

**Ovipositional Responses of the Pulse Beetle, *Bruchus chinensis* (Coleoptera: Bruchidae) to Extracts and Compounds of *Capparis decidua***

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Extracts of *Capparis decidua* stems and flowers showed insecticidal and oviposition inhibitory activities against *Bruchus chinensis*. The LC<sub>50</sub> values of these extracts were found to increase with the increase in the polarity of the extract at different exposure periods. For instance, after 96 h, the LC<sub>50</sub> values were found to be 3.619, 7.319, and 10.151  $\mu\text{g}$  for CD1, CD2, and CD3, respectively. Extract CD7 was effective only at higher doses. The toxicity was found to be dose- and time-dependent. The females laid lesser number of eggs, when exposed to sublethal doses of different extracts and pure compounds, as compared to control. The maximum oviposition deterrence index was found for extract CD1 followed in decreasing order by CD2, CD3, and CD7. From extract CD1, two compounds were isolated and characterized as triacontanol (C1) and 2-carboxy-1,1-dimethylpyrrolidine (C2). When the females were exposed to sublethal doses of these compounds, they laid lesser number of eggs as compared to the control. C2 was found to have a slightly greater oviposition inhibition effect than C1. From fraction CD7, one novel compound labeled as CDF1 has been isolated and identified as 6-(1-hydroxy-non-3-enyl)tetrahydropyran-2-one. CDF1 has also shown insecticidal and oviposition inhibitory activities against *B. chinensis* at low concentrations.

**KEYWORDS:** *Bruchus chinensis*; *Capparis decidua*; oviposition deterrence index; triacontanol; 2-carboxy-1,1-dimethylpyrrolidine; 6-(1-hydroxy-non-3-enyl)tetrahydropyran-2-one

**INTRODUCTION**

The pulse beetle, *Bruchus chinensis*, is a serious pest of stored food grains and causes damage to cowpea, gram, soybean, and pulses. This pest is distributed worldwide and is commonly found in India. The grubs cause serious damage to the grains by eating out the entire content of the grain, leaving behind the empty shell or the seed coat. The overall damage, caused by the insect pests worldwide, is estimated to be 10–40% annually (1). Various synthetic pesticides, in the form of fumigants, sprays, and dusts, have been used in the past, to protect the field crops as well as the stored grains. Some of these methods are not successful because they result in a high rate of migration of insects in the form of eggs, pupae, or adults during transportation from field to warehouses (2). Moreover, the synthetic compounds can undergo bioaccumulation and may cause environmental hazards and various biochemical and behavioral changes in animals and humans (3–5). Therefore, in order to develop safe alternatives that are nontarget friendly, many botanical resources have been examined with respect to

its chemical constituents in the past. Several plant extracts have shown insecticidal properties against insect pests of field crops (6) as well as of stored grains (7, 8). Essential oils have also been known to play an important role in this regard and have proved to possess repellent, insecticidal, and developmental inhibitory activities (9–13). Besides these, toxic effects of some individual constituents of essential oils (14, 15) and many microbial metabolites (16, 17) have also been reported.

We, in continuation to our phytochemical studies on various Indian medicinal plants (18), have now come across *Capparis decidua*, which belongs to the family Capparidaceae (19). The plants of this genus are reported to possess medicinal properties besides insecticidal properties (20). *C. decidua* is well-known for curing a variety of ailments such as toothache, cough, asthma, intermittent fever, and rheumatism (21). Its seeds showed antibacterial activity against *Vibrio cholerae* *ogava*, *inaba*, and *etor* (22), while its fruit has been used in antidiabetic formulations (23). The phytochemical examination of this plant in the past has led to the isolation of a wide spectrum of physiologically active compounds, mainly spermidine alkaloids (24) and glucosinolates (25). However, there is no report to date with respect to the insecticidal properties of this plant, even though it is not attacked by any insect. We, therefore, thought

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Table 1. LD<sub>50</sub> Values of Different *C. deciddua* Extracts at Different Exposure Periods to *B. chinensis*

stimulus	solubility	LC <sub>50</sub> values at different exposure periods ( <i>P</i> > 0.05) <sup>a</sup>		LCL <sup>b</sup>	UCL <sup>b</sup>	<i>g</i> value <sup>c</sup>	<i>t</i> ratio <sup>c</sup>	slope values <sup>c</sup>	heterogeneity <sup>c</sup>
		h	μg						
CD1	CHCl <sub>3</sub>	24	14.146	8.86	74.52	0.357	3.280	1.376	0.22
		48	9.546	6.39	44.80	0.440	2.953	1.112	0.32
		72	6.958	5.04	16.13	0.482	2.687	0.989	0.35
		96	3.619	1.92	5.13	0.419	3.027	1.103	0.43
CD2	CH <sub>3</sub> OH (hot)	24	14.368	12.23	19.74	0.195	4.442	2.682	0.29
		48	12.161	10.42	15.65	0.214	4.237	2.444	0.23
		72	9.631	8.01	11.39	0.212	4.252	2.401	0.33
		96	7.319	5.48	8.57	0.191	4.481	2.569	0.36
CD3	CH <sub>3</sub> OH (hot)	24	16.246	13.57	24.05	0.197	4.419	2.788	0.23
		48	14.354	11.78	23.20	0.292	3.624	2.089	0.26
		72	11.834	9.84	16.36	0.229	3.582	2.020	0.23
		96	10.151	8.52	12.21	0.221	4.172	2.359	0.35
CD7	CHCl <sub>3</sub> + CH <sub>3</sub> OH	24	118.586	80.32	383.69	0.304	3.556	1.478	0.21
		48	82.272	60.80	175.12	0.268	3.668	1.425	0.26
		72	62.488	47.14	109.44	0.305	3.551	1.328	0.33
		96	48.781	35.96	71.20	0.229	3.586	1.324	0.37

<sup>a</sup> LC<sub>50</sub> values represent lethal concentrations 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> *g* value, *t* ratio, slope value, and heterogeneity were significant at all probability levels (90, 95, and 99%). *g* value, correlation between initial and final mortality; *t* ratio, difference in degree of freedom at 0.5, 0.05, and 0.005 levels; slope value, shows the average between LC<sub>80</sub> and LC<sub>20</sub>, from which LC<sub>50</sub> value is calculated; and heterogeneity value, shows the effect of active fraction on both susceptible and tolerant insects among all of the treated insects.

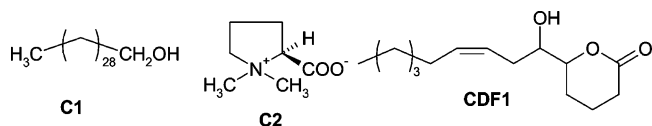
of carrying out bioassay-guided fractionation of extracts of its various parts, in order to find compound(s) responsible for insecticidal activity.

## MATERIALS AND METHODS

**Insects.** *B. chinensis*, a pulse beetle, was used to examine the activity of different extracts of *C. deciddua* and its pure compounds. For this purpose, adult insects of *B. chinensis* were collected from the stored grain house and their cultures were maintained in the laboratory at 28 ± 2 °C, 75 ± 5 relative humidity, and a photoperiod of 12:12 (L:D) h. The insects were reared on gram seeds at 10–12% moisture content.

**Extraction of Plant Materials.** Stems and flowers of *C. deciddua* were collected from Agra in Uttar Pradesh, India. These were separately shaved, dried, and subsequently pulverized to obtain a fine powder. The powdered stems (200 g) were then extracted using CHCl<sub>3</sub>/MeOH (1:1), cold MeOH, and hot MeOH sequentially to obtain dry extracts CD1 (14 g), CD2 (5 g), and CD3 (2 g), respectively. Its flowers (40 g) were only extracted with CHCl<sub>3</sub>/MeOH (1:1) to obtain dry extract CD7 (3 g).

**Isolation and Characterization of Active Components from Extract CD1.** The solvent free extract CD1 from *C. deciddua* stems was found to be a mixture of several components of varying polarity on thin-layer chromatography (TLC) and, therefore, was fractionated by column chromatography. The column was prepared in petroleum ether using silica gel (60–80) as an adsorbent and eluted with petroleum ether/chloroform, chloroform, and chloroform/methanol mixtures of increasing polarity. Twelve fractions were obtained, and similar ones were combined on the basis of their TLC behavior. Two compounds, labeled as **C1** and **C2**, were isolated in pure form from these fractions by further using column chromatography.



Compound **C1** (25 mg) was obtained from the fractions eluted with petroleum ether/chloroform (80:20, 60:40). Compound **C2** (50 mg) was obtained from chloroform/methanol (80:20) eluates and purified by column chromatography. It gave an orange color with Dragendroff's reagent, thereby showing its alkaloid nature.

Compound **C1** was obtained as a white solid on crystallization with methanol, with a mp 82–85 °C. Its <sup>1</sup>H NMR spectrum indicated it to be aliphatic in nature. The absence of a molecular ion peak in the mass spectrum and the presence of a peak at *m/z* 420 for (M<sup>+</sup> – H<sub>2</sub>O) indicated that it contained a hydroxyl group and was therefore analyzed for C<sub>30</sub>H<sub>62</sub>O. The presence of a hydroxyl group was further supported by its <sup>13</sup>C NMR spectrum, which showed a signal at δ 63.04 for the carbon carrying the hydroxyl group. The presence of a hydroxyl group was further confirmed by its IR spectrum, which showed a broad absorption band at 3398 cm<sup>-1</sup>. Its <sup>1</sup>H NMR spectrum exhibited signals for the presence of a terminal methyl group at δ 0.88 and a chain of methylene protons at δ 1.26. A triplet at δ 3.63 showed the presence of a methylene linked to the oxygen. All of these spectral data, when coupled together, indicated compound **C1** to be a saturated primary alcohol containing C<sub>30</sub> carbon atoms. On the basis of the above spectral studies, compound **C1** was characterized as triacontanol. The proposed structure was confirmed by analyzing its acetate **C1a**, prepared by acetylating **C1** with Ac<sub>2</sub>O/pyridine under dry conditions.

The IR spectrum of **C1a** showed an absorption at 1731 cm<sup>-1</sup> confirming the formation of an ester. Its <sup>1</sup>H NMR showed a singlet at δ 2.04 integrating for three protons and a triplet at δ 4.05 integrating for two protons, corresponding to the acetyl group and α-hydroxymethylene, respectively. Thus, on the basis of the spectral data of **C1** and its acetate **C1a**, compound **C1** was identified as triacontanol.

**Triacontanol (C1).** White solid, mp 82–85 °C (26). IR ν<sub>max</sub> (KBr): 3398, 2918, 2849, 1463, 1360, 1061, 720 cm<sup>-1</sup>. <sup>1</sup>H NMR (δ, CDCl<sub>3</sub>, 300 MHz): 0.88 (t, 3H, –CH<sub>3</sub>), 1.26 (brs, 54H), 1.54 (m, 2H, –CH<sub>2</sub>CH<sub>2</sub>OH), 3.63 (t, 2H, –CH<sub>2</sub>OH). <sup>13</sup>C NMR (δ, CDCl<sub>3</sub>, 75.47 MHz): 14.01 (–CH<sub>3</sub>), 25.67–32.76 (–CH<sub>2</sub>), 63.04 (–CH<sub>2</sub>OH). EIMS *m/z* (%): 420 (M<sup>+</sup> – 18, 36), 392 (8), 364 (5), 167 (18), 153 (20), 125 (95), 57 (100).

**Triacontanol Acetate (C1a).** White solid, mp 68–70 °C. IR ν<sub>max</sub> (KBr): 2917, 2849, 1731, 1473, 1463, 1367, 1239, 1043, 756, 729, 719 cm<sup>-1</sup>. <sup>1</sup>H NMR (δ, CDCl<sub>3</sub>, 300 MHz): 0.88 (t, 3H, –CH<sub>3</sub>), 1.27 (brs, 54H), 1.61 (m, 2H, H-2), 2.04 (s, 3H, –COCH<sub>3</sub>), 4.05 (t, 2H, –CH<sub>2</sub>O).

Compound **C2** was isolated from chloroform/methanol fraction (80:20) of the extract **CD1** and purified by column chromatography as a white solid, with a mp of 268 °C. It was hygroscopic in nature. It gave an orange color with Dragendroff's reagent, which is a positive test for the presence of an alkaloid. Its IR spectrum showed a characteristic absorption at 1621 cm<sup>-1</sup>, indicating the presence of a carboxyl group. It did not give acetate when treated with acetic anhydride and pyridine,

**Table 2.** LD<sub>50</sub> Values of Compounds Isolated from Extracts CD1 and CD7 at Different Exposure Periods to *B. chinensis*

compound	LC <sub>50</sub> values at different exposure periods ( <i>P</i> > 0.05) <sup>a</sup>		LCL <sup>b</sup>	UCL <sup>b</sup>	<i>g</i> value <sup>c</sup>	<i>t</i> ratio <sup>c</sup>	slope values <sup>c</sup>	heterogeneity <sup>c</sup>
	h	μg						
C1	24	17.314	11.07	86.53	0.229	3.646	2.382	0.26
	48	12.662	9.57	56.35	0.243	3.458	2.683	0.21
	72	9.049	7.49	24.92	0.265	4.174	2.252	0.23
	96	6.913	4.37	10.21	0.214	3.593	2.925	0.22
C2	24	19.783	15.66	25.97	0.395	3.831	3.321	0.39
	48	15.664	12.84	20.39	0.274	4.668	2.424	0.23
	72	12.731	10.32	15.44	0.312	3.784	2.643	0.37
	96	9.619	7.96	12.29	0.291	4.395	2.265	0.33
CDF1	24	16.124	24.92	13.61	0.239	3.749	2.398	0.32
	48	12.096	19.03	8.76	0.263	3.979	2.871	0.27
	72	9.445	12.99	6.71	0.295	3.574	2.657	0.33
	96	8.022	11.22	4.71	0.342	4.293	3.198	0.37

<sup>a</sup> LC<sub>50</sub> values represent lethal concentrations 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> *g* value, *t* ratio, slope value, and heterogeneity were significant at all probability levels (90, 95, and 99%). *g* value, correlation between initial and final mortality; *t* ratio, difference in degree of freedom at 0.5, 0.05, and 0.005 levels; slope value, shows the average between LC<sub>80</sub> and LC<sub>20</sub>, from which LC<sub>50</sub> value is calculated; and heterogeneity value, shows the effect of active fraction on both susceptible and tolerant insects among all of the treated insects.

**Table 3.** Effect of Different *C. decidua* Extracts on Oviposition Behavior of *B. chinensis*

stimulus <sup>a</sup>	dose used (μg)	no. of eggs laid per insect mean ± SE	% eggs laid per insect mean ± SE	%ODI <sup>b</sup> mean ± SE	<i>F</i> value <sup>c</sup> at df 3 and 20
CD1	0.875	8.35 ± 0.22	76.60 ± 2.08	13.34 ± 1.33	183.62
	1.750	6.85 ± 0.21	62.83 ± 2.01	22.92 ± 1.48	
	2.625	4.10 ± 0.21	39.13 ± 1.91	45.11 ± 1.92	
CD2	1.0	8.80 ± 0.16	72.72 ± 1.37	16.69 ± 1.14	193.70
	3.0	7.20 ± 0.20	59.49 ± 1.69	25.47 ± 1.31	
	5.0	4.95 ± 0.29	40.49 ± 2.41	41.18 ± 2.43	
CD3	1.0	9.05 ± 0.24	73.57 ± 2.17	14.56 ± 1.32	165.08
	3.0	7.98 ± 0.22	64.89 ± 1.86	21.37 ± 1.37	
	5.0	5.36 ± 0.22	43.62 ± 1.86	39.98 ± 1.78	
CD7	6.0	9.70 ± 0.20	83.18 ± 1.74	9.23 ± 1.03	108.98
	12.0	8.83 ± 0.22	75.49 ± 1.88	14.99 ± 1.33	
	18.0	6.20 ± 0.24	52.97 ± 2.11	30.88 ± 1.82	

<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%).

indicating the absence of free -NH- or -OH groups in the molecule. It gave a molecular ion peak at *m/z* 144 in its mass spectrum and, hence, has been assigned the molecular formula as C<sub>7</sub>H<sub>13</sub>NO<sub>2</sub>. Its <sup>1</sup>H NMR spectrum displayed two singlets at δ3.14 and δ3.31, each integrating for three protons, and was assigned for two methyl groups present on nitrogen atom. This was further supported by the peaks at δ46.80 and δ53.17 in its <sup>13</sup>C NMR spectrum. Because these N-methyls appeared in the downfield region, nitrogen is of quaternary nature and bears a positive charge. Furthermore, its <sup>1</sup>H NMR spectrum showed a triplet for one proton at δ4.01 and was assigned for the proton α to the carboxylic group and quaternary N-methyl group. Remaining methylenes were observed at δ3.67 and δ3.71 and were assigned for H-5 and H-5' and others at δ2.49 and 2.32 for H-3 and H-3', respectively, along with a multiplet at δ2.13 for H-4 protons. <sup>13</sup>C DEPT 135 and <sup>1</sup>H-<sup>1</sup>H COSY spectra were also recorded to completely identify its structure. On the basis of the above-mentioned spectral data, compound **C2** was identified as 2-carboxy-1,1-dimethylpyrrolidine.

**2-Carboxy-1,1-dimethylpyrrolidine (C2).** White solid, mp 268 °C (26). It gave a single spot on TLC, *R<sub>f</sub>* = 0.5 using chloroform/methanol (60:40) as the developing solvent. IR ν<sub>max</sub> (KBr): 3401, 1621, 1531, 1479, 1400, 1368, 1326, 1002, 961 cm<sup>-1</sup>. <sup>1</sup>H NMR (δ, CD<sub>3</sub>OD, 300 MHz): 2.13 (m, 2H, H-4), 2.32 (m, 1H, H-3'), 2.49 (m, 1H, H-3), 3.14 (s, 3H, N-CH<sub>3</sub>), 3.31 (s, 3H, N-CH<sub>3</sub>), 3.67 (m, 1H, H-5), 3.71

**Table 4.** Effect of Compounds Isolated from Extracts CD1 and CD7 on Oviposition Behavior of *B. chinensis*

compd <sup>a</sup>	dose used (μg)	no. of eggs laid per insect mean ± SE	% eggs laid per insect mean ± SE	% ODI <sup>b</sup> mean ± SE	<i>F</i> value <sup>c</sup> at df 3 and 20
C1	3.0	8.92 ± 0.25	70.34 ± 2.14	13.21 ± 1.37	166.79
	6.0	7.22 ± 0.30	48.44 ± 2.59	23.49 ± 1.98	
	9.0	4.35 ± 0.18	36.58 ± 1.57	45.56 ± 1.65	
C2	3.0	8.30 ± 0.20	76.86 ± 1.67	17.48 ± 1.18	224.61
	6.0	5.72 ± 0.19	62.21 ± 1.60	34.82 ± 1.48	
	9.0	4.32 ± 0.29	37.50 ± 2.48	46.73 ± 2.73	
CDF1	1.8	12.3 ± 0.345	67.30 ± 1.885	46.27 ± 1.085	159.08
	2.4	9.9 ± 0.608	54.55 ± 3.325	54.52 ± 2.208	
	3.0	5.9 ± 0.257	32.69 ± 1.407	69.73 ± 1.103	

<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%).

(m, 1H, H-5'), 4.01 (t, 1H, H-2). <sup>13</sup>C NMR (δ, CD<sub>3</sub>OD, 75.47 MHz): 20.22 (C-4), 27.23 (C-3), 46.80 (N-CH<sub>3</sub>), 53.17 (N-CH<sub>3</sub>), 68.43 (C-5), 78.17 (C-2). <sup>13</sup>C NMR DEPT 135 (δ, CD<sub>3</sub>OD, 75.47 MHz): 20.21 (-CH<sub>2</sub>), 27.02 (-CH<sub>2</sub>), 46.73 (-CH<sub>3</sub>), 53.09 (-CH<sub>3</sub>), 68.42 (-CH<sub>2</sub>), 78.14 (-CH). EIMS *m/z* (%): 144 (M<sup>+</sup>, 2), 117 (7), 115 (6), 101 (5), 99 (3), 85 (7), 59 (100), 55 (13), 45 (42), 43 (70).

Extracts CD2 and CD3 when examined on TLC were found to contain a mixture of highly polar inseparable components and therefore could not be further fractionated with respect to their active constituents.

Solvent free extract CD7 from *C. decidua* flowers was also subjected to column chromatography using silica gel (60-80) as an adsorbent, and the column was eluted with petroleum ether/chloroform, chloroform, and chloroform/methanol mixtures of increasing polarity. One major compound CDF1 (15 mg) was isolated from the chloroform fraction of the extract.

Compound CDF1 was obtained as a colorless oil. Its IR spectrum showed a characteristic absorption of a six-membered lactone at 1744 cm<sup>-1</sup> along with a peak of a hydroxyl group at 3427 cm<sup>-1</sup>. It did not form any acetate under normal conditions (Ac<sub>2</sub>O/pyridine) indicating thereby that the hydroxyl group was either secondary or tertiary. It gave a molecular ion peak at *m/z* 240 that corresponded to the molecular formula C<sub>14</sub>H<sub>24</sub>O<sub>3</sub>. Two characteristic peaks at *m/z* 213 and 111 were observed in its EIMS, as a result of fragmentation of δ-lactones and allylic cleavage, respectively. Its <sup>1</sup>H NMR spectrum displayed two multiplets at δ4.13 and δ4.28, each integrating for one proton, with a

coupling constant of 12 Hz, and were assigned to two methines adjacent to oxygen in the molecule. The carbons carrying oxygen were observed at  $\delta$ 63.39 and 70.19 in the  $^{13}\text{C}$  NMR spectrum. Also, there was a triplet at  $\delta$ 2.33 integrating for two protons and was assigned to  $\alpha$ -methylene protons to the carbonyl group. Its  $^1\text{H}$  NMR also displayed a multiplet at  $\delta$ 5.34 for the two olefinic protons. Also, there was a multiplet at  $\delta$ 1.29 integrating for six protons and it corresponded to three methylenes in the molecule. All of this spectral data showed compound CDF1 to contain an unsaturated six-membered lactone ring and was therefore assigned constitution as 6-(1-hydroxy-non-3-enyl)tetrahydropyran-2-one.

The proposed structure was also biogenetically feasible as it could have been possibly derived from 5Z,8Z-tetradecadienoic acid, commonly known as goshuic acid. Selective epoxidation of the double bond at the C-5 position of the acid, followed by epoxide ring opening and subsequent lactonization with the loss of a water molecule, can lead to the formation of CDF1. Thus, from correlating spectral studies and plausible biogenesis pathways, the structure of CDF1 was proposed as 6-(1-hydroxy-non-3-enyl)tetrahydropyran-2-one.

**6-(1-Hydroxy-non-3-enyl)tetrahydropyran-2-one (CDF1).** Colorless oil; gave a single spot on TLC,  $R_f = 0.7$  in chloroform. IR  $\nu_{\text{max}}$  (KBr): 3427, 2920, 2852, 1744, 1463, 1377, 1164, 1099, 721  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR ( $\delta$ ,  $\text{CDCl}_3$ , 300 MHz): 0.96 (t, 3H, H-10'), 1.29–1.33 (6H,  $-\text{CH}_2$ ), 1.96 (m, 2H, allylic  $-\text{CH}_2$ ), 2.33 (t, 2H,  $-\text{CH}_2\text{CO}-$ ), 2.77 (m, 2H, H-3'), 4.13 (dd, 1H,  $J = 4.3$  and 12 Hz, H-2'), 4.28 (dd, 1H,  $J = 6.2$  and 12 Hz, H-1'), 5.34 (m, 2H,  $=\text{CH}$ ).  $^{13}\text{C}$  NMR ( $\delta$ ,  $\text{CDCl}_3$ , 75.47 MHz): 14.45, 23.05, 25.23, 29.50, 29.64, 30.06, 31.94, 32.29, 34.43, 63.39, 70.19, 129.37, 131.51, 176.24. EIMS  $m/z$  (%): 240 ( $\text{M}^+$ , 14), 213 (10), 202 (14), 186 (20), 170 (15), 158 (22), 141 (30), 127 (18), 111 (32), 99 (44), 97 (56), 85 (64), 71 (100), 43 (30).

**Bioassay Protocol.** The toxicity of different extracts and compounds isolated from it was tested against *B. chinensis*. Ten adult insects were taken from laboratory culture and placed in glass culture tubes (10 cm ht.  $\times$  4 cm diameter) along with ten grains of gram. Filter paper strips (1  $\text{cm}^2$ ) treated with different doses of extracts/pure compounds, dissolved in their concerned solvents, were then placed at the bottom of the culture tubes, and the open end was closed by the cotton sheet. The coated filter paper strip with either the extract or the compound was air-dried before application. In control, the filter paper strip was treated only with the solvent used for dissolving extract/pure compound. For each fraction and compound, four different doses were used, and for each dose, six replicates were set. Mortality was recorded after 24, 48, 72, and 96 h.

The oviposition inhibition assay was studied by repeating the above procedure. For each chemical stimulus, three different doses were used, and for each dose, six replicates were set. The number of eggs laid was recorded after 96 h.

**Data Analysis.** The  $\text{LC}_{50}$  values were calculated by POLO program (27). The efficacy of the test stimuli was compared with control on the basis of oviposition deterrence index (ODI). The ODI of females for the test stimuli was calculated as  $100(A - B)/(A + B)$ , where A and B are the number of eggs laid in the control and in the test, respectively. One-way analysis of variance was performed between different doses of stimulus and number of eggs laid (28).

## RESULTS AND DISCUSSION

The extracts CD1, CD2, and CD3 were quite effective at lower doses while CD7 was effective at higher doses. The  $\text{LC}_{50}$  values for different extracts at different exposure periods are given in **Table 1**. From extract CD1, two compounds, namely, triacontanol (**C1**) and 2-carboxy-1,1-dimethylpyrrolidine (**C2**), have been isolated and were found to be toxic for the adult insects (**Table 2**). The  $\text{LC}_{50}$  values at different exposure periods were found to be lower for **C1** as compared to **C2**.

A novel compound, **CDF1**, isolated from fraction CD7, has also shown insecticidal activity. The mortality rate was found to increase with an increase in dose, and the  $\text{LC}_{50}$  values decreased at different graded exposure periods indicating that the response was dose- and time-dependent. The index of

significance of potency estimation,  $g$  value, indicated that the mean value is within the limits at all probabilities (90, 95, and 99%) as it is less than 0.5. Values of the  $t$  ratio greater than 1.96 indicated that the regression was significant. The steep slope values indicated that even small increase in the dose causes high mortality. Values of the heterogeneity factor less than 1.0 denoted that in the replicate test of random samples, the doses response lines would fall within 95% confidence limits and thus the model fits the data adequately.

When the newly emerged adult insects of *B. chinensis* were exposed to sublethal doses of different extracts and compounds, they laid a lesser number of eggs, as comparison to the control, i.e., when no extract or compound was used (**Tables 3** and **4**). The maximum percent oviposition deterrence index (%ODI  $\pm$  SE) at highest sublethal doses was observed in the case of CD1 ( $45.11 \pm 1.92$ ) followed in decreasing order by CD2 ( $41.18 \pm 2.43$ ), CD3 ( $39.98 \pm 1.78$ ), and CD7 ( $30.88 \pm 1.82$ ), respectively. When the insects were exposed to sublethal doses of pure compounds obtained from the extract CD1, the maximum oviposition deterrence index was observed for C2 ( $46.73 \pm 2.73$ ) followed by C1 ( $45.56 \pm 1.65$ ).  $F$  values at  $df$  3 and 20 were highly significant for all stimuluses at all probability levels (90, 95, and 99%) indicating that all stimuli affected the oviposition behavior of the pest. **CDF1** has also shown oviposition inhibition in *B. chinensis*. The maximum %ODI ( $69.73 \pm 1.103$ ) has been recorded for **CDF1** (**Table 4**).

The above study showed clearly that different extracts as well as the compounds isolated from *C. decidua* can significantly kill or influence the egg delivery response of *B. chinensis*. This also indicated that insects have susceptibility and oviposition deterrence to biochemical extracts and compounds of *C. decidua*, which can be used for the disruption of egg lying in the field and stored grain godowns to reduce the pest population.

Furthermore, isolation and characterization of some other minor components of the rest of extracts of *C. decidua* will certainly provide complete insight into the total pesticidal activity of *C. decidua* and will help in the preparation of formulations against the pulse beetle *B. chinensis* in future.

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Received for review March 25, 2006. Revised manuscript received October 19, 2006. Accepted November 6, 2006. We are thankful to Council of Scientific and Industrial Research (CSIR) for the financial assistance.

JF0608367