution and are common pests of several stored commodities. Both larvae and adults cause damage. Although these insects are incapable of feeding on sound grains, they inflict considerable damage to flour and flour products and also to grains damaged by other pests (Mamun *et al.*, 2009; Atwal, 1993). Hence any management strategy should be aimed at avoiding or reducing the infestation in storage.

Throughout history, plant products have been successfully exploited as insecticides, insect repellents and insect antifeedants (Dethier *et al.*, 1960; Schoonhoven, 1982; Mordue (Luntz), 1998). The insecticidal and acaricidal properties of a number of plants have been discovered long ago, and some of the plants can compete with synthetic means of control (Hedin and Holligworth, 1997). They degrade rapidly and therefore are considered safer to the environment than the synthetically produced fertilizers, pesticides and chemicals. Especially remarkable are tropical plants, from which hundreds of products of secondary metabolism with insecticidal properties have been extracted (Hiiesaar *et at.*, 2001; Parugrug and Roxas, 2008). Effectiveness of many plant derivatives against stored product insects has already been demonstrated (Miah *et al.*, 1996; Gupta *et al.*, 1988; Delobel and Melonga, 1987; Weaver *et al.*, 1991; Xie *et al.*, 1995; Mondal *et al.*, 1989; Pereira and Wohlgeuth, 1982; Mekanjuola, 1989). They are environmentally less harmful than synthetic pesticides

and act in many insects in different ways ( Schmutterer, 1990; Luik, 1994).

Pesticides derived from plants have the potential to play a major role in pest management in sustainable agriculture production. *Sapium indicum* Willd (Common name is Hurmui or Batul) is an evergreen tree found in the Sundarbans and Barishal district and it belongs to the family Euphorbiaceae. Fruits are used as a fish poison. Seeds contain a yellowish oil (57%) and unsaponing matter (0.62%). Fruits contain an aesculatin which has been isolated in a crystalline form. Two crystalline substances of the empirical formulae C<sub>21</sub>H<sub>36</sub>O<sub>3</sub> (a lactone) and C<sub>33</sub>H<sub>51</sub>O (an alcohol) have been isolated from the bark (Anon., 1972). The latex is reported to be poisonous and it causes blisters (Anon., 1972). The antifungal activity of *S. indicum* leaf has also been reported (Miah *et al.*, 1990). The research work was undertaken to determine the action of *S. indicum* seed extracts on the mortality and growth of *T. castaneum* and *T. confusum*, which are two important parameters for assessing the rate of infestation and control of the beetles.

## 2. Materials and Methods

### 2.1 Process of Extraction

*S. indicum* seeds were collected and dried in a well-ventilated room under shade and finally dried in an oven at 40 °C for 12 hours. Dried seeds were powdered in a grinding machine. Extractions were done serially with three solvents (Petroleum ether, Acetone and Methanol) for separating different compounds. Extracted liquids of different solvents

were dried in a rotary evaporator and then collected in small reagent bottles and preserved at 4 °C in a refrigerator.

## 2.2 Maintenance of Stock Culture and Preparation of Doses

Stock cultures of *T. castaneum* and *T. confusum* were maintained in plastic containers (1200 ml) and subcultures in beakers (1000 ml) with the food medium at 30°C ±0.5 °C in an incubator. A standard mixture of whole wheat flour with powdered dry yeast in a ratio of 19:1 was used as the food medium (Park, 1962). Residual film technique (Busvine, 1971) was used to test the mortality of larvae and adults of *T. castaneum* and *T. confusum*. The doses 78.60, 157.19, 314.38, 471.57 and 628.76 µg/cm<sup>2</sup> were used against both the larvae and adults. The doses were prepared by mixing the requisite quantities of extracted materials with 10 ml acetone or methanol. Methanol was used in case of methanolic extract because that extract does not dissolve properly in acetone.

## 2.3 Dose Mortality Experiment on Larvae and Adult

For each test dose 01 ml liquid was dropped on a petridish (9.5 cm dia.). After drying 40 larvae (16-day old) were released in each petridish with three replications for the insect species separately. A control batch was also maintained with the same number of insects having the solvent only. Similarly one-to two-week old adult beetles were used including the control (only solvent). The same number of adults were released in each petridish and the experiment was replicated thrice at 30°C ± 0.5 °C. The doses were calculated by measuring the weight of extracted materials (µg) in 01 ml of the solvent divided by the surface area of the petridish and converted into µg/cm<sup>2</sup>. Mortality was assessed after 24-48- and 72- hour post treatment. Mortality was corrected using Abbott's formula (Abbott, 1925) and LD<sub>50</sub> values were calculated by probit analysis (Busvine, 1971).

## 2.4 Preparation of Food Medium and Experimental Design

For another experiment adults of *T. castaneum* and *T. confusum* were collected from the stock culture and a large number of beetles were put on a thin film of wholemeal flour previously passed through a 60-mesh sieve in a beaker for egg collection. Eggs were collected on the following day and were incubated at 30°C ± 1.5 °C. To assess the growth and development of treated insects five different doses of acetone extract were used, viz. 1000, 2000, 4000, 8000 and 16000 ppm. The required quantities of the extracts were mixed with 15g of standard food and 15ml acetone for each dose. These were dried by fanning, kept in an incubator at 30°C for 24 hrs and then put in a blender for proper mixing. One hundred neonate larvae for each dose were collected with a fine camel hair brush and placed in a beaker (500 ml) containing fresh or treated medium and secured at the top with coarse cloth. Food was changed at 5-day intervals to avoid conditioning of the medium by the larvae. One hundred newly hatched larvae were maintained as the control on a medium treated with acetone only. The experiment was replicated thrice.

## 2.5 Assessment of Larval Growth and Development.

Growth of the larvae was assessed at 10 days and at maturity (16 days old). Ten days old larvae were collected by sieving, washed from adhered flour particles and were individually weighed on an electronic balance. Similarly mature larvae were collected and weighed. The larval period was noted and freshly formed pupae were weighed and counted, and placed in separate Petridish for adult emergence. The pupal period was recorded. Freshly eclosed adults were weighed and counted in each treatment. All the experiments were conducted at 30°C ±1 °C in an incubator. The co-efficient of variation (CV) and growth index (GI) of *T. castaneum* and *T. confusum* on different doses were calculated. The following formula was used for calculating the growth index (Saxena, 1969):

$$G.I. = \frac{\text{Adult recovery}\%}{\text{Total larval and pupal period}}$$

## 3. Results and Discussion

### 3.1 Contact Toxicity

The results of the contact toxicity of different solvent extracts *S. indicum* seed on *T. castaneum* and *T. confusum* larvae and adults are given in Table 1 and 2 and shown in Figs. 1 and 2. Mortality of the larvae and adults in both the species in different solvent extracts were observed. The AC extract was found to be the most toxic against the larvae and adults of both the insect species and the ME extract produced no mortality.

The present result is in conformity with the result of Rahman *et al.* (1999) who reported the highest repellency in acetone extract of Neem followed by *S. indicum* and *Curcuma longa* against *Sitophilus oryzae*. The results receive support from the result of Kamruzzaman *et al.* (2005) who reported a moderate repellent effect of *S. indicum* leaf and seed extract against the tenebrionid *Alphitobius diaperinus*.

### 3.2 Growth Inhibitory Effect

The mean weight of larvae, pupae and adults of *T. castaneum* and *T. confusum* treated with acetonic extract of *S. indicum* seed (SSE) are in Table 3 and illustrated in Fig. 3. Different doses of SSE extract significantly reduced the weight of 10day old and mature larvae; male and female pupae (P< 0.001) and adults of both the sexes (P< 0.01) of *T. castaneum*. With the increase in dose of SSE, reduction in the weight was increased at all the stages, the lowest and highest being observed at 16,000 ppm and 0 (control) respectively. Similarly SSE also reduced the weight of 10 day old larvae, mature larvae, male and female pupae and adults of both the sexes of *T. confusum* significantly (P< 0.001).

The effect of SSE on larval and pupal periods of *T. castaneum* and *T. confusum* are presented in Table 4 and shown in Fig. 4. It was observed that different doses of SSE prolonged the larval and pupal period of *T. castaneum* in comparison with the control (P< 0.001). Similarly, a prolonged larval period was observed in treated *T. confusum*

( $P < 0.001$ ) but the pupal period was not significantly affected. The highest and lowest developmental periods were recorded at 16,000 ppm and 0 (control) respectively.

The pupal recovery (%), adult emergence (%) and the growth index of the beetle were decreased with an increase in the doses of SSE in both the beetle species (Table 5 and Fig. 5). Mondal *et al.*, (1989) also reported a reduced larval weight in *T. castaneum* by *Nicotiana*, *Azadirachta*, *Datura* and *Vitex*. According to Mukherjee and Ramachandran (1989), Azadirachtin incorporated in wheat flour reduced the growth and survival of *T. castaneum* larvae. Jilani *et al.* (1988) also observed significant reduction in body weight of *T. castaneum* larvae, pupae and adults by turmeric, sweet flag and neem oil. Again, Khalequzzaman *et al.* (2004) reported prolonged larval and pupal periods of *T. castaneum* through the use of methanolic extract of the yellow oleander seed.

The high contact mortality and significantly decreased growth and developments of *T. castaneum* and *T. confusum* following treatments with the *S. indicum* seed extractives are very much to be solicited from the point of phytochemical control of the pests.

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**Table 1:** Toxicity of *Sapium indicum* Willd. seed extracts on *T. castaneum* and *T. confusum* larvae.

Species	Exposure period	Solvents used	LD <sub>50</sub> (mg/cm <sup>2</sup> )	Fiducially limits		Regression equation	χ <sup>2</sup> for hetero-geneity
				Lower	Upper		
<i>T. castaneum</i>	24 hours	*PE	—	—	—	—	—
		AC	361.13	327.24	389.53	Y= - 1.97 + 2.73X	2.53
		ME	—	—	—	—	—
	48 hours	PE	816.14	458.30	1453.39	Y= 1.17 + 1.32X	8.57
		AC	262.10	203.99	336.76	Y= - 1.28 + 2.60	18.26
		ME	—	—	—	—	—
	72 hours	PE	304.42	267.63	346.28	Y= 0.180 + 1.94X	7.36
		AC	1173.98	141.41	214.06	Y= - 0.048 + 2.25X	8.24
		ME	—	—	—	—	—
<i>T. confusum</i>	24 hours	PE	—	—	—	—	—
		AC	368.70	333.48	407.66	Y= - 1.93 + 2.70X	0.685
		ME	—	—	—	—	—
	48 hours	PE	823.30	596.50	1136.32	Y= 0.877 + 1.41X	3.58
		AC	293.70	262.73	328.32	Y= - 0.709 + 2.31X	5.90
		ME	—	—	—	—	—
	72 hours	PE	340.60	254.69	455.50	Y= 0.503 + 1.78X	12.33
		AC	190.32	153.76	235.58	Y= 0.269 + 2.08X	7.91
		ME	—	—	—	—	—

PE=Petroleum ether, AC=Acetone, ME=Methanol, HAT=Hours after treatment

**Table 2:** Toxicity of *Sapium indicum* Willd. seed extracts on *T. castaneum* and *T. confusum* adults.

Species	Exposure period	Solvents used	LD <sub>50</sub> (mg/cm <sup>2</sup> )	Fiducial limits		Regression equation	χ <sup>2</sup> for hetero-geneity
				Lower	Upper		
<i>T. castaneum</i>	24 hours	*PE	—	—	—	—	—
		AC	220.07	201.77	240.02	Y= - 2.87 + 3.36X	7.66
		ME	—	—	—	—	—
	48 hours	PE	—	—	—	—	—
		AC	170.29	143.96	201.44	Y= - 2.52 + 3.37X	9.63
		ME	—	—	—	—	—
	72 hours	PE	—	—	—	—	—
		AC	74.34	45.45	121.59	Y= 0.476 + 2.42X	18.60
		ME	—	—	—	—	—
<i>T. confusum</i>	24 hours	PE	—	—	—	—	—
		AC	320.29	257.67	398.13	Y= - 2.41 + 2.96X	16.70
		ME	—	—	—	—	—
	48 hours	PE	671.77	560.58	805.01	Y= - 1.45 + 2.28X	7.23
		AC	228.03	165.79	313.62	Y= - 1.87 + 2.91X	33.56
		ME	—	—	—	—	—
	72 hours	PE	501.32	393.16	639.23	Y= - 2.86 + 2.91X	14.74
		AC	155.90	112.97	215.13	Y= - 0.455 + 2.49X	21.13
		ME	—	—	—	—	—

PE=Petroleum ether, AC=Acetone, ME=Methanol, HAT=Hours after treatment



**Table 3:** Effect of *S. indicum* seed extracts on the growth of *Tribolium*, spp.

Species	Treatment (ppm)	Mean Weight ± S.E. (mg). (N=30)					
		10 days old larvae	Mature larvae	Pupae (male)	Pupae (female)	Adult (male)	Adult (female)
T. castaneum	0(Control)	1.05±0.04	2.44±0.05	2.36±0.03	2.49±0.03	1.90±0.06	2.02±0.06
	1000	0.56±0.03	2.06±0.03	2.23±0.04	2.41±0.02	1.82±0.04	1.91±0.04
	2000	0.51±0.02	1.98±0.04	2.13±0.06	2.35±0.05	1.74±0.04	1.85±0.04
	4000	0.45±0.03	1.87±0.04	2.07±0.04	2.28±0.03	1.67±0.05	1.78±0.04
	8000	0.38±0.04	1.78±0.04	1.98±0.04	2.23±0.03	1.62±0.04	1.73±0.06
	16000	0.32±0.04	1.58±0.04	1.89±0.06	2.15±0.04	1.56±0.03	1.66±0.06
	C.D. at	5% Level	0.106	0.131	0.142	0.112	0.144
	1% Level	0.150	0.183	0.199	0.157	0.202	0.218
T. confusum.	0(Control)	0.91±0.01	2.94±0.02	2.82±0.04	2.85±0.04	2.27±0.04	2.34±0.03
	1000	0.77±0.05	2.66±0.03	2.62±0.03	2.64±0.04	1.92±0.04	1.93±0.05
	2000	0.68±0.01	2.52±0.04	2.51±0.04	2.54±0.03	1.77±0.05	1.79±0.02
	4000	0.58±0.01	2.46±0.04	2.38±0.04	2.42±0.04	1.70±0.03	1.73±0.03
	8000	0.49±0.02	2.25±0.06	2.25±0.03	2.33±0.04	1.59±0.02	1.67±0.02
	16000	0.42±0.01	2.07±0.04	2.08±0.04	2.08±0.04	1.53±0.05	1.57±0.02
	C.D. at	5% Level	0.073	0.125	0.122	0.121	0.156
	1% Level	0.103	0.175	0.171	0.169	0.176	0.173

**Table 4:** Effect of *S. indicum* seed extracts on the duration of larval and pupal period of *Tribolium* spp. (days).

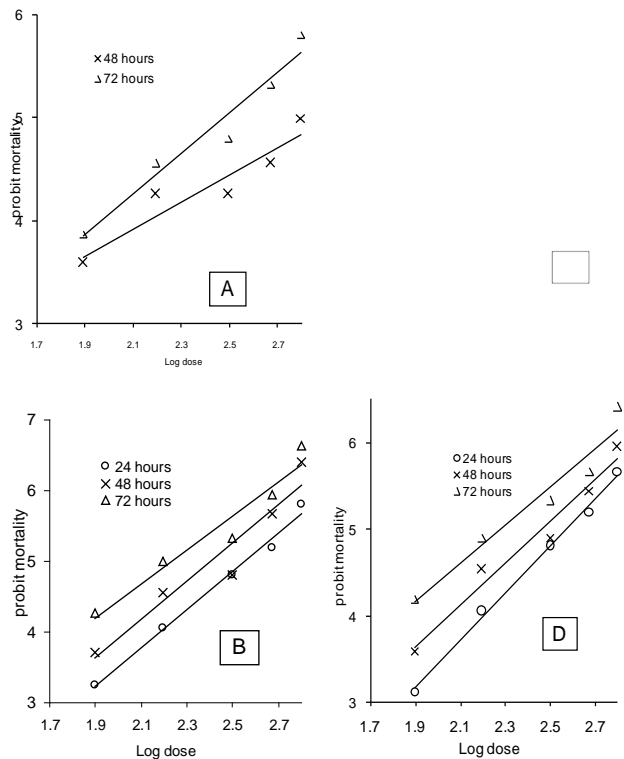
Species	Treatment (ppm)	Larval period				Pupal period			
		Total no.	Mean ± S.E	C. V. (%)	t-value	Total no.	Mean ± S.E	C. V. (%)	t-value
T. castaneum	0(Control)	278	20.10 ± 0.46	2.58	-	272	7.46 ± 0.08	1.89	-
	1000	240	21.92 ± 0.39	3.10	3.71*	235	8.50 ± 0.17	3.53	4.74**
	2000	229	22.23 ± 0.43	3.38	4.11*	227	8.68 ± 0.16	3.27	5.96**
	4000	223	23.38 ± 0.75	5.56	4.41*	211	8.78 ± 0.16	3.14	6.67**
	8000	185	23.87 ± 0.46	3.36	6.98**	182	8.90 ± 0.13	2.45	8.52**
	16000	157	26.68 ± 0.47	3.08	12.03**	155	9.18 ± 0.20	3.86	7.58**
T. confusum	0(Control)	280	19.36 ± 0.37	3.32	-	274	7.87 ± 0.41	9.01	-
	1000	230	22.03 ± 0.48	3.80	4.43*	205	8.23 ± 0.32	6.69	0.71 <sup>NS</sup>
	2000	200	23.82 ± 0.86	6.17	5.18**	185	8.43 ± 0.15	2.98	1.44 <sup>NS</sup>
	4000	188	27.99 ± 1.33	8.21	7.19**	175	8.60 ± 0.31	6.15	1.45 <sup>NS</sup>
	8000	182	29.62 ± 0.67	3.89	14.01**	152	8.73 ± 0.15	2.88	2.21 <sup>NS</sup>
	16000	156	30.34 ± 0.46	2.61	18.76**	148	9.00 ± 0.29	5.55	2.30 <sup>NS</sup>

\*, \*\* Significant at 5% and 1% respectively; NS-Not significant.

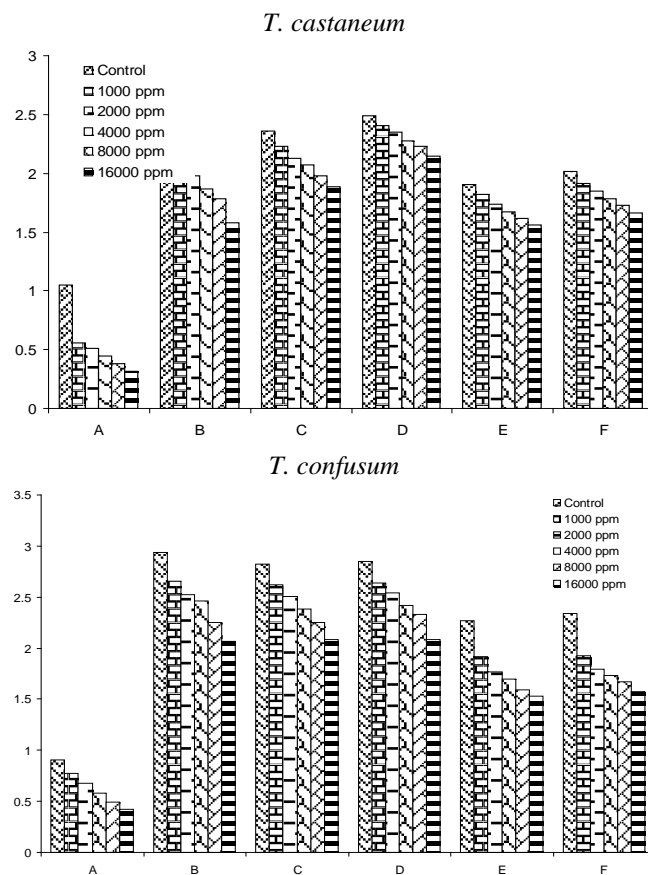
**Table 5:** Effect of *S. indicum* seed extracts on pupal recovery and adult eclosion of *Tribolium* spp.

Species	Treatment (ppm)	No. of larvae used	Pupation (%)	d-value	Adult emergence (%)	d-value	Growth index, G.I.
T. castaneum	0(Control)	300	278 (92.67)	-	272 (90.67)	-	3.27
	1000	300	240 (80)	4.60**	235 (78.33)	4.24**	2.57
	2000	300	229 (76.33)	4.85**	227 (75.67)	4.37**	2.45
	4000	300	223 (74.33)	5.21**	211 (70.33)	5.62**	2.19
	8000	300	185 (61.67)	8.22**	182 (60.67)	7.77**	1.80
	16000	300	157 (52.33)	10.02**	155 (51.67)	9.67**	1.44
T. confusum.	0(Control)	300	280 (93.33)	-	274 (91.33)	-	3.34
	1000	300	230(76.67)	5.88**	205(68.33)	7.32**	2.26
	2000	300	200(66.67)	7.29**	185(61.67)	7.63**	1.91
	4000	300	188(62.67)	7.86**	175(58.33)	8.25**	1.59
	8000	300	182(60.67)	8.23**	152(50.67)	10.02**	1.32
	16000	300	156(52.00)	10.25**	148(49.33)	10.28**	1.25

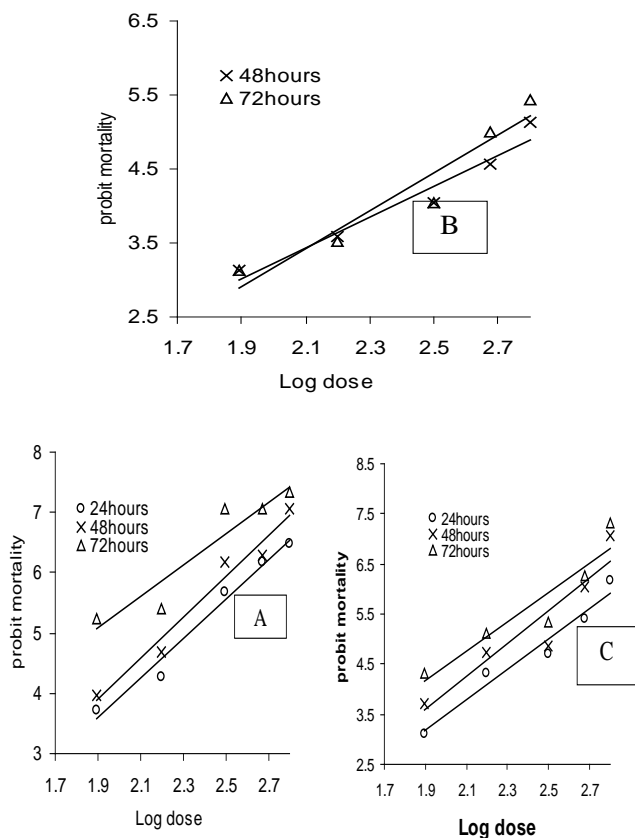
\*\* Significant at 1%; d = Standardized normal deviate



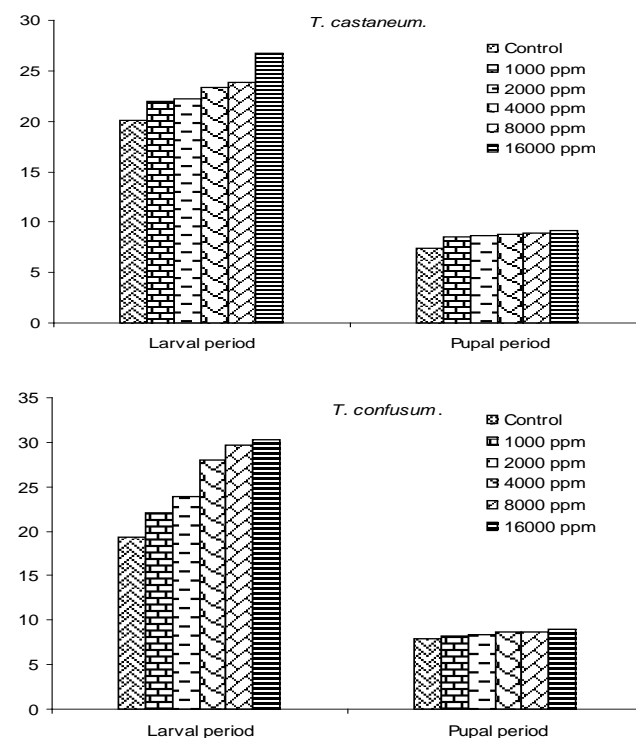
**Figure 1:** Regression lines of probit mortality on log doses of *S. indicum* seed extracts with different solvents on larvae of *T. castaneum* (A: Petroleum ether, B: Acetone) and *T. confusum* (C: Petroleum ether, D: Acetone).



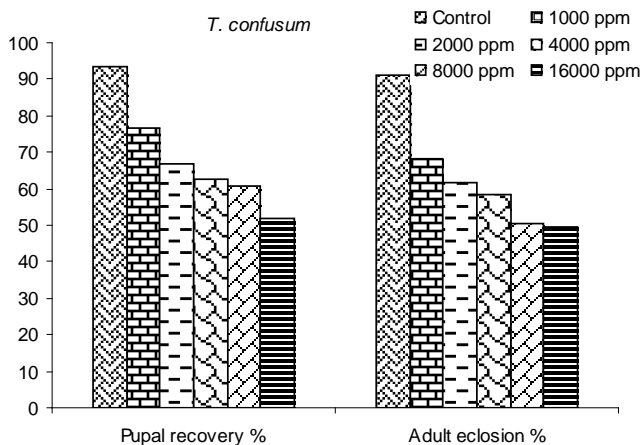
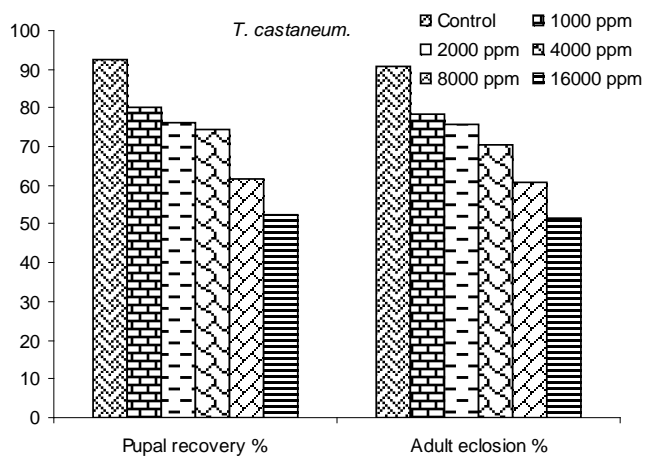
**Figure 3:** Effect of *S. indicum* seed extracts on the growth (weight) of *T. castaneum* and *T. confusum* (A: 10-day old larvae; B: mature leavae; C: male pupae; D: female pupae; E: male adult; F: female adult).



**Figure 2:** Regression lines of probit mortality on log doses of *S. indicum* seed extracts with different solvents on adults of *T. castaneum* (A: Acetone) and *T. confusum* (B: Petroleum ether, C: Acetone).



**Figure 4:** Effect of *S. indicum* seed extracts on the duration (days) of larval and pupal periods of *T. castaneum* and *T. confusum*.



**Figure 5:** Effect of *S. indicum* seed extracts on pupal recovery and adult eclosion (%) of *T. castaneum* and *T. confusum*.