



# Insecticidal potential of aqueous and solvent extracts of *Cassia fistula*, *Cleome viscosa* and *Capparis decidua* against *Callosobruchus chinensis* L. (Coleoptera: Bruchidae)

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## Abstract

In the present investigation different bioassays were conducted to evaluate insecticidal potential of *Cassia fistula*, *Cleome viscosa*, *Capparis decidua* extracts against the pulse beetle *Callosobruchus chinensis*. Various solvent extracts such as acetone, chloroform, petroleum ether, methanol, hexane and water were prepared and used to expose the insects. These have shown very high insecticidal potential as LD<sub>50</sub> obtained in each solvent extract was very low. In addition both solvent and aqueous extracts significantly repelled large no. of insects at a very low dose. When beetles were exposed with sub-lethal doses of above extracts it also significantly inhibited oviposition in *C. chinensis* as the % ODI (oviposition deterrence index) was obtained very high i.e. 56.31% to 85.51%. The study showed that solvent extracts of these plants showed 90-100% mortality that may be due to presence of toxic components in above plant species which can be used to control insects if isolated and used in pure form.

**Key words:** *Capparis deciduas*, *Cassia fistula*, *Cleome viscosa*, *Callosobruchus chinensis*, Oviposition inhibition.

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## 1. Introduction

The pulse beetle *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) is a highly destructive insect pest of stored legumes. It infests food grains in godowns and makes major losses to food grain quality. During rainy season their fourth instar larva become highly active and causes heavy infestation to legume seeds. For control of *Callosobruchus chinensis* different synthetic pesticides were used in store houses, but these chemicals have shown very good results in beginning later on ceased to show toxic effects. Later on, insects have acquired wider resistance and start resurging in large numbers. Hence, new safe alternatives of these synthetic pesticides were explored in form of bio-organic pesticides. These are proved environmentally much safer than synthetic pesticides. However, natural plant products [1, 2] such as essential oils [3] and bio-organic compounds [4] were found to be much safer and toxic to control insect pests. These have shown very high mortality in stored grain pests [5] and efficiently control grain damage and seed weight loss [6]. However, plant species selected for study possess very high insecticidal activity and belong to different families. *Cassia fistula* commonly known as Amaltash belongs to family Caesalpiniaceae [7]. It is tree grown as ornamental. The pulp of fruit is used as a purgative and laxative. It's flowers are yellow in colour and are used as bile protective and used as stomach and skin ailments.

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Seeds are used against nematode worms. *Cleome viscosa* Linn. belongs to family Capparidaceae. It is a common rainy season herb with sticky shoot and yellow flowers. The seeds are used in curries. The seeds and leaves are also medicinally used. The seeds are stimulant and carminative.

*Capparis decidua* belongs to the family Capparidaceae and is an indigenous medicinal plant, commonly known as 'Kureel' in Hindi. It is a densely branching shrub with scanty, small, caduceus leaves. Barks, leaves and roots of *C. decidua* have been claimed to relieve variety of ailments such as toothache, cough, asthma, intermittent fever and rheumatism [8]. Extracts and pure compounds isolated from *C. deciduas* were found more effective against drug resistant human pathogenic bacterial strains [9]. From the above plant species both solvent and aqueous extracts prepared and tested against *C. chinensis* to control its infestation in the field as well as in laboratory. For this purpose, various bioassays were conducted to assess insecticidal, repellent and oviposition inhibitory action of plant species were determined at different doses and exposure periods in stored product beetle *C. chinensis*.

## 2. Material and Methods

### 2.1. Insect rearing

Adult insects of *Callosobruchus chinensis* were collected from the food grain store houses available in local market in Gorakhpur. The beetles were reared on healthy and clean gram (*Cicer aromaticum*) seeds in glass jars. One thousand insects were released in 1 kg of gram seeds capped with muslin cloth for ventilation. Culture was maintained in laboratory under controlled temperature ( $28 \pm 2$  C), relative humidity ( $75 \pm 5$  % RH) and a photoperiod of 12: 12 (L:D) h in B.O.D. Insects were reared in glass jars on gram seeds and each time early age beetles were used for the experiments.

### 2.2. Preparation of extract

Stems of *Capparis decidua* were collected from different places of western part of India especially from state of Rajasthan, while *Cassia fistula* pods and *Cleome viscosa* seeds were collected from the botanical garden of Deen Dayal Upadhyay Gorakhpur University, Uttar Pradesh, India. Specimens were identified by applying standard taxonomic key specially by observing inflorescence and family formula with the help of a taxonomic expert. Fresh plant material was used to prepare extracts. Plant material was dried, chopped, grounded and milled to make powder in domestic grinder. The seeds of *Cleome viscosa* capsules (fruits) and legumes of *Cassia fistula* were collected from the University campus, dried in shade and milled to make powder in an electric grinder. While stem of *C. decidua* was collected and chopped in to small pieces, dried and pulverized to make fine powder in an electric grinder. The powdered stem (200 g) was then extracted with various solvent (800 ml for each) according to their polarity. While seed (200 g) and legume powder (200 g) was used to make extract according to the same method. Extracts were allowed to evaporate in a SpeedVac vacuum concentrators to get residue. It was dried and weighed and re-dissolved in known volume of different solvents. Dissolved residues were stored in cold at 4°C for experimental purpose.

### 2.3. Dose-response determination

Adults of *C. chinensis* were exposed with various increasing concentrations of each plant extracts separately. For this purpose, separate filter paper strips ( $1 \text{ cm}^2$ ) were coated with different concentrations of plant extracts and placed in the glass culture tubes and open ends were plugged with cotton balls. The coated filter paper strips were air-dried before application. Only solvent treated filter paper strips were strips used to set control. Ten adult insects were released culture in glass culture tubes (10 cm Height  $\times$  4 cm diameter). For each extract, five different concentrations were used and for each concentration six replicates were set. Mortality in *C. chinensis* was recorded after 24 hr in presence and absence of various plants extracts separately. For observation of feeding inhibition responses in insects known volume of each plant extract was coated on Whatman filter paper strips ( $42 \mu$  micron,  $1 \text{ cm}^2$ ), and placed in a tri-arm repellency apparatus in the centre from open side of one arm. Ten adult beetles were released inside this arm with the help of aspirator tube and plugged with cotton. Gram seeds (20 in number) were kept inside from

remaining open sides of the two arms. Number of repelled insects in presence of each plant extract were counted after 30 min and five different concentrations (1.0, 2.0, 4.0, 8.0 and  $16.0 \mu\text{l}$ ) of each plant were used. For determination of oviposition deterrence in *C. chinensis* sub lethal concentrations (20, 40 and 60% of  $\text{LD}_{50}$ ) of each plant extract were coated separately on filter paper strips ( $1 \times 1 \text{ cm}$ ) and provided to insects. Six replicates were set for each plant extract. The number of eggs laid recorded after 96 hrs and %ODI (Oviposition Deterrence Index) was calculated.

### 2.4. Statistical analysis

$\text{LD}_{50}$  values of each solvent and aqueous extracts were calculated by applying POLO programme [10]. The efficacy of the test stimuli was compared with control on the basis of oviposition deterrence index (ODI). The %ODI of females was calculated as  $100 (A - B)/(A + B)$ , A and B being the number of eggs in the control and test, respectively. Repellency in various plant extracts was calculated on the basis of insects repelled in presence of each extract. Data was analyzed to have mean  $\pm$  SE of each concentration used to deter feeding in beetles.

## 3. Results

All the extracts isolated from *C. viscosa* and *C. fistula* have shown very low  $\text{LD}_{50}$  values i.e. 4.60, 2.56, 1.85, 5.61, 0.97 and  $0.414 \mu\text{g}/\text{mg}$  in acetone, chloroform, petroleum ether, methanol, hexane and water extracts, respectively in *Cleome viscosa*. Its upper and lower confidence limits were found between 9.10-2.75, 6.47-1.369, 9.28-0.822, 20.95-2.65, 1.94-0.58 and 0.75-0.24 for the above extracts (Table 1). Contrary to this, both solvent and aqueous extracts from *Cassia fistula* were found more toxic to *C. chinensis* have shown lower  $\text{LD}_{50}$  values in comparison to *Cleome viscosa*.  $\text{LD}_{50}$  values obtained in solvent and aqueous extracts of *Cassia fistula* were 0.99, 0.49, 0.39, 0.51, 0.16 and  $2.57 \mu\text{g}/\text{mg}$  for acetone, chloroform, petroleum ether, methanol, hexane and water extracts, respectively. The upper and lower confidence limits were found in the range of 2.16-0.59, 1.65-0.25, 2.09-0.17, 0.83-0.35, 0.31-0.08 and  $4.01-1.84 \mu\text{g}/\text{mg}$  in *Cassia fistula* extract (Table- 1). Similarly *Capparis decidua* extract have shown very high insecticidal activity. Extracts of *C. decidua* have shown 0.44, 0.63, 0.78, 1.34, 0.43 and  $0.55 \mu\text{g}/\text{mg}$   $\text{LD}_{50}$  values in acetone, chloroform, petroleum ether, methanol, hexane and water extracts, respectively. The upper and lower confidence limits were found in a range of 0.81-0.25, 1.10-0.37, 1.22-0.55, 2.023-0.96, 0.67-0.31 and 1.84-0.29 (Table- 1). Hexane extract of *C. fistula* showed least  $\text{LD}_{50}$  value i.e.  $0.16 \mu\text{g}/\text{gm}$  body weight. Contrary to this *Capparis decidua* aqueous extracts has shown  $\text{LD}_{50}$  value  $0.55 \mu\text{g}/\text{gm}$  body weight (Table 1).

Same extract have also shown very high antifedent activity. *Cleome viscosa* and *Cassia fistula* solvent extracts have shown very high repellency i.e. 66-80% on *C. chinensis* at a very low concentration (Table 2a, 2b). Aqueous extracts of *Cassia fistula* have also shown 80% repellency at  $0.80 \mu\text{g}$  concentrations (Table 2b). Besides this, both solvent and aqueous extracts of *C. decidua* have shown very good repellency (Table 2c). Maximum repellency was obtained in petroleum ether and methanol of same extract i.e. 83.33% while acetone,

**Table 1.** LD<sub>50</sub> of different extracts of *Cleome viscosa* *Cassia fistula* and *Capparis decidua* against *C. chinensis*

Extracts	hr	LD <sub>50</sub> Values (µg/gm) (p<0.05)	LCL	UCL	t-ratio	Slope Value	Heterogeneity	Chi-test
<i>Cleome viscosa</i>								
Acetone	24	4.6	2.75	9.10	8.315	2.075	2.389	11.945
Chloroform	24	2.56	1.369	6.47	8.17	1.77	2.929	14.646
Petroleum ether	24	1.85	0.822	9.28	6.96	1.264	3.01	15.07
Methanol	24	5.61	2.65	20.95	7.28	1.458	3.14	15.728
Hexane	24	0.969	0.585	1.946	8.11	1.865	2.06	10.308
Water	24	0.414	0.237	0.755	4.995	1.753	2.19	10.97
<i>Cassia fistula</i>								
Acetone	24	0.99	0.599	2.168	6.74	1.253	1.087	5.434
Chloroform	24	0.490	0.255	1.658	6.46	1.154	1.659	8.29
Petroleum ether	24	0.391	0.172	2.099	7.59	1.486	3.836	19.18
Methanol	24	0.510	0.356	0.831	6.88	1.327	0.32	3.162
Hexane	24	0.161	0.088	0.310	8.936	2.034	2.98	14.916
Water	24	2.578	1.84	4.017	7.131	1.45	0.491	2.457
<i>Capparis decidua</i>								
Acetone	24	0.441	0.254	0.810	8.973	2.145	2.739	13.696
Chloroform	24	0.632	0.374	1.10	8.84	2.03	2.323	11.615
Petroleum ether	24	0.782	0.551	1.220	7.146	1.298	0.823	4.115
Methanol	24	1.34	0.961	2.023	7.299	1.381	0.437	2.187
Hexane	24	0.439	0.313	0.679	7.259	1.382	0.999	4.994
Water	24	0.551	0.290	1.845	6.678	1.218	1.68	8.40

<sup>a</sup> LD50 values represents lethal dose that cause 50% mortality in the test insects. <sup>b</sup>LCL and UCL mean lower confidence limit and upper confidence limit respectively. <sup>c</sup> t- ratio, slope-value and heterogeneity were significant at all probability levels (90, 95 and 99%). t-ratio, difference in degree of freedom at 0.5, 0.05 and 0.005 levels; slope-value shows the average between LD<sub>50</sub> and LD<sub>80</sub>, from which LD<sub>50</sub> value is calculated; and heterogeneity value, shows the effect of active extraction both susceptible and tolerant insects among all of the treated insects.

**Table 2a.** Repellency of various extracts prepared from *Cleome viscosa* against the Pulse beetle, *Callosobruchus chinensis*

Extract	Dose (µg)	% repellency	Mean ±SE	Variance	F-Test
Acetone	0.125	23.33	2.3±0.33	18.709	0.0416
	0.250	36.66	3.6±0.33		
	0.500	46.66	4.6±0.33		
	1.0	60.00	6.0±0.00		
	2.0	70.00	7.0±0.00		
Chloroform	0.075	30.00	3.0±0.57	18.911	0.0741
	0.150	33.33	3.3±0.33		
	0.30	53.33	5.3±0.88		
	0.60	70.00	7.0±0.00		
	1.20	80.00	8.0±0.00		
Petroleum ether	0.05	33.33	3.3±0.33	18.332	0.0271
	0.10	30.00	3.0±1.00		
	0.20	46.66	4.6±0.33		
	0.40	63.33	6.3±0.88		
	0.80	66.66	6.6±0.33		
Methanol	0.126	23.33	2.3±0.88	18.641	0.0361
	0.256	40.00	4.0±1.15		
	0.504	40.00	4.0±0.0		
	1.008	66.66	6.6±0.33		
	2.016	63.33	6.3±0.33		
Hexane	0.025	26.66	2.6±0.33	18.988	0.0398
	0.050	26.66	2.6±0.88		
	0.100	50.00	5.0±0.57		
	0.200	46.00	4.6±1.20		
	0.400	70.00	7.0±0.57		
Water	0.025	6.00	0.6±0.33	22.004	0.0017
	0.050	16.00	1.6±0.33		
	0.10	23.00	2.3±0.33		
	0.20	26.66	2.6±0.33		
	0.40	33.33	2.3±0.33		

\*Two choice repellency bioassay was performed for each fraction. \*\* Repellency for each plant extract was tested three times for each concentration

**Table 2b.** Repellency of various solvent and aqueous extracts of *Cassia fistula* against the Pulse beetle, *Callosobruchus chinensis*.

Extract	Dose ( $\mu\text{g}$ )	% repellency	Mean $\pm$ SE	Variance	F-Test
Acetone	0.02	23.33	2.3 $\pm$ 0.33	19.525	0.0595
	0.04	30.00	3.0 $\pm$ 0.57		
	0.08	36.66	3.6 $\pm$ 0.33		
	0.160	46.66	4.6 $\pm$ 0.33		
	0.032	76.66	7.6 $\pm$ 0.33		
Chloroform	0.01	30.00	3.0 $\pm$ 1.52	18.798	0.0287
	0.02	30.00	3.0 $\pm$ 0.57		
	0.04	33.33	3.3 $\pm$ 0.33		
	0.08	56.66	5.6 $\pm$ 0.33		
	0.160	66.66	6.6 $\pm$ 0.33		
Petroleum ether	0.01	30.00	3.0 $\pm$ 0.57	18.430	0.0438
	0.02	36.66	3.6 $\pm$ 0.33		
	0.04	53.33	5.3 $\pm$ 0.33		
	0.08	63.33	6.3 $\pm$ 0.33		
	0.160	76.66	7.6 $\pm$ 0.33		
Methanol	0.01	16.66	1.6 $\pm$ 0.33	20.857	0.153
	0.02	23.33	2.3 $\pm$ 0.88		
	0.04	33.33	3.3 $\pm$ 0.88		
	0.08	70.00	7.0 $\pm$ 0.57		
	0.160	76.66	7.6 $\pm$ 0.33		
Hexane	0.01	30.00	3.0 $\pm$ 0.57	18.913	0.0607
	0.02	30.00	3.0 $\pm$ 1.52		
	0.04	50.00	5.0 $\pm$ 2.08		
	0.08	56.66	5.6 $\pm$ 1.45		
	0.160	80.00	8.0 $\pm$ 0.57		
Water	0.05	16.66	1.6 $\pm$ 0.33	19.378	0.0957
	0.10	23.33	2.3 $\pm$ 0.33		
	0.20	40.00	4.0 $\pm$ 0.57		
	0.40	60.00	6.0 $\pm$ 0.57		
	0.80	80.00	8.0 $\pm$ 0.57		

\*Two choice repellency bioassay was performed for each fraction. \*\* Repellency for each plant extract was tested three times for each concentration

**Table 2c.** Repellency of various solvent and aqueous extracts of *Capparis decidua* against the Pulse beetle, *Callosobruchus chinensis*

Extract	Dose ( $\mu\text{g}$ )	% repellency	Mean $\pm$ SE	Variance	F-Test
Acetone	0.025	23.34	2.3 $\pm$ 0.33	18.981	0.0424
	0.050	26.67	2.6 $\pm$ 0.33		
	0.10	53.34	5.3 $\pm$ 1.45		
	0.20	53.34	5.3 $\pm$ 0.33		
	0.40	66.67	6.6 $\pm$ 0.33		
Chloroform	0.04	20.00	2.0 $\pm$ 0.57	18.693	0.0416
	0.08	40.00	4.0 $\pm$ 1.52		
	0.16	50.00	5.0 $\pm$ 1.15		
	0.32	56.67	5.6 $\pm$ 0.88		
	0.64	70.00	7.0 $\pm$ 0.57		
Petroleum ether	0.02	20.00	2.0 $\pm$ 0.57	19.849	0.110
	0.04	30.00	3.0 $\pm$ 0.57		
	0.08	46.67	4.6 $\pm$ 0.33		
	0.160	60.00	6.0 $\pm$ 0.57		
	0.032	83.33	8.3 $\pm$ 0.33		
Methanol	0.035	40.00	4.0 $\pm$ 1.15	19.267	0.0974
	0.070	30.00	3.0 $\pm$ 0.57		
	0.140	40.00	4.0 $\pm$ 0.57		
	0.280	76.67	7.6 $\pm$ 0.33		
	0.560	83.33	8.3 $\pm$ 0.33		
Hexane	0.01	13.33	1.3 $\pm$ 0.33	20.606	0.105
	0.02	23.33	2.3 $\pm$ 0.33		
	0.04	33.33	3.3 $\pm$ 0.66		
	0.08	56.67	5.6 $\pm$ 0.88		
	0.160	73.33	7.3 $\pm$ 0.33		
Water	0.01	26.67	2.6 $\pm$ 0.33	19.378	0.0957
	0.02	30.00	3.0 $\pm$ 0.57		
	0.04	50.00	5.0 $\pm$ 0.57		
	0.08	80.00	8.0 $\pm$ 0.57		
	0.160	70.00	7.0 $\pm$ 0.57		

\*Two choice repellency bioassay was performed for each fraction. \*\* Repellency for each plant extract was tested three times for each concentration.

**Table 3a.** Efficacy of various solvent extracts of *Cleome viscosa* on oviposition behavior of *Callosobruchus chinensis*

Extract used	Dose applied	Mean no. of eggs laid per insect Mean $\pm$ SE	% eggs laid per insect Mean $\pm$ SE	%ODI <sup>B</sup>	F-value <sup>C</sup> At df 1 and 7
<i>Cleome viscosa</i>					
Acetone	20% of LD <sub>50</sub>	17.70 $\pm$ 0.378	72.24 $\pm$ 1.555	15.77	34.51
	40% of LD <sub>50</sub>	3.70 $\pm$ 0.288	15.20 $\pm$ 1.186	73.59	
	60% of LD <sub>50</sub>	1.90 $\pm$ 0.173	7.80 $\pm$ 0.713	85.51	
Chloroform	20% of LD <sub>50</sub>	15.33 $\pm$ 0.466	63.00 $\pm$ 1.919	22.69	11.41
	40% of LD <sub>50</sub>	7.30 $\pm$ 0.230	30.00 $\pm$ 0.946	53.84	
	60% of LD <sub>50</sub>	1.63 $\pm$ 0.405	6.69 $\pm$ 1.168	87.44	
Petroleum ether	20% of LD <sub>50</sub>	15.33 $\pm$ 0.548	63.00 $\pm$ 2.255	22.69	71.47
	40% of LD <sub>50</sub>	8.70 $\pm$ 0.288	35.75 $\pm$ 1.186	47.32	
	60% of LD <sub>50</sub>	6.30 $\pm$ 0.346	26.89 $\pm$ 1.426	58.06	
Methanol	20% of LD <sub>50</sub>	18.46 $\pm$ 0.463	75.87 $\pm$ 1.904	13.71	20.75
	40% of LD <sub>50</sub>	13.53 $\pm$ 0.480	55.61 $\pm$ 1.977	28.52	
	60% of LD <sub>50</sub>	13.00 $\pm$ 0.665	53.43 $\pm$ 2.735	30.35	
Hexane	20% of LD <sub>50</sub>	20.03 $\pm$ 0.611	82.32 $\pm$ 2.514	9.69	60.90
	40% of LD <sub>50</sub>	16.16 $\pm$ 0.348	66.42 $\pm$ 1.432	20.15	
	60% of LD <sub>50</sub>	2.03 $\pm$ 0.176	8.34 $\pm$ 0.726	84.59	
Water	20% of LD <sub>50</sub>	22.86 $\pm$ 0.523	93.95 $\pm$ 2.153	3.11	349.20
	40% of LD <sub>50</sub>	13.16 $\pm$ 0.260	54.08 $\pm$ 1.070	29.79	
	60% of LD <sub>50</sub>	6.46 $\pm$ 0.600	26.55 $\pm$ 2.469	58.03	

<sup>A</sup>The chemical stimulus was coated on the Whatmann filter paper stripes (1 cm<sup>2</sup>) in the oviposition inhibition test. <sup>B</sup> the ODI% was calculated as 100(A-B)/ A+B, where A and B represent the number of eggs laid in the control and in the test respectively. <sup>C</sup> F-values were significant at all probability levels (90, 95 and 99%).

**Table 3b.** Efficacy of various solvent extracts of *Cassia fistula* on oviposition behavior of *Callosobruchus chinensis*

Extract used	Dose applied	Mean no. of eggs laid per insect Mean $\pm$ SE	% eggs laid per insect Mean $\pm$ SE	%ODI B	F-value C At df 1 and 7
Acetone	20% of LD <sub>50</sub>	19.60 $\pm$ 0.378	80.55 $\pm$ 1.554	10.76	116.73
	40% of LD <sub>50</sub>	15.70 $\pm$ 0.692	64.52 $\pm$ 2.849	21.55	
	60% of LD <sub>50</sub>	9.83 $\pm$ 0.696	40.41 $\pm$ 2.862	42.44	
Chloroform	20% of LD <sub>50</sub>	18.20 $\pm$ 0.513	74.80 $\pm$ 2.109	14.41	80.46
	40% of LD <sub>50</sub>	10.30 $\pm$ 0.585	42.33 $\pm$ 2.408	40.51	
	60% of LD <sub>50</sub>	7.06 $\pm$ 0.523	29.04 $\pm$ 2.153	55.02	
Petroleum ether	20% of LD <sub>50</sub>	14.36 $\pm$ 0.44	59.04 $\pm$ 1.813	25.76	21.53
	40% of LD <sub>50</sub>	9.50 $\pm$ 0.680	39.04 $\pm$ 2.796	43.83	
	60% of LD <sub>50</sub>	8.70 $\pm$ 0.757	35.75 $\pm$ 3.113	47.32	
Methanol	20% of LD <sub>50</sub>	7.43 $\pm$ 0.896	30.54 $\pm$ 3.686	53.76	14.46
	40% of LD <sub>50</sub>	5.50 $\pm$ 0.680	22.60 $\pm$ 2.796	63.12	
	60% of LD <sub>50</sub>	4.10 $\pm$ 0.230	16.84 $\pm$ 0.949	71.15	
Hexane	20% of LD <sub>50</sub>	16.70 $\pm$ 0.529	68.63 $\pm$ 2.175	18.59	236.41
	40% of LD <sub>50</sub>	8.13 $\pm$ 0.348	33.42 $\pm$ 1.429	49.90	
	60% of LD <sub>50</sub>	2.53 $\pm$ 0.592	10.40 $\pm$ 2.435	81.16	
Water	20% of LD <sub>50</sub>	15.53 $\pm$ 0.721	63.84 $\pm$ 2.968	22.07	23.91
	40% of LD <sub>50</sub>	4.06 $\pm$ 0.417	16.70 $\pm$ 1.715	68.02	
	60% of LD <sub>50</sub>	3.43 $\pm$ 0.491	14.10 $\pm$ 2.018	75.28	

<sup>A</sup>The chemical stimulus was coated on the Whatmann filter paper stripes (1 cm<sup>2</sup>) in the oviposition inhibition test. <sup>B</sup> the ODI% was calculated as 100(A-B)/ A+B, where A and B represent the number of eggs laid in the control and in the test respectively. <sup>C</sup> F-values were significant at all probability levels (90, 95 and 99%).

**Table 3c.** Efficacy of various solvent extracts of *Capparis decidua* on oviposition behavior of *Callosobruchus chinensis*

Extract used	Dose applied	Mean no. of eggs laid per insect Mean $\pm$ SE	% eggs laid per insect Mean $\pm$ SE	%ODI B	F-value C At df 1 and 7
Acetone	20% of LD <sub>50</sub>	10.30 $\pm$ 0.346	42.33 $\pm$ 1.426	40.51	106.18
	40% of LD <sub>50</sub>	7.40 $\pm$ 0.585	30.41 $\pm$ 2.408	53.35	
	60% of LD <sub>50</sub>	4.33 $\pm$ 0.348	17.80 $\pm$ 1.429	69.78	
Chloroform	20% of LD <sub>50</sub>	4.33 $\pm$ 0.529	39.04 $\pm$ 2.175	43.83	8.58
	40% of LD <sub>50</sub>	8.80 $\pm$ 0.378	36.16 $\pm$ 1.555	46.87	
	60% of LD <sub>50</sub>	7.86 $\pm$ 0.960	27.94 $\pm$ 3.951	56.31	
Petroleum ether	20% of LD <sub>50</sub>	5.50 $\pm$ 0.642	22.60 $\pm$ 2.641	63.12	6.29
	40% of LD <sub>50</sub>	4.03 $\pm$ 0.405	16.57 $\pm$ 1.665	71.57	
	60% of LD <sub>50</sub>	2.93 $\pm$ 0.433	12.05 $\pm$ 1.779	78.50	
Methanol	20% of LD <sub>50</sub>	10.96 $\pm$ 0.463	45.07 $\pm$ 1.901	37.88	191.49
	40% of LD <sub>50</sub>	6.46 $\pm$ 0.545	26.57 $\pm$ 2.242	58.03	
	60% of LD <sub>50</sub>	2.43 $\pm$ 0.480	9.99 $\pm$ 1.976	81.83	
Hexane	20% of LD <sub>50</sub>	9.67 $\pm$ 0.520	40.13 $\pm$ 2.137	42.73	66.22
	40% of LD <sub>50</sub>	6.43 $\pm$ 0.409	26.43 $\pm$ 1.685	58.30	
	60% of LD <sub>50</sub>	4.46 $\pm$ 0.384	18.35 $\pm$ 1.581	69.16	
Water	20% of LD <sub>50</sub>	17.46 $\pm$ 0.643	71.78 $\pm$ 2.646	16.43	40.58
	40% of LD <sub>50</sub>	7.46 $\pm$ 0.284	30.68 $\pm$ 1.167	53.06	
	60% of LD <sub>50</sub>	5.46 $\pm$ 0.617	22.46 $\pm$ 2.536	63.34	

<sup>A</sup>The chemical stimulus was coated on the Whatmann filter paper stripes (1 cm<sup>2</sup>) in the oviposition inhibition test. <sup>B</sup> the ODI% was calculated as 100(A-B)/ A+B, where A and B represent the number of eggs laid in the control and in the test respectively. <sup>C</sup> F-values were significant at all probability levels (90, 95 and 99%).

chloroform, hexane and aqueous extracts have shown more than 67% repellency at higher dose. It was also found that the percent repellency was dose and time dependent (Table 2a-2c). Besides toxic and repellent action solvent and aqueous extracts of all three plant species exhibited very high oviposition inhibitory activity (Table 3a-3c). However, 60% of LD<sub>50</sub> of petroleum ether and methanol extracts have shown 78.50 and 81.83% oviposition deterrence in *C. chinensis*, while 60% of LD<sub>50</sub> of acetone, chloroform and hexane extract of *Cleome viscosa* have shown 85.51, 87.44 and 84.59 %ODI, respectively. Methanol, hexane and water extract of *Cassia fistula* have shown higher % ODI i.e. 71.15, 81.16 and 75.28 at 60% of LD<sub>50</sub> (Table 3b).

#### 4. Discussion

Results obtained in the present investigation clearly demonstrate that both solvent and aqueous extracts of *Cleome viscosa*, *C. fistula* and *C. decidua* are highly toxic to *C. chinensis* as each extract exhibited very high mortality in *C. chinensis*. Each extract has shown very low LD<sub>50</sub> value. However, maximum toxicity was obtained in water extract of *C. viscosa* i.e. 0.41µg/mg, while its hexane extract has shown 0.96µg/gm LD<sub>50</sub> value. Similarly solvent extracts of *C. fistula* have shown LD<sub>50</sub> in a range of 0.161-2.578µg/gm while aqueous extracts 2.57µg/gm (Table 1). Similar trends of toxic potential was obtained in *C. decidua* as all the solvent extracts have shown LD<sub>50</sub> value in the range of 0.43-1.34µg/gm. Its water extract has shown LD<sub>50</sub> value 0.55µg/gm (Table 1). *Artemisia princepi* and *Cinnamomum camphora* (L) have shown insecticidal and repellent activity against *Sitophilus oryzae* and *Bruchus rugimanus* [11] while *Melia dubia* has shown growth inhibitory and antifedent activity against *Spodoptera litura* and *Helicoverpa armigera* larvae [2]. Similar results were obtained in chemical constituents of *Foeniculum vulgare* [12] and Japanese mint (*Mentha arvensis*) [13]. *Azadirachta indica* against adults of *S. oryzae*, *T. castenum* and *Rhizopertha dominica* (F) [14].

Similarly both solvent and aqueous extracts from *C. viscosa*, *C. fistula* and *C. decidua* extracts have shown enormous mortality and very high repellency in *C. chinensis* adults (Table 2a-2c). Maximum percent repellency was obtained in chloroform extract of *C. viscosa* i.e. 80% while hexane and water extract of *C. fistula* at 1.20 µg/gm dose have shown 80% repellency. *C. decidua* petroleum ether and methanol extracts have shown 83.0% repellency while its water extract have shown 80% repellency at 0.32 µg concentration. In bio-assays when adults of *C. chinensis* were exposed to sublethal doses of different extracts significantly repelled large number of insects in comparison to control (Table 2a-2c). From the results the steep slope values obtained in mortality indicated that a small dose of plant extracts can kill large population of *C. chinensis*. These values fall within 95.0% confidence limit and thus the model fits the data adequately. Besides this, number of insects repelled and F-values calculated indicate that dose responses were worked well to repel significantly more number of insects at a very small dose. It shows presence of few active components present in various plants extracts. Similar insecticidal activity is reported in essential oils and its constituents against household [15, 16] and field crop insects [17, 18]. Few essential oil constituents such as d-limonene, linalool and terpenols [19, 20] and diallyl disulphides have shown potent toxic and feeding deterrent

activity against stored grain pests i.e. *S. oryzae* (L) and *Tribolium castenum* (Herbst) [21].

When adult beetles were exposed to sub-lethal dose of different plant extract, these significantly inhibited oviposition in insects and block the emergence of F<sub>1</sub> individuals from exposed eggs. Effect of solvent extracts of *C. viscosa*, *C. fistula* and *C. decidua* on oviposition behavior on *C. chinensis* is given in the Table 3a-3c. From the result it was found that toxicity, repellent and oviposition inhibition in *C. chinensis* were dose and time dependent. A slight increase in concentration of natural extract increased the percent mortality and oviposition inhibition in adult beetles. Similarly active ingredients isolated from certain botanicals have shown very high toxicity [22-24] and adversely affect fecundity, egg to adult survival and progeny production in *C. chinensis* [2, 25-26]. *Curcuma longa* and *Lippia alba* essential oils have shown similar oviposition inhibition and egg hatching suppression activity against *C. maculates*, *C. chinensis* and *T. castenum* [27]. The treatments with bio-insecticides also significantly cut down the grain damage and seed weight loss done by beetles in stored grains [6]. This activity is due to presence of some chemicals present in the various plant parts which successfully inhibit oviposition [28] and show wider repellent responses in beetles to deter them from feeding [29-32]. Besides this, ethyl formate was also found effective against stored grain insects when combines with carbon dioxide [33]. Similarly few pesticides such as (S)-hydroprene and cyfluthrin [34], acrolein vapors [35] and allyl acetate [36] as a fumigant were found effective against stored grain insects. Similarly heat treatment and high temperature exposure also showed very high mortality in pupae and adults of *Tribolium castaneum* [37].

#### 5. Conclusion

However, in insects oviposition is influenced by type of chemical, functional group occur in volatile natural product in stored conditions. On the basis of toxic and repellent action generated by fumigants these are considered as most effective method in which fumigant creates a poisonous atmosphere for the insect pest, which not only kill the adult insects but inhibit oviposition in susceptible insects and disallow emergence of F<sub>1</sub> individuals by blocking the development. Similarly, volatile oils evaporate to form poisonous environment in store houses and kill large number of stored grain insects. In the present study plant extracts have largely repelled beetles and killed them by contact poisoning. It was observed that toxicity of solvent extracts more efficiently acted upon both pest larvae and pupae of *C. chinensis*. Besides this, a significant reduction was observed in insect's fecundity, egg to adult survival and adult progeny production [35]. Further, toxic, repellent and oviposition inhibition response were found to be dose and time dependent. It was also proved by F- ratio obtained between dose and oviposition responses. Therefore, it can be concluded that above plant species can be used for isolation of bio-pesticides to control pulse beetles. For this purpose, constituent's level study along with structure activity relationships of natural products are to be required. Certainly active components from prepared plant species would show wider insecticidal performance and efficacy not only against *C. chinensis* but also against all other legume pests.

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