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Insecticidal potential of *Capparis decidua* on biochemical and enzymatic parameters of *Tribolium castaneum* (Herbst)

Ravi Kant UPADHYAY
email: rkupadhya@yahoo.com

Neeraj YADAV Shoeb AHMAD

Department of Zoology, D D U Gorakhpur University,
Gorakhpur 273009, U.P. India

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Abstract. An insecticidal effect of solvent extract of *Caparis decidua* was exercised. Six extract, acetone, chloroform, petroleum ether, methanol, hexane and water has shown very low LD_{50} values i. e. 1.5 μg/gm, 1.2 μg/gm, 1.57 μg/gm, 0.3 μg/gm and 2.0 μg/gm. These solvent extracts have exerted toxic effects on biochemical and enzymatic parameters of *Tribolium castaneum* extracts of *C. decidua* has shown very potent activity against *T. castaneum* when insects were treated with 40% and 80% of 24-hLD50. Hexane extract has displayed a significant (p ¡ 0.05) inhibition in the level of glycogen (34.12%), protein (44.19%), DNA (41.46%), RNA (33.33%) and amino acid (30.63%). It also inhibit the activity of acid phosphatase (55.88%), alkaline phosphatase (72.90%), glutamate pyruvate transaminase (69.45%) and glutamate oxaloacetate transaminase (77.02%), lactic dehydrogenase (85.50%) and acetylcholinesterase (69.85) at a very low concentrations after 16 h treatment.

Keywords: *Tribolium castanem*, *Capparis decidua* natural pesticides, protein, amino acid glycogen, ACP, ALP

1 Introduction

Beetles (Coleoptera) and moths (Lepidoptera) are stored grain insect pest (Lepidoptera) which cause heavy loses and damage to food grains. Of these beetles are for more diversified and are highly destructive stored grain insects in comparison to moths. Both grubs and adult insects attack the stored food material while among the moth, only the caterpillars are harmful life stage that causes the damage. Besides, there are certain insect pests which do not bread in stored grains but their presence in the stores is harmful because they generate filth, noxious smell and debris. These insects are cockroaches, ants, crickets, silverfiches, pscolids, and *T. castaneum*. Few mites also cause infestation in grain flour and other stored products. Few major stored grain pests are *Sitophilus oryzae* Linn. (Rice weevil), *Trogaderma grariarium* (Kuapre beetle), *Rhizopertha dominca* (Fabr), *Tribolium castaneum* (Herbst) (Rust red flour beetle), *Sitotraga cerealella* [1]. Grain and flour moth, Bruchus chinensis (Pulse beetle). Among all the stored grain insects Tribolium is a dangerous stored grain pest that damages food grains, occurs in storehouses and godowns and has a worldwide distribution [2]. It eat the entire content of the grain and leave the hollow shell of grain behind [3]. Therefore, control of stored grain insects is highly essential. For this purpose framers and ware house owners have used synthetic chemicals mainly fumigants to control surging population of stored grain insects. But with the time due to repetitive use of synthetic pesticides, insects become resistant and are resurging enormously. Besides this, synthetic chemicals were proved highly toxic to non-tagret organisms, entered in the food chain and put adverse impact on the environment [4]. Hence, their use should be restricted to minimum. Thus, insect pests have developed resistance to many commercially available synthetic pesticides [5, 6] hence, new safe alternatives are being searched in form of bio-organic pesticides [7].

However, present plant species '*Capparis decidua*' selected for investigation possess very high insecticidal activity and belong to 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]. The powdered fruit of *C. decidua* is used in anti-diabetic formulations [9]. From the above plant species both solvent and aqueous extracts prepared and tested. In the present study, insecticidal effects of *C. decidua* and its mixtures were observed on biochemical and enzymatic parameters of *Tribolium castaneum*.

2 Materials and methods

Insect culture

Adult insects of *Tribolium castaneum* (Herbst) were collected from the food grain store houses available in local market in Gorakhpur. The beetles were reared on healthy, clean and un-infested wheat seeds in glass jars and capped with muslin cloth for ventilation. Culture was maintained in laboratory under controlled temperature (28 ± 2°C), relative humidity (75 ± 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.

Collection of plant material

Stems of *Capparis decidua* were collected from different places of western part of India especially from state of Rajasthan. 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.

Preparation of extracts

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 gm) was then extracted with various solvent according to their polarity. Extracts were allowed to evaporate in a speed vac 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 temperature for experimental purpose.

Toxicity bio-assays

Adults of *Tribolium castaneum* were exposed with various increasing concentrations of each plant extracts separately. For this purpose, separate filter paper strips (1 cm^2) were coated with different concentrations of plant extracts were 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 papers were strips used to set control. Ten adult insects were released culture in glass culture tubes (10 cm Height × 4 cm diameter). For each extract, five different concentrations were used and for

each concentration six replicates were set. Mortality in Tribolium castaneum was recorded after 24 hr in presence and absence of various plants extracts separately. LD_{50} values were determined by Probit method [10]. LD50 values were calculated in μg/gm body weight of the insect.

Determination of glycogen

Glycogen contents were measured according to method of Dubois et al. [11]. For this purpose 500 mg of *T. castaneum* were homogenized in 2 ml of 5% Tri-chloro acetic acid with the help of glass-glass homogenizer and centrifuged. Optical density of the reactant was read at 530 nm. Glycogen contents in unknown (supernatant) were calculated by using standard curve drawn with known amount of glucose. The blank was set by taking 0.50 ml of 5% TCA and 6 ml of concentrate H_2SO_4. The amount of glycogen was expressed in gm/100gm of body weight of *T. castaneum.* Three treatments were performed at three trials. Data obtained was statistically analyzed by using ANOVA method.

Determination of total free amino acid

Level of free amino acids was determined following Spies [12]. A total 500 mg of *T. castaneum* were homogeninzed in 2 ml of 95% ethyl alcohol. Homogenate was centrifuged at 15,000 $\times$ g for 20 minutes and supernatant was separated. For estimation of total free amino acids 0.1 ml of supernatant was taken and to it 0.1 ml of distilled water and 2.0 ml Ninhydrin reagent were mixed. The reaction mixture was kept in boiling water for 15 minutes. A total of 2 ml of 5.0 % ethyl alcohol was added to the above boiled mixture. A violet color was developed in the reaction mixture which was measured at 575 nm. For calculating the total free amino acid content standard curve was prepared by using known amount of glycine and was expressed in gm/100gm body weight of *T. castaneum.* Three replicates were used and data is statistically analyzed by ANOVA method.

Determination of nucleic acids

Level of nucleic acids in the whole body extracts of *T. castaneum* was estimated according to method of Scheidner [13]. For this purpose a total 500 mg of *T. castaneum* were fed with 40% and 80% of LD50 of different solvent extracts of C. decidua separately. Insects were scarified and homogenized in 5%TCA with glass-glass homogenizer at 15,000 $\times$ g for 25 minutes.

DNA estimation

For DNA estimation, 0.2 ml of supernatant was taken and it was diluted by adding 3.8 ml of distilled water. Then 4.0 ml of diphenylamine reagent (1 gm of diphenylamine, 100 glacial acetic acid and 2.5 ml of conc. H_2SO_4) were added to it. The mixtures were kept in boiling water bath for 10 minutes. A blue color was developed in the solution which is measured at 595 nm (O.D.).

RNA estimation

For RNA estimation 0.2 ml of supernatant was taken and it was diluted by adding 4.8 ml of distilled water. Now 2 ml of orcinol reagent (1 gm orcinol, 100 ml conc. HCl and 0.5 gm ferric acid) was added to it. The solution was kept in boiling water bath for 10 minutes, a green color was developed, which was measured at 660 nm. In both cases three replicates were set and data obtained was statistically analyzed by ANOVA method.

Determination of total protein

Total proteins of *T. castaneum* were estimated according to Lowry et al. [14]. For this purpose 500 mg of *T. castaneum* were treated with 40% and 80% of LD_{50} of different solvent extracts of C. decidua. These treated *T. castaneum* were homogenized in 4.0 ml of 10% TCA with the help of glass-glass homogenizer. The obtained homogenate was centrifuged at 15,000 × g for 15 minutes. Each experiment was performed three times. Standard curve was prepared by using 10 μg, 20 μg, 40 μg, 80 μg and 100μg of Bovine serum albumen. Data obtained was statistically analyzed by ANOVA method.

In vivo Determination of enzymatic parameters

To observe the effect on enzymatic parameters 500 mg of adult termite workers were provided sub-lethal doses (40% and 80% of LD_{50}) of different solvent extract of *C. deciduas* was provided. Insects were sacrificed at the 4 h interval up to 16 h for measurement of various enzyme levels. Insects were homogenized in phosphate saline buffer (pH 6.9) in a glass-glass homogenizer and centrifuged at 4°C for 25 minutes at 15,000 × g. Supernatant was isolated in a glass tube and used as enzyme source.

Determination of acid and alkaline phosphatase

Level of alkaline phophatase level was determined according to the method of Bergmeyer [15]. For this purpose 500 mg of *T. castaneum* were homogenized in 1 ml of PBS buffer at 4°C and centrifuged at 15,000 × g for 15 min. A 0.2 ml of supernatant was taken in a test tube and 1.0 ml of acid buffer substrate solution was added. Contents were mixed thoroughly and incubated for 30 minutes at 37°C. Now 4.0 ml of 0.10N NaOH solution was added to the incubation mixture. Similarly, for determination of ALP, 0.10 ml of supernatant was taken in a test tube and 1.0 ml of alkaline buffer substrate was mixed with it. The mixture was mixed thoroughly and incubated for 30 minutes at 37°C. Now 5.0 ml of 0.02 N NaOH was added to the incubation mixture. The reaction was stopped by adding excess of NaOH. The p-nitrophenol formed as result of hydrolysis of p-nitrophenyl phosphate gave a yellow colour with NaOH. Optical density was measured at 420 nm. Standard curve was drawn with the help of different concentrations of p-nitrophenol. Enzyme activity was expressed as μ moles of p-nitrophenol formed /30min/mg protein.

Determination of lactic dehydrogenase

Activity of lactic dehydrogenase was measured according to the method of Annon [16]. For this purpose, 100 mg of insects were homogenized in 1.0 ml of 0.1 M phosphate buffer (pH 7.5) in ice bath and centrifuged at 10000 × g for 30 minutes in cold centrifuge at 4°C. Supernatant was used as enzyme source. For determination of enzyme activity 0.05 ml of enzyme source was added to 0.50 ml of pyruvate substrate. Now the contents were incubated at 37°C for 45 minutes. Now 0.50 ml of 2,4- dinitrophenyl hydrazine solution was added and the contents were mixture and kept at the room temperature. After 20 minutes, 5.0 ml of 0.4 N NaOH was mixed and left for 30 minutes at room temperature. The optical density was measured at 540 nm and it was converted to LDH unit by drawing a standard curve. Enzyme activity has been expressed as μ moles of pyruvate reduced/45min/mg protein.

Determination of glutamate pyruvate transaminase and glutamic–oxaloacetic transaminase

GPT and GOT activity was measured according to the method of Reitman and Frankel [17]. A total of 500 mg T. castaneum were homogenized in 2 ml ice cold PBS buffer and centrifuged at 15,000 × g for 15 min at 4°C. For determining the activity of GPT, 0.10 ml of enzyme source was taken and

0.50 ml of GPT substrate. Similarly, for determination of GOT, 0.10 ml of enzyme source was taken and 0.50 ml of GOT substrate was added to it. Now 0.50 ml of 2, 4-dinitrophenyl hydrazine solution was added and contents were left stand for 15 minutes at room temperature. Then 5.0 ml of 0.4 N NaOH was added and mixed well and allowed to stand at room temperature for 20 minutes. The optical density was read at 505 nm after setting the blank. Standard curve was prepared by using oxaloacetic acid as working standard. The enzyme activity was expressed in units of glutamate pyruvate transaminase or glutamate oxaloacetate transaminase activity/ hr/mg protein

Determination of acetylcholinesterase

Acetylcholinesterase activity was determined according to the method of Ellman et al. [18]. For this purpose 500 mg treated *T. castaneum* were homogenized 50 mM phosphate buffer (pH 8) in ice bath and centrifuged at 1000 × g for 30 minutes in cold centrifuge at 4°C. To the supernatant 0.10 ml (5 × 10-4 M) of freshly prepared acetylcholinethioiodide solution, 0.05 ml of DTNB reagent (chromogenic agent) and 1.45 ml of PBS (pH 6.9) were added. The changes in optical density were monitored at 412 nm regularly for three minutes at 25°C. Enzyme activity has been expressed as moles 'SH' hydrolysed per minute per mg protein.

Statistical analysis

The LD_{50} for each extract was determined by using Probit analysis. Mean, standard deviation, standard error and Student t-test were applied [19].

3 Results

Toxicity determination

The solvent extracts of *C. deciduas* have shown potent toxicity against the insect *T. castaneum* as have shown very low LD50 i.e. 1.5 μg/gm, 1.2μg/gm, 1.2 μg/gm, 1.57 μg/gm, 0.3 μg/gm and 2.0 μg/gm of body weight of *T. castaneum* for acetone, chloroform, petroleum ether, methanol, hexane and water extracts respectively (Table 1).

Table 1: LD_{50} of different extracts of *C. deciduas* against *T. castaneum* (Herbst)

Solvent extract	LD_{50} (μg/gm)	UCL	LCL	Slope function
Acetone	1.5	2.724	0.825	1.98
Chloroform	1.2	2.198	0.655	2.00
Petroleum ether	1.2	2.313	0.622	2.12
Methanol	1.57	2.902	0.849	2.02
Hexane	0.3	0.562	0.160	2.05
Water	2.0	6.296	2.541	1.68

Determination of bio-molecules

Treatment of *T. castaneum* with sub-lethal concentration of *C. decidua* acetone, chloroform, petroleum ether, methanol hexane and water extracts have significantly depleted the glycogen content up to 36.66%, 52.09%, 47.76%, 58.08%, 34.12% and 60.84% after 16 hr. (Table2-7). Same extracts have also retard the protein synthesis and cut down it's level up to 57.44%, 46.36%, 47.57%, 46.45%, 44.19 and 54.85%. Similarly, the solvent extracts significantly inhibited the DNA content up to 58.15%, 46.96%, 42.93%, 50.08% 41.46, 54.85% and the RNA was inhabited up to 26.20% 46.82%, 61.27%, 33.33%, 47.39% and 33.91% In a similar way the amino acid content was also found to be reduced up to 52.05%, 41.76%, 28.08%, 53.87%, 30.63% and 46% (Table 2-7).

Determination of enzymes

Significant alteration in the activity of certain metabolic enzymes of *T. castaneum* was found with respect to treatment with sub-lethal concentration of different solvent extracts of *C. decidua*. Hexane extract has shown higher inhibitory activity against the enzymes and significantly reduced the body content of ACP (55.88%), ALP (72.90%), GPT (69.45%), GOT (77.02%), LDH (86.74%), and AChE (69.85%). Contrary to this, aqueous extract have shown lower activity against the enzymes and have shown lesser inhibition i. e. 81.54%, 87.37%, 94.36%, 92.74%, 96.69% and 91.58% in ACP, ALP, GPT, GOT, LDH and AChE contents (Table 8-13). Meanwhile, acetone chloroform petroleum ether and methanol have shown moderate activity against these enzymes, data presented in tables 8-13.

Table 2: Effect of 40% and 80% of LD_{50} of *Capparis decidua* acetone fraction on glycogen, protein, DNA, RNA and amino acid of *Tribolium castaneum* (Herbst).

Parameters	0 (Control)	Time (in h)							
		4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen	2.01±0.04 (100)	1.413±0.055 (70.29)	1.203±0.026 (59.85)	1.340±0.032 (66.66)	1.157±0.014 (57.56)	1.230±0.023 (61.19)	0.957±0.015 (47.61)	0.900±0.017 (44.77)	0.737±0.023 (36.66)
Protein	9.187±0.070 (100)	9.097±0.038 (99.02)	8.057±0.096 (87.70)	8.210±0.021 (89.36)	7.750±0.046 (84.36)	7.787±0.052 (84.76)	6.037±0.135 (65.71)	5.717±0.059 (62.23)	5.277±0.106 (57.44)
D.N.A.	0.545±0.001 (100)	0.517±0.0038 (94.84)	0.442±0.0014 (81.08)	0.472±0.0056 (86.59	0.418±0.0032 (76.68)	0.210±0.005 (75.95)	0.366±0.0014 (67.14)	0.339±0.0018 (62.19)	0.317±0.0063 (58.15)
R.N.A.	0.519±0.007 (100)	0.354±0.0011 (68.21)	0.326±0.0038 (62.81)	0.315±0.0056 (60.69)	0.262±0.0050 (50.48)	0.210±0.005 (40.46)	0.193±0.0029 (37.19)	0.174±0.0061 (33.53)	0.136±0.0041 (26.20)
Amino acid	0.826±0.005 (100)	0.778±0.0022 (94.18)	0.723±0.0061 (87.53)	0.611±0.002 (73.97)	0.520±0.0072 (62.95)	0.556±0.002 (67.31)	0.488±0.0076 (59.08)	0.478±0.0027 (57.87)	0.430±0.005 (52.05)

Values are mean ±SE of three replicates

Table 3: Effect of 40% and 80% of LD_{50} of *Capparis decidua* chloroform fraction on glycogen, protein, DNA, RNA and amino acid of *Tribolium castaneum* (Herbst).

Parameters	0 (Control)	Time (in h)							
		4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen	2.01±0.04 (100)	1.867±0.029 (92.88)	1.677±0.026 (83.43)	1.603±0.033 (79.75)	1.487±0.030 (73.98)	1.517±0.020 (75.47)	1.190±0.017 (59.20)	1.157±0.026 (57.56)	1.047±0.070 (52.09)
Protein	9.187±0.070 (100)	8.303±0.122 (90.38)	7.420±0.108 (80.77)	7.510±0.112 (81.75)	6.140±0.076 (66.83)	6.757±0.098 (73.55)	5.437±0.064 (59.18)	5.530±0.097 (60.19)	4.260±0.095 (46.36)
D.N.A.	0.545±0.001 (100)	0.479±0.0029 (87.870	0.430±0.0033 (78.88)	0.441±0.0048 (80.90)	0.381±0.0044 (69.89)	0.385±0.0015 (70.63)	0.364±0.0026 (66.77)	0.303±0.0018 (55.58)	0.256±0.0037 (46.96)
R.N.A.	0.519±0.007 (100)	0.439±0.0035 (84.59)	0.399±0.383 (76.88)	0.419±0.0037 (80.73)	0.383±0.0033 (73.80)	0.367±0.0067 (70.71)	0.339±0.004 (65.32)	0.311±0.0021 (59.92)	0.243±0.0035 (46.82)
Amino acid	0.826±0.005 (100)	0.682±0.0012 (82.56)	0.460±0.0030 (55.68)	0.538±0.0011 (65.13)	0.427±0.002 (51.69)	0.475±0.0017 (57.50)	0.375±0.0015 (45.40)	0.387±0.0025 (46.85)	0.345±0.0006 (41.76)

Values are mean ±SE of three replicates

Table 4: Effect of 40% and 80% of LD_{50} of *Capparis decidua* petroleum ether fraction on glycogen, protein, DNA, RNA and amino acid of *Tribolium castaneum* (Herbst)

Parameters	0 (Control)	Time (in h)							
		4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen	2.01±0.04 (100)	1.710±0.017 (85.07)	1.463±0.035 (72.78)	1.463±0.023 (72.78)	1.263±0.018 (62.83)	1.317±0.024 (65.52)	1.160±0.023 (57.71)	1.190±0.023 (59.20)	0.960±0.015 (47.76)
Protein	9.187±0.070 (100)	8.453±0.070 (92.01)	7.503±0.084 (81.67)	7.247±0.110 (78.88)	6.290±0.078 (68.47)	6.747±0.062 (73.44)	5.327±0.11 (57.98)	5.447±0.090 (59.29)	4.370±0.0095 (47.57)
D.N.A.	0.545±0.001 (100)	0.368±0.0035 (67.51)	0.340±0.0023 (62.37)	0.335±0.0024 (61.45)	0.293±0.0024 (53.75)	0.325±0.0043 (59.62)	0.256±0.0014 (46.96)	0.268±0.0012 (49.16)	0.234±0.0023 (42.93)
R.N.A.	0.519±0.007 (100)	0.514±0.0075 (99.04)	0.449±0.0044 (86.51)	0.468±0.0026 (90.17)	0.403±0.0047 (77.65)	0.429±0.0029 (82.66)	0.359±0.004 (69.17)	0.362±0.0072 (69.75)	0.318±0.0043 (61.27)
Amino acid	0.826±0.005 (100)	0.558±0.0042 (67.55)	0.501±0.0027 (60.65)	0.420±0.0034 (50.84)	0.315±0.0044 (38.13)	0.357±0.0024 (43.22)	0.283±0.0036 (34.26)	0.263±0.0018 (31.84)	0.232±0.0046 (28.08)

Values are mean ±SE of three replicates

Table 5: Effect of 40% and 80% of LD_{50} of *Capparis decidua* methanol fraction on glycogen, protein, DNA, RNA and amino acid of *Tribolium castaneum* (Herbst).

Parameters	0 (Control)	Time (in h)							
		4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen	2.01±0.04 (100)	1.673±0.0176 (83.23)	1.423±0.032 (70.79)	1.504±0.042 (74.82)	1.347±0.035 (67.01)	1.297±0.014 (64.52)	1.217±0.032	1.183±0.018 (58.85)	1.167±0.041 (58.08)
Protein	9.187±0.070 (100)	7.840±0.081 (85.34)	7.030±0.089 (76.52)	6.823±0.090 (74.27)	6.277±0.066 (68.32)	5.760±0.046 (62.70)	5.183±0.081 (56.42)	4.080±0.092 (44.41)	4.267±0.093 (46.45)
D.N.A.	0.545±0.001 (100)	0.461±0.0018 (84.57)	0.431±0.001 (79.08)	0.413±0.0021 (75.77)	0.363±0.0029 (66.60)	0.333±0.0018 (61.09)	0.319±0.0024 (58.52)	0.307±0.0035 (56.32)	0.273±0.0018 (50.08)
R.N.A.	0.519±0.007 (100)	0.352±0.0023 (67.82)	0.313±0.0052 (60.30)	0.322±0.0033 (62.04)	0.259±0.004 (49.90)	0.244±0.0021 (47.01)	0.228±0.0043 (43.93)	0.210±0.0038 (40.46)	0.173 ±0.0032 (33.33)
Amino acid	0.826±0.005 (100)	0.695±0.0026 (84.140	0.675±0.0017 (81.71)	0.603±0.0048 (73.00)	0.559±0.0024 (67.67)	0.546±0.0012 (66.10)	0.505±0.001 (61.13)	0.583±0.0014 (58.47)	0.445±0.0026 (53.87)

Values are mean ±SE of three replicates

Table 6: Effect of 40% and 80% of LD_{50} of *Capparis decidua* hexane fraction on glycogen, protein, DNA, RNA and amino acid of *Tribolium castaneum* (Herbst)

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen	2.01±0.04 (100)	1.323±0.026 (65.82)	1.213±0.058 (60.35)	1.237±0.026 (61.54)	0.970±0.0029 (48.26)	1.147±0.064 (57.06)	0.767±0.014 (38.16)	1.140±0.032 (56.71)	0.700±0.032 (34.12)
Protein	9.187±0.070 (100)	7.537±0.081 (65.82)	7.347±0.052 (79.97)	7.163±0.067 (77.97)	6.803±0.066 (74.05)	6.900±0.053 (75.11)	5.250±0.032 (57.15)	4.630±0.061 (50.40)	4.060±0.070 (44.19)
D.N.A.	0.545±0.001 (100)	0.362±0.0023 (66.41)	0.313±0.0029 8	0.307±0.0014 (56.32)	0.273±0.0013 (50.08)	0.275±0.0018 (50.45)	0.254±0.0026 (46.60)	0.251±0.007 (46.04)	0.226±0.0073 (41.46)
R.N.A.	0.519±0.007 (100)	0.469±0.0049 (90.36)	0.396±0.0022 (76.30)	0.430±0.003 (82.85)	0.349±0.004 (67.24)	0.384±0.006 (73.98)	0.313±0.0018 (60.30)	0.356±0.0046 (68.59)	0.246±0.0038 (47.39)
Amino acid	0.826±0.005 (100)	0.807±0.004 (97.69)	0.667±0.0024 (80.75)	0.722±0.0012 (87.40)	0.469±0.0024 (56.78)	0.496±0.002 (60.04)	0.346±0.0011 (41.89)	0.292±0.0023 (35.35)	0.253±0.0029 (30.63)

Values are mean ±SE of three replicates

Table 7: Effect of 40% and 80% of LD_{50} of *Capparis decidua* aqueous fraction on glycogen, protein, DNA, RNA and amino acid of *Tribolium castaneum* (Herbst).

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen	2.01±0.04 (100)	1.843±0.026 (91.69)	1.740±0.023 (86.56)	1.670±0.0173 (83.08)	1.583±0.024 (78.75)	1.390±0.063 (69.15)	1.417±0.026 (70.49)	1.313±0.030 (65.32)	1.223±0.050 (60.84)
Protein	9.187±0.070 (100)	7.060±0.099 (76.85)	6.857±0.071 (74.64)	6.257±0.078 (69.19)	5.683±0.046 (61.86)	5.170±0.043 (56.27)	4.383±0.037 (47.71)	4.643±0.075 (50.54)	3.817±0.078 (54.85)
D.N.A.	0.545±0.001 (100)	0.517±0.004 (94.84)	0.482±0.0063 (88.79)	0.457±0.0029 (83.84)	0.413±0.0047 (75.76)	0.393±0.0014 (72.09)	0.341±0.0021 (62.56)	0.319±0.0029 (58.52)	0.299±0.0049 (54.85)
R.N.A.	0.519±0.007 (100)	0.425±0.0055 (81.88)	0.378±0.0037 (72.83)	0.369±0.0052 (71.09)	0.307±0.0009 (59.15)	0.309±0.0035 (59.53)	0.244±0.0032 (47.01)	0.245±0.0047 (47.20)	0.176±0.0046 (33.91)
Amino acid	0.826±0.005 (100)	0.609±0.0012 (83.53)	0.613±0.0067 (74.21)	0.585±0.0015 (70.82)	0.459±0.0024 (55.57)	0.466±0.0045 (56.41)	0.437±0.0032 (52.90)	0.413±0.0068 (50.00)	0.380±0.0026 (46.00)

Values are mean ±SE of three replicates

Table 8: Effect of 40% and 80% of LD_{50} of *Capparis decidua* acetone fraction on ACP, ALP, GPT, GOT, LDH and AChE of *Tribolium castaneum* (Herbst)

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	2.346±0.003 (100)	2.220±0.05 (94.62)	2.124±0.022 (90.53)	2.018±0.0024 (86.01)	1.921±0.057 (81.88)	1.918±0.013 (81.75)	1.917±0.0233 (81.71)	1.915±0.0083 (81.62)	1.913±0.012 (81.54)
ALP	1.853±0.047 (100)	1.692±0.015 (91.31)	1.586±0.021 (85.59)	1.517±0.0057 (81.86)	1.502±.0.0081 (80.05)	1.481±0.011 (79.11)	1.466±0.041 (79.11)	1.424±0.003 (76.84)	1.419±0.0013 (76.57)
GPT	4.289±0.0046 (100)	4.045±0.017 (94.31)	4.039±0.0083 (94.17)	4.031±0.0018 (93.98)	4.027±0.0012 (93.89)	4.005±0.012 (93.37)	3.912±0.003 (91.21)	3.909±0.002 (91.14)	3.886±0.0013 (90.60)
GOT	3.117±0.0012 (100)	2.949±0.022 (94.61)	2.892±0.045 (92.78)	2.849±0.004 (91.40)	2.839±0.0024 (91.08)	2.821±0.031 (90.50)	2.816±0.01 (90.34)	2.807±0.012 (90.05)	2.801±0.022 (89.86)
LDH	8.316±0.0022 (100)	8.297±0.019 (99.77)	8.287±0.012 (99.65)	8.281±0.0087 (99.57)	8.259±0.015 (99.31)	8.251±0.024 (99.21)	8.241±0.05 (99.09)	8.131±0.0017 (97.77)	8.001±0.011 (96.21)
AChE	0.962±0.0009 (100)	0.918±0.012 (95.42)	0.911±0.0011 (94.69)	0.909±0.031 (94.49)	0.891±0.035 (92.61)	0.879±0.0014 (91.37)	0.852±0.007 (88.56)	0.846±0.0013 (87.94)	0.831±0.003 (86.38)

Values are mean ±SE of three replicates

Table 9: Effect of 40% and 80% of LD_{50} of *Capparis decidua* chloroform fraction on ACP, ALP, GPT, GOT, LDH and AChE of *Tribolium castaneum* (Herbst)

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	2.346±0.003 (100)	2.198±0.0017 (93.69)	2.104±0.016 (89.68)	2.008±0.0019 (85.59)	1.891±0.018 (80.60)	1.788±0.003 (76.21)	1.751±0.0013 (74.63)	1.735±0.001 (73.95)	1.723±0.021 (73.44)
ALP	1.853±0.047 (100)	1.611±0.0053 (86.94)	1.566±0.016 (84.51)	1.502±0.0012 (81.05)	1.479±.008 (79.81)	1.471±0.0031 (79.38)	1.455±0.006 (78.52)	1.413±0.05 (76.25)	1.411±0.0023 (76.14)
GPT	4.289±0.0046 (100)	3.895±0.007 (90.81)	3.777±0.008 (88.06)	3.661±0.0018 (85.35)	3.437±0.002 (80.13)	3.405±0.012 (79.38)	3.212±0.023 (74.88)	3.189±0.013 (74.35)	3.127±0.0043 (72.90)
GOT	3.117±0.0012 (100)	2.909±0.02 (93.32)	2.812±0.015 (90.21)	2.809±0.0019 (90.01)	2.793±0.002 (89.60)	2.721±0.013 (87.29)	2.696±0.011 (86.49)	2.687±0.0021 (86.20)	2.485±0.022 (79..72)
LDH	8.316±0.0022 (100)	8.167±0.019 (98.20)	8.127±0.021 (97.72)	8.101±0.0021 (97.41)	7.959±0.015 (95.70)	7.851±0.016 (94.40)	7.641±0.011 (91.88)	7.432±0.037 (89.36)	7.111±0.031 (85.50)
AChE	0.962±0.0009 (100)	0.903±0.011 (93.86)	0.891±0.0022 (92.61)	0.885±0.031 (91.99)	0.871±0.025 (90.54)	0.857±0.0023 (89.08)	0.841±0.019 (87.42)	0.836±0.0013 (86.90)	0.811±0.008 (84.30)

Values are mean ±SE of three replicates

Table 10: Effect of 40% and 80% of LD_{50} of *Capparis decidua* petroleum ether fraction on ACP, ALP, GPT, GOT, LDH and AChE of *Tribolium castaneum* (Herbst)

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	2.346±0.003 (100)	2.028±0.0017 (86.44)	1.865±0.016 (79.49)	1.856±0.0019 (79.11)	1.674±0.018 (71.35)	1.629±0.0001 (69.43)	1.612±0.0013 (68.71)	1.595±0.0012 (67.98)	1.583±0.012 (67.47)
ALP	1.853±0.047 (100)	1.511±0.05 (81.54)	1.502±0.036 (81.05)	1.459±0.0022 (78.73)	1.439±.0081 (77.65)	1.421±0.0011 (76.68)	1.401±0.036 (75.60)	1.391±0.015 (75.06)	1.365±0.03 (73.66)
GPT	4.289±0.0046 (100)	3.811±0.007 (88.85)	3.711±0.01 (86.34)	3.632±0.0083 (84.68)	3.412±0.012 (79.55)	3.398±0.0021 (79.22)	3.201±0.0021 (74.63)	3.179±0.002 (74.11)	3.109±0.033 (72.48)
GOT	3.117±0.0012 (100)	2.801±0.0009 (89.86)	2.792±0.03 (89.57)	2.768±0.0019 (88.80)	2.756±0.002 (88.41)	2.702±0.033 (86.68)	2.659±0.001 (85.30)	2.652±0.003 (85.08)	2.414±0.002 (77.44)
LDH	8.316±0.0022 (100)	8.066±0.0019 (96.99)	8.048±0.012 (96.77)	8.035±0.0083 (96.62)	7.788±0.045 (93.65)	7.771±0.016 (93.44)	7.523±0.011 (90.46)	7.369±0.017 (88.61)	7.242±0.011 (87.08)
AChE	0.962±0.0009 (100)	0.811±0.013 (84.30)	0.781±0.0011 (81.18)	0.772±0.031 (80.24)	0.765±0.045 (79.52)	0.731±0.014 (75.98)	0.712±0.019 (74.01)	0.703±0.03 (73.07)	0.687±0.011 (71.41)

Values are mean ±SE of three replicates

Table 11: Effect of 40% and 80% of LD_{50} of *Capparis decidua* methanol fraction on ACP, ALP, GPT, GOT, LDH and AChE of *Tribolium castaneum* (Herbst)

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	2.346±0.003 (100)	2.118±0.002 (90.28)	2.004±0.04 (85.42)	1.898±0.009 (80.90)	1.787±0.018 (76.17)	1.766±0.0013 (75.27)	1.731±0.0023 (73.78)	1.715±0.002 (73.10)	1.703±0.011 (72.59)
ALP	1.853±0.047 (100)	1.587±0.015 (85.64)	1.546±0.016 (83.43)	1.492±0.005 (80.51)	1.468±.0009 (79.22)	1.454±0.03 (78.46)	1.447±0.016 (78.08)	1.403±0.05 (75.71)	1.391±0.0023 (75.06)
GPT	4.289±0.0046 (100)	3.825±0.006 (89.18)	3.717±0.001 (86.66)	3.641±0.0081 (84.89)	3.417±0.0032 (79.66)	3.401±0.021 (79.23)	3.209±0.003 (74.81)	3.181±0.0032 (74.16)	3.116±0.0013 (72.65)
GOT	3.117±0.0012 (100)	2.813±0.02 (90.24)	2.802±0.015 (89.89)	2.779±0.009 (89.15)	2.764±0.0012 (88.67)	2.711±0.0013 (86.97)	2.676±0.01 (85.85)	2.666±0.034 (85.53)	2.425±0.021 (77.79)
LDH	8.316±0.0022 (100)	8.086±0.0019 (97.23)	8.059±0.032 (96.90)	8.041±0.0031 (96.69)	7.869±0.05 (94.62)	7.848±0.016 (94.37)	7.591±0.002 (91.28)	7.397±0.0009 (88.94)	7.261±0.0021 (87.31)
AChE	0.962±0.0009 (100)	0.887±0.012 (92.20)	0.872±0.0011 (90.64)	0.862±0.005 (89.60)	0.853±0.01 (88.66)	0.846±0.004 (87.94)	0.839±0.009 (87.21)	0.822±0.013 (85.44)	0.801±0.001 (83.26)

Values are mean ±SE of three replicates

Table 12: Effect of 40% and 80% of LD_{50} of *Capparis decidua* hexane fraction on ACP, ALP, GPT, GOT, LDH and AChE of *Tribolium castaneum* (Herbst)

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	2.346±0.003 (100)	1.877±0.001 (80.00)	1.745±0.006 (74.38)	1.564±0.009 (66.66)	1.446±0.008 (61.63)	1.421±0.0013 (60.57)	1.382±0.0031 (58.90)	1.355±0.001 (57.75)	1.311±0.05 (55.88)
ALP	1.853±0.047 (100)	1.482±0.01 (79.97)	1.442±0.02 (77.78)	1.431±0.0012 (77.22)	1.425±.0001 (76.90)	1.412±0.021 (76.20)	1.391±0.006 (75.06)	1.372±0.03 (74.04)	1.351±0.0023 (72.90)
GPT	4.289±0.0046 (100)	3.802±0.007 (88.64)	3.703±0.009 (86.33)	3.522±0.008 (82.11)	3.401±0.0012 (79.29)	3.359±0.015 (78.31)	3.195±0.003 (74.49)	3.172±0.002 (73.95)	2.979±0.0013 (69.45)
GOT	3.117±0.0012 (100)	2.783±0.032 (89.28)	2.751±0.009 (88.25)	2.658±0.0019 (85.27)	2.639±0.0022 (84.66)	2.621±0.013 (84.08)	2.619±0.04 (84.02)	2.602±0.001 (83.47)	2.401±0.032 (77.02)
LDH	8.316±0.0022 (100)	7.969±0.0001 (95.82)	7.849±0.012 (94.38)	7.735±0.008 (93.01)	7.728±0.006 (92.92)	7.702±0.016 (92.61)	7.513±0.011 (90.34)	7.319±0.0009 (88.01)	7.214±0.0011 (86.74)
AChE	0.962±0.0009 (100)	0.781±0.011 (81.18)	0.774±0.0081 (80.45)	0.766±0.01 (79.62)	0.754±0.015 (78.37)	0.711±0.004 (73.90)	0.703±0.009 (73.07)	0.685±0.0013 (71.20)	0.672±0.021 (69.85)

Values are mean ±SE of three replicates

Table 13: Effect of 40% and 80% of LD_{50} of *Capparis decidua* aqueous fraction on ACP, ALP, GPT, GOT, LDH and AChE of *Tribolium castaneum* (Herbst)

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	2.346±0.003 (100)	2.240±0.004 (95.48)	2.134±0.0061 (90.96)	2.038±0.002 (86.87)	1.941±0.003 (82.77)	1.938±0.013 (82.60)	1.931±0.023 (82.31)	1.925±0.038 (82.02)	1.913±0.019 (81.54)
ALP	1.853±0.047 (100)	1.792±0.01 (96.70)	1.786±0.006 (96.38)	1.717±0.005 (92.66)	1.686±.0001 (90.98)	1.671±0.0009 (90.17)	1.666±0.04 (89.90)	1.624±0.031 (87.64)	1.619±0.0002 (87.37)
GPT	4.289±0.0046 (100)	4.145±0.007 (96.64)	4.139±0.005 (96.50)	4.131±0.0031 (96.31)	4.127±0.0021 (96.22)	4.115±0.011 (95.94)	4.112±0.0013 (95.87)	4.109±0.002 (95.80)	4.056±0.013 (94.36)
GOT	3.117±0.0012 (100)	3.019±0.002 (96.85)	3.006±0.002 (96.43)	2.949±0.003 (94.61)	2.915±0.005 (93.51)	2.911±0.031 (93.39)	2.906±0.021 (93.23)	2.897±0.0034 (92.94)	2.891±0.012 (92.74)
LDH	8.316±0.0022 (100)	8.301±0.019 (99.81)	8.297±0.012 (99.77)	8.285±0.0083 (99.62)	8.269±0.001 (99.43)	8.256±0.006 (99.27)	8.248±0.03 (99.18)	8.149±0.017 (97.99)	8.041±0.031 (96.69)
AChE	0.962±0.0009 (100)	0.938±0.012 (97.50)	0.926±0.001 (96.25)	0.919±0.031 (65.53)	0.911±0.024 (94.69)	0.909±0.0023 (94.49)	0.901±0.04 (93.65)	0.896±0.0023 (93.13)	0.881±0.006 (91.58)

Values are mean ±SE of three replicates

4 Discussion

The present investigation clearly demonstrates that both solvent and aqueous extracts of *C. decidua* are highly toxic to *T. castaneum*. Each extract has shown very low LD50 value. However, maximum toxicity was obtained in hexane extract of *C. decidua* i.e. 0.3 μg/mg, while acetone, chloroform, petroleum ether methanol and water extracts has shown 1.5 μg/gm, 1.2 μg/gm, 1.2 μg/gm, 1.57 μg/gm and 2.0 μg/gm $LD_{50}50$ value. (Table 1). Similarly, *Artemisia princepi* and *Cinnamomum camphora* (L) have shown potent toxic activity against *Sitophilus oryzae* and *Bruchus rugimanus* [20] having low LD_{50} value. Chemical constituents of Foeniculum vulgare [21] and Japanese mint (Mentha arvensis) [22] have successfully control the damage caused by *S. oryzae, T. castenum* and *Rhizopertha dominica* (F) [23].

Extract from *C. deciduas* has potentially reduced the body content of glycogen highest in hexane extract i.e 34.12%. This may be due to Depletion of glycogen indicates more and more utilization of food reserves to cope up the insecticide induced stress [24]. This decrease in glycogen level may be due to high release of glucagon, corticosteroids and catecholamines which stimulate glucose production to combat energy demand. Normally in the body free glycogen floats in the haemolymph/ blood that after breakdown help to maintain glucose level in hemolymph. These changes provide ample stimulus for glycogenolysis in insect tissues and rapid utilization of glycogen units in response to stress caused by pesticide treatment [25]. Highest reduction in DNA i. e. 41.46%, RNA i. e. 33.33% and protein i. e. 44.19% was also reported in hexane extract (Table 2-7). Similarly protein and nucleic acid synthesis may also block at cellular level and catabolism get increased which results into low availability of proteins and nucleic acid. [26, 27].

Similar results were reported in *Pimpla turionella* wasp when its larvae, pupae and adult females were treated with cypermethrin. Cypermethrin affected the level of glycogen, protein and lipid [28]. Similarly cypermethirn decrease the protein level in Spodoptera litrua larvae in comparison to control [29]. Few organophosphorus insecticides such as chloropyrifos, thiamethoxam, fipronil, and malathion caused significant depletion in total protein in haemolymph and fat body of silk worm Bombyx mori [30]. Normally in the body free glycogen floats in the haemolymph/ blood that after breakdown help to maintain glucose level in blood. These changes provide ample stimulus for glycogenolysis in insect tissues and rapid utilization of glycogen units in response to stress caused by pesticide treatment [25].

In the present investigation hexane extract of C. decidua worked as enzyme inhibitor and check the activity of ACP (55.88%), ALP (72.90%), GPT (69.45%), GOT (77.02%), LDH (85.50) and AChE (69.85) (Table 8-13). Similar effects were obtained in *T. castaneum* treated with sub-lethal concentration malathion and permethrin combinations that have check the activity of activities of acetylcholine esterase, carbohydrate-metabolizing enzyme- lactate dehydrogenase, protein-metabolizing enzymes (GPT and GOT), as well as acid and basic phosphatases (ACP and ALP) [26]. Acid and alkaline phosphatase has been studied as enzymes significant in detoxification. The compound isolated from *C. deciduas* inhibits the phosphatase enzymes and made its body defense week.

Moreover, both GPT and GOT also play an important role in protein metabolism and were inhibited by the C. decidua extracts that affected the level of glutamate pyruvate transaminase (GPT) and glutamate oxaloacetate transaminase (GOT) in treated insects [31]. However, fat body and heamolymph exhibit higher glutamate oxaloacetate transaminase activity than the glutamate pyruvate transaminase. Hence, the level of heamolymph aminotransferase gets significantly decreased. Similarly an increase in glycogenesis causes a significant decrease in free amino acid level [26]. Therefore, a sharp decrease or increase in the level of above enzymes effect oxygen consumption in insects. However, inhibition of phosphatase and lactic dehydrogenase level shows tissue necrosis in insects [32]. However, this imbalance in enzyme level indicates inhibition of important metabolic pathways [33]. Similar effects on phosphatases activity were observed in Pectinophora gossypiella after insecticide treatment [34]. Hence, all significant changes in the level of ALP, ACP, GPT, GOT, LDH and AchE indicate very high insecticidal activity of the *C. decidua* extracts towards the *T. castaneum*. However, it can be concluded that *C. decidua* possess few active ingredients that might be highly effective against stored grain insects. It is proved by the results that these ingredients cause high lethality in *T. castaneum* at a very low dose and caused significant inhibition of metabolic enzymes. Therefore, it is recommended that *C. decidua* active ingredients could be used for preparation of herbal insecticidal formulation to control stored grain insects.

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