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Toxic effects of solvent and aqueous extracts of *Cassia alata* against bio-molecules and enzymatic parameters of *Callosobruchus chinensis* L. (Coleoptera: Bruchidae:)

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ABSTRACT

In the present investigation toxicity of different solvent extracts of Cassia alata fruits was tested on adults of Callosobruchus chinensis and toxic effects were enumerated on certain bio-molecules at an interval of 4hr, 8hr, 12hr and 16hr following sub-lethal exposures. Six different solvent extracts acetone, chloroform, petroleum ether, methanol, hexane and water were prepared and their LD_{50} values were determined as 0.840µg/gm, 0.510µg/gm, 0.590µg/gm, 1.57µg/gm 0.310 µg/gm and 2.50µg/gm respectively. After exposure of acetone, chloroform, petroleum ether, methanol hexane and water extracts the glycogen content was found to be significantly depleted 39.71%, 37.94%, 41.52%, 33.00%, 45.35%, 44.55&%. Similarly, it cut the protein level up to 49.97%, 49.48%, 58.02%, 49.84%, 58.42% and 68.50%. It has shown depletions in DNA level, 58.95%, 43.44%, 46.85%, 47.97%, 50.86% and 53.38%. RNA content was also found to be reduced51.65%, 62.02%, 67.77%, 63.10%, 58.51% and 82.13%. A significant ($p<0.05$) depletion in amino acid content was recorded8.81%, 59.03%, 59.58%, 58.59%, 71.10% and 81.09% in comparison to control. However, a slight decrease (74.67%, 58.09%, 74.92%, 66.50%, 58.17% and 58.33%) in lipid level heave also been reported. Almost all extract have act potentially against enzyme, but hexane extract has performed more efficiently and displayed inhibition in ACP (58.52%), ALP (75.39%), GPT (71.86%,), GOT (79.52%), LDH (86.90%,) and AChE (71.65%). Contrarily, aqueous extract have shown lower activity against both bio-molecule and enzymatic parameters, while acetone, chloroform, petroleum ether and methanol have also shown inhibitory activity against these bio-molecules and metabolic enzymes having moderate activity. Hexane extract has shown higher lethality that indicate that it may have more active compounds

Key words: *Callosobruchus chinensis* ,*Cassia alata*, enzyme inhibition, ACP, ALP

INTRODUCTION

The pulse beetle *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) is the most widespread and a dreadful pest of stored legumes. It infests food grains and makes major losses to food grain and its quality. During rainy season its fourth instar larvae become highly active and causes heavy infestation to legume seeds. For control present beetle species 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 and in turn insects have acquired wider resistance start resurging in large numbers. Besides this, synthetic insecticides kill non-target organisms and persist in the environment as residues for longer period, poison the food chain and impose adverse effects on the environment and organisms. 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 such as essential oils [1, 2] and bio-organic compounds (3) were tested safer and toxic to control insect pests. These have shown very high mortality in stored grain pests [4] and efficiently control grain damage [5].

However, present plant species selected for study possess very high insecticidal activity and belong to different families. *Cassia alata* commonly known as Amaltash belongs to family Caeselpinaceae. It is tree grown as ornamental. The pulp of fruit is used as a purgative and laxative. It's flowers are yellow in color and are used as bile protective and used as stomach and skin aliments. Seeds are used against nematode worms. From the above plant species both solvent and aqueous extracts prepared and tested its toxicity against certain bio-molecules and metabolic enzymes

MATERIALS AND METHODS

Insect culture

Adult insects of *Callosbruchus chinensis* L. 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 ± 2^0C), 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

Fruits *Cassia alata* of 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

Fruits of *C. chinensis* 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 4^0 C temperature for experimental purpose.

Toxicity bio-assays

Adults of *C. chinensis* 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 X 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. LD_{50} values were determined by Probit method [6]. LD_{50} 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., [7]. For this purpose 500 mg of *C. chinensis* were homogenized in 2ml of 5% Tri-chloro acetic acid with the help of glass-glass homogenizer and centrifuged. Optical density of the reactant was read at 530nm. Glycogen contents in unknown (supernatant) were calculated by using standard curve drawn with known amount of glucose. The blank was set by taking 0.50ml of 5% TCA and 6 ml of concentrate H_2SO_4. The amount of glycogen was expressed in gm/100gm of body weight of *C. chinensis*. 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, [8]. A total 500 mg of *C. chinensis* were homogeninzed in 2 ml of 95% ethyl alcohol. Homogenate was centrifuged at 15,000 X 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 *C. chinensis*. 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 *C. chinensis* was estimated according to method of Scheidner [9]. For this purpose a total 500 mg of *C. chinensis* were fed with 40% and 80% of LD_{50} of different solvent extracts of *C. alata* separately. Insects were scarified and homogenized in 5%TCA with glass-glass homogenizer at 15,000Xg 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 4.8ml of distilled water. Now 2ml 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 660nm. In both cases three replicates were set and data obtained was statistically analyzed by ANOVA method.

Determination of total protein

Total proteins of *C. chinensis* were estimated according to Lowry et al., [10]. For this purpose 500mg of *C. chinensis* were treated with 40% and 80% of LD_{50} of different solvent extracts of *C. alata* These treated *C. chinensis* were homogenized in 4.0 ml of 10% TCA with the help of glass-glass homogenizer. The obtained homogenate was centrifuged at 15,000Xg 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.

Determination of Total lipid

Level of total lipid in whole body extracts of *C. chinensis* was estimated according to method of Floch et al., [11]. A total of 500 mg of insects homogenized in 5 ml of chloroform and methanol mixture (2:1 v/v). Total lipid contents were weighted at the end and expressed in gm/100gm body weight of insect. Three replicates were set and data was statistically analyzed by ANOVA method.

***In vivo* Determination of enzymatic parameters**

To observe the effect on enzymatic parameters 500 mg of *C. chinensis* were provided sub-lethal doses (40% and 80% of LD_{50}) of different solvent extract of *C. alata* 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 ^{0}C for 25 minutes at 15,000 X 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, [12]. For this purpose 500 mg of *C. chinensis* were homogenized in 1 ml of PBS buffer at 4 ^{0}C and centrifuged at 15,000 X 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 ^{0}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 ^{0}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, [13]. 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 X g for 30 minutes in cold centrifuge at 4 ^{0}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 ^{0}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, [14]. A total of 500 mg *C. chinensis* were homogenized in 2 ml ice cold PBS buffer and centrifuged at 15,000 X g for 15 min at4 ^{0}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., [15]. For this purpose 500mg treated *C. chinensis* were homogenized 50 mM phosphate buffer (pH 8) in ice bath and centrifuged at 1000 X g for 30 minutes in cold centrifuge at 4 ^{0}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 ^{0}C. Enzyme activity has been expressed as µ 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 [16].

RESULTS

The solvent extracts of *C. alata* have shown potent toxicity against the insect *C. chinensis* as have shown very low LD_{50} i.e. 1.5 µg/gm, 1.2µg/gm, 1.2 µg/gm, 1.57 µg/gm, 03 µg/gm and 2.0

µg/gm of body weight of *C. chinensis* for acetone, chloroform, petroleum ether, methanol, hexane and water extracts respectively (Table 1)

Table 1: LD_{50} of different extracts of *Cassia alata* against *Callosobruchus chinensis*

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

Exposure of sub-lethal concentrations of *C. alata* acetone, chloroform, petroleum ether, methanol hexane and water extracts have significantly ($p<0.05$) depleted the glycogen, protein, DNA, RNA amino acids and lipid profile after 16 hr. Glycogen content of insect body was significantly cut down 39.71%, 37.94%, 41.52%, 33.00%, 45.35%, 44.55&% following acetone, chloroform, petroleum ether, methanol, hexane and aqueous extracts of *C. alata* (Table 2-7). In a similar consequence protein level was also found to be reduced 49.97%, 49.48%, 58.02%, 49.84%, 58.42% and 68.50% after the application of same extracts respectively (Table 2-7). Further, depletions were also observed in DNA (58.95%, 43.44%, 46.85%, 47.97%, 50.86% and 53.38%) and RNA (51.65%, 62.02%, 67.77%, 63.10%, 58.51% and 82.13%) (Table 2-7). These extracts have also been found to have an inhibitory activity against amino acids that is why the amino acid content was found to lower (58.81%, 59.03%, 59.58%, 58.59%, 71.10% and 81.09%) in comparison to control (Table 2-7). A similar dose of same extracts *C. alata* extract caused very slight decrease in lipid contents after 4 to 8 hr of treatment. Later on it was found to be significantly ($p< 0.05$) decreased (74.67%, 58.09%, 74.92%, 66.50%, 58.17% and 58.33%) in other successive treatments (Table 2-7)

Determination of enzymes

Sub-lethal dose of acetone, chloroform, petroleum ether, methanol hexane and water extracts of *C. alata* have displayed toxic effects on certain vital enzyme that play important role in metabolic activities of insect body. Acid phosphatase enzyme was depleted 85.40%, 76.91%, 70.66%, 76.02%, 58.52% and 84.95% (Table 8-13). Similarly alkaline phosphatase content was cut down 79.18%, 78.73%, 76.17%, 77.62%, 75.39% and 88.95% (Table 8-13). In a similar consequence a significant ($p<0.05$) reduction was observed in GPT (93.75%, 75.44%, 75.00%, 75.17%,71.86%, and 96.28%) and GOT (92.77%, 82.31%, 79.96%, 80.32%, 79.52% and 88.47%) enzymes (Table8-13). Similarly lactic dehydrogenase activity was found to be reduced 96.38%, 85.66%, 87.24%, 87.47%, 86.90%, and 96.14% (Table 8-13). These extracts have shown a potent toxic effect on neurologically active enzymes AChE activity and block its activity up to 88.59%, 86.46%, 73.24%, 85.39%, 71.65% and 92.00% in caparison to control (Table 8-13)

Table 2: Effect of 40% and 80% of LD_{50} of *Cassia alata* acetone fraction on glycogen, protein, DNA, RNA amino acid and lipid of *Callosobruchus chinensis*

Parameters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen (µg/gm)	2.148±0.0046 (100)	1.975±0.010 (91.94)	1.911±0.0047 (88.96)	1.713±0.0058 (79.74)	1.640±0.0095 (76.34)	1.184±0.0042 (55.12)	0.954±0.0035 (44.41)	0.976±0.013 (45.43)	0.853±0.0056 (39.71)
Protein (µg/gm)	10.512±0.0031 (100)	9.6827±0.0075 (92.08)	9.4313±0.0047 (89.69)	8.275±0.0054 (78.70)	7.5430±0.0052 (71.73)	7.6417±0.0037 (72.67)	6.4213±0.0029 (61.07)	6.999±0.013 (66.56)	5.2543±0.0045 (49.97)
DNA (µg/gm)	0.924±0.0042 (100)	0.8355±0.0071 (90.42)	0.8008±0.0053 (86.66)	0.7479±0.0054 (80.94)	0.7140±0.0074 (77.27)	0.6321±0.004 (68.41)	0.5875±0.0002 (63.58)	0.5509±0.0061 (59.62)	0.5447±0.0059 (58.95)
RNA (µg/gm)	0.6848±0.0002 (100)	0.5348±0.0082 (78.09)	0.4858±0.0082 (70.94)	0.4480±0.0053 (65.42)	0.40±0.0062 (58.41)	0.4164±0.0023 (60.81)	0.3728±0.002 (54.49)	0.3725±0.0037 (54.39)	0.3537±0.0067 (51.65)
Amino acid (µg/gm)	0.9113±0.007 (100)	0.8113±0.007 (89.02)	0.810±0.004 (88.88)	0.710±0.0011 (77.94)	0.653±0.0025 (71.65)	0.695±0.0024 (76.26)	0.548±0.0023 (60.13)	0.568±0.0041 (62.33)	0.536±0.0043 (58.81)
Lipid (µg/gm)	1.212±0.0029 (100)	1.506±0.0035 (124.25)	1.604±0.0023 (132.35)	1.303±0.0024 (107.51)	1.403±0.0024 (115.76)	1.003±0.0018 (82.76)	1.108±0.0023 (91.42)	0.806±0.0029 (66.42)	0.905±0.0018 (74.67)

Values are mean ±SE of three replicates

Table 3: Effect of 40% and 80% of LD_{50} of *Cassia alata* chloroform fraction on glycogen, protein, DNA, RNA amino acid and lipid of *Callosobruchus chinensis*

Parameters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen (µg/gm)	2.148±0.0046 (100)	1.780±0.011 (82.86)	1.730±0.0076 (80.53)	1.674±0.0066 (77.92)	1.646±0.0058 (76.62)	1.394±0.0056 (64.89)	1.236±0.0046 (57.53)	0.871±0.0055 (40.54)	0.815±0.0049 (37.94)
Protein (µg/gm)	10.512±0.0031 (100)	8.832±0.0087 (83.99)	8.067±0.0082 (76.72)	7.8657±0.0049 (74.80)	7.225±0.0072 (68.71)	6.6227±0.0035 (62.98)	6.2433±0.0024 (59.37)	5.2685±0.0068 (50.10)	5.2031±0.0075 (49.48)
DNA (µg/gm)	0.924±0.0042 (100)	0.9126±0.0044 (98.76)	0.849±0.0044 (91.88)	0.8061±0.0031 (87.24)	0.7647±0.0044 (82.75)	0.5813±0.007 (62.91)	0.5316±0.0023 (57.53)	0.4689±0.0069 (50.74)	0.4014±0.0059 (43.44)
RNA (µg/gm)	0.6848±0.0002 (100)	0.6269±0.005 (91.55)	0.6065±0.0058 (88.57)	0.5720±0.0046 (83.53)	0.5631±0.004 (82.23)	0.5446±0.0024 (79.53)	0.5045±0.0017 (73.67)	0.4342±0.0084 (63.41)	0.4247±0.0041 (62.02)
Amino acid (µg/gm)	0.9113±0.007 (100)	0.840±0.0046 (92.17)	0.814±0.003 (89.32)	0.728±0.0043 (79.88)	0.697±0.0061 (76.48)	0.6826±0.004 (74.90)	0.568±0.0029 (62.33)	0.645±0.0043 (70.77)	0.538±0.0043 (56.03)
Lipid (µg/gm)	1.212±0.0029 (100)	1.207±0.0024 (99.58)	1.304±0.0031 (107.57)	1.005±0.0017 (82.92)	1.1107±0.0045 (91.64)	0.805±0.0021 (66.42)	0.908±0.0026 (74.92)	0.608±0.0012 (50.17)	0.704±0.0017 (58.09)

Values are mean ±SE of three replicates

Table 4: Effect of 40% and 80% of LD_{50} of *Cassia alata* petroleum ether fraction on glycogen, protein, DNA, RNA amino acid and lipid of *Callosobruchus chinensis*

Parameters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen (µg/gm)	2.148±0.0046 (100)	1.861±0.0087 (86.63)	1.771±0.0077 (82.44)	1.713±0.004 (79.74)	1.519±0.003 (70.71)	1.449±0.0048 (67.45)	1.345±0.0045 (62.61)	0.924±0.0066 (43.01)	0.892±0.0099 (41.52)
Protein (µg/gm)	10.512±0.0031 (100)	9.390±0.0055 (89.30)	8.9003±0.007 (84.64)	8.433±0.0079 (80.20)	7.434±0.0081 (70.69)	7.3207±0.0018 (69.62)	6.171±0.002 (58.69)	6.7856±0.007 (64.53)	8.101±0.0063 (58.02)
DNA (µg/gm)	0.924±0.0042 (100)	0.7892±0.0052 (85.41)	0.7135±0.0065 (77.21)	0.5683±0.0079 (61.50)	0.4827±0.0061 (52.24)	0.4860±0.0002 (52.59)	0.4467±0.0024 (48.34)	0.4708±0.0053 (50.95)	0.4329±0.0035 (46.85)
RNA (µg/gm)	0.6848±0.0002 (100)	0.5443±0.0053 (79.48)	0.5067±0.006 (73.99)	0.5184±0.0074 (75.70)	0.5038±0.0058 (73.57)	0.5064±0.0023 (73.95)	0.4811±0.0017 (70.26)	0.4842±0.0069 (70.71)	0.4641±0.0037 (67.77)
Amino acid (µg/gm)	0.9113±0.007 (100)	0.892±0.0040 (97.88)	0.829±0.0024 (90.97)	0.773±0.0074 (84.82)	0.691±0.0043 (75.82)	0.669±0.0024 (0.0024)	0.662±0.0026 (72.64)	0.554±0.0021 (60.79)	0.543±0.0035 (59.58)
Lipid (µg/gm)	1.212±0.0029 (100)	1.415±0.0032 (116.75)	1.509±0.0014 (124.51)	1.307±0.0028 (107.84)	1.411±0.006 (116.42)	1.110±0.0026 (91.59)	1.205±0.0029 (99.42)	0.805±0.0035 (66.42)	0.902±0.0014 (74.92)

Values are mean ±SE of three replicates

Table 5: Effect of 40% and 80% of LD_{50} of *Cassia alata* methanol fraction on glycogen, protein, DNA, RNA amino acid and lipid of *Callosobruchus chinensis*

Parameters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen (µg/gm)	2.148±0.0046 (100)	1.884±0.0069 (87.70)	1.739±0.0097 (80.95)	1.837±0.011 (85.51)	1.682±0.0064 (78.29)	1.505±0.0058 (70.06)	1.385±0.0052 (64.47)	0.805±0.0048 (37.47)	0.709±0.004 (33.00)
Protein (µg/gm)	10.512±0.0031 (100)	8.734±0.0073 (83.06)	8.2003±0.0001 (77.98)	7.112±0.0092 (67.63)	7.084±0.0057 (67.37)	6.7603±0.002 (64.29)	6.297±0.0031 (63.05)	5.506±0.0095 (52.36)	5.2405±0.0075 (49.84)
DNA (µg/gm)	0.924±0.0042 (100)	0.8329±0.0071 (90.14)	0.7898±0.005 (85.47)	0.7037±0.0063 (76.15)	0.6179±0.0081 (66.87)	0.6439±0.0017 (69.66)	0.5362±0.0035 (58.03)	0.4493±0.0063 (48.62)	0.4433±0.0064 (47.97)
RNA (µg/gm)	0.6848±0.0002 (100)	0.5659±0.0051 (82.64)	0.5378±0.0064 (78.53)	0.4962±0.0064 (72.46)	0.4723±0.0043 (68.97)	0.4793±0.0017 (69.99)	0.4493±0.0017 (65.61)	0.4548±0.0081 (66.41)	0.4321±0.0054 (63.10)
Amino acid (µg/gm)	0.9113±0.007 (100)	0.815±0.0035 (89.43)	0.733±0.0048 (80.43)	0.786±0.0043 (86.25)	0.693±0.0049 (76.04)	0.692±0.0026 (75.93)	0.578±0.0035 (63.42)	0.558±0.0023 (61.23)	0.534±0.0026 (58.59)
Lipid (µg/gm)	1.212±0.0029 (100)	1.313±0.0024 (108.34)	1.405±0.0029 (115.93)	1.107±0.0029 (91.34)	1.204±0.0023 (99.34)	0.806±0.0029 (66.50)	0.907±0.0043 (74.84)	0.711±0.0058 (58.66)	0.806±0.0035 (66.50)

Values are mean ±SE of three replicates

Table 6: Effect of 40% and 80% of LD_{50} of *Cassia alata* hexane fraction on glycogen, protein, DNA, RNA amino acid and lipid of *Callosobruchus chinensis*

Parameters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen (µg/gm)	2.148±0.0046 (100)	1.986±0.0053 (92.44)	1.3882±0.0064 (87.61)	1.753±0.0059 (81.60)	1.776±0.0081 (82.67)	1.692±0.0043 (78.76)	1.538±0.0075 (71.59)	0.979±0.010 (45.57)	0.974±0.0072 (45.35)
Protein (µg/gm)	10.512±0.0031 (100)	9.772±0.0064 (92.93)	9.533±0.0058 (90.66)	8.834±0.0074 (84.01)	8.6244±0.0065 (82.02)	7.5573±0.0029 (71.87)	7.2543±0.0027 (68.99)	6.5943±0.0069 (62.71)	6.1426±0.0041 (58.42)
DNA (µg/gm)	0.924±0.0042 (100)	0.8657±0.0067 (86.74)	0.8376±0.0032 (90.64)	0.7452±0.0055 (80.69)	0.7264±0.0054 (78.61)	0.6283±0.003 (67.99)	0.5656±0.0023 (61.21)	0.5319±0.0055 (57.56)	0.47±0.0023 (50.86)
RNA (µg/gm)	0.6848±0.0002 (100)	0.594±0.0055 (86.74)	0.5337±0.0096 (77.94)	0.5138±0.0076 (75.03)	0.4533±0.008 (66.19)	0.4827±0.0011 (70.49)	0.4355±0.0017 (63.60)	0.4013±0.0075 (50.60)	0.4007±0.0045 (58.51)
Amino acid (µg/gm)	0.9113±0.007 (100)	0.865±0.0035 (94.92)	0.820±0.0035 (89.98)	0.720±0.0029 (79.00)	0.668±0.003 (73.30)	0.693±0.0026 (76.04)	0.655±0.0026 (71.87)	0.675±0.004 (74.07)	0.648±0.0037 (71.10)
Lipid (µg/gm)	1.212±0.0029 (100)	1.308±0.0042 (107.92)	1.406±0.0031 (116.00)	1.210±0.0058 (99.83)	1.306±0.0035 (107.75)	0.915±0.0033 (75.49)	1.004±0.0023 (82.84)	0.604±0.002 (49.84)	0.705±0.0035 (58.17)

Values are mean ±SE of three replicates

Table 7: Effect of 40% and 80% of LD_{50} of *Cassia alata* aqueous fraction on glycogen, protein, DNA, RNA amino acid and lipid of *Callosobruchus chinensis*

Parameters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen (µg/gm)	1.923±0.004 (89.51)	1.916±0.0064 (89.18)	1.923±0.004 (89.51)	1.732±0.0064 (80.62)	1.811±0.0077 (84.30)	1.557±0.0056 (72.48)	1.626±0.0052 (75.69)	0.883±0.0047 (41.10)	0.957±0.0077 (44.55)
Protein (µg/gm)	9.328±0.0083 (88.73)	9.152±0.0045 (87.03)	9.328±0.0083 (88.73)	8.5093±0.011 (80.92)	8.9023±0.0058 (84.66)	7.1617±0.0055 (68.11)	7.324±0.0023 (69.65)	7.1577±0.0023 (68.07)	7.2033±0.0027 (68.50)
DNA (µg/gm)	0.8999±0.006 (97.39)	0.8365±0.0073 (90.53)	0.8999±0.006 (97.39)	0.7062±0.0068 (76.42)	0.7112±0.0059 (76.97)	0.5931±0.001 (64.11)	0.5893±0.0018 (63.77)	0.4649±0.0041 (50.31)	0.4933±0.0067 (53.38)
RNA (µg/gm)	0.6371±0.0065 (93.03)	0.6263±0.0049 (91.46)	0.6371±0.0065 (93.03)	0.5697±0.0055 (83.19)	0.5907±0.0024 (86.26)	0.5484±0.0023 (80.08)	0.5727±0.0017 (83.63)	0.515±0.0078 (75.20)	0.5624±0.0067 (82.13)
Amino acid (µg/gm)	0.813±0.0059 (89.21)	0.799±0.0040 (87.67)	0.813±0.0059 (89.21)	0.752±0.0033 (82.52)	0.759±0.0032 (83.28)	0.743±0.0026 (81.53)	0.751±0.003 (82.41)	0.733±0.004 (80.43)	0.739±0.0049 (81.09)
Lipid (µg/gm)	1.408±0.0035 (116.17)	1.514±0.0023 (124.92)	1.408±0.0035 (116.17)	1.304±0.0023 (107.59)	1.207±0.0035 (99.58)	1.005±0.0035 (82.92)	0.904±0.0002 (74.59)	0.808±0.0046 (66.67)	0.707±0.0036 (58.33)

Values are mean ±SE of three replicates

Table 8: Effect of 40% and 80% of LD_{50} of *Cassia alata* acetone fraction on ACP, ALP, GPT, GOT, LDH and AChE of *Callosobruchus chinensis*

Parameters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	2.240±0.004 (100)	2.220±0.05 (99.10)	2.124±0.022 (95.50)	2.018±0.0024 (90.08)	1.921±0.057 (85.75)	1.918±0.013 (85.62)	1.917±0.0233 (85.58)	1.915±0.0083 (85.49)	1.913±0.012 (85.40)
ALP	1.792±0.01 (100)	1.692±0.015 (94.41)	1.586±0.021 (88.50)	1.517±0.0057 (84.65)	1.502±.0.0081 (83.81)	1.481±0.011 (82.64)	1.466±0.041 (81.80)	1.424±0.003 (79.46)	1.419±0.0013 (79.18)
GPT	4.145±0.007 (100)	4.045±0.017 (97.58)	4.039±0.0083 (97.44)	4.031±0.0018 (97.24)	4.027±0.0012 (97.15)	4.005±0.012 (96.62)	3.912±0.003 (94.37)	3.909±0.002 (94.30)	3.886±0.0013 (93.75)
GOT	3.019±0.002 (100)	2.949±0.022 (97.68)	2.892±0.045 (95.79)	2.849±0.004 (94.36)	2.839±0.0024 (94.03)	2.821±0.031 (93.44)	2.816±0.01 (93.27)	2.807±0.012 (92.97)	2.801±0.022 (92.77)
LDH	8.301±0.019 (100)	8.297±0.019 (99.95)	8.287±0.012 (99.83)	8.281±0.0087 (99.75)	8.259±0.015 (99.49)	8.251±0.024 (99.39)	8.241±0.05 (99.27)	8.131±0.0017 (97.95)	8.001±0.011 (96.38)
AChE	0.938±0.012 (100)	0.918±0.012 (97.86)	0.911±0.0011 (97.12)	0.909±0.031 (96.90)	0.891±0.035 (94.98)	0.879±0.0014 (93.71)	0.852±0.007 (90.83)	0.846±0.0013 (90.19)	0.831±0.003 (88.59)

Values are mean ±SE of three replicates

Table 9: Effect of 40% and 80% of LD_{50} of *Cassia alata* chloroform fraction on ACP, ALP,GPT, GOT, LDH and AChE of *Callosobruchus chinensis*

Parameters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	2.240±0.004 (100)	2.198±0.0017 (98.12)	2.104±0.016 (93.92)	2.008±0.0019 (89.64)	1.891±0.018 (84.41)	1.788±0.003 (79.82)	1.751±0.0013 (78.16)	1.735±0.001 (77.45)	1.723±0.021 (76.91)
ALP	1.792±0.01 (100)	1.611±0.0053 (89.89)	1.566±0.016 (87.38)	1.502±0.0012 (83.81)	1.479±.008 (82.53)	1.471±0.0031 (82.08)	1.455±0.006 (81.19)	1.413±0.05 (78.85)	1.411±0.0023 (78.73)
GPT	4.145±0.007 (100)	3.895±0.007 (93.96)	3.777±0.008 (91.12)	3.661±0.0018 (88.32)	3.437±0.002 (82.91)	3.405±0.012 (82.14)	3.212±0.023 (77.49)	3.189±0.013 (76.93)	3.127±0.0043 (75.44)
GOT	3.019±0.002 (100)	2.909±0.02 (96.35)	2.812±0.015 (93.14)	2.809±0.0019 (93.04)	2.793±0.002 (92.51)	2.721±0.013 (90.12)	2.696±0.011 (89.30)	2.687±0.0021 (89.00)	2.485±0.022 (82.31)
LDH	8.301±0.019 (100)	8.167±0.019 (98.38)	8.127±0.021 (97.90)	8.101±0.0021 (97.59)	7.959±0.015 (95.88)	7.851±0.016 (94.57)	7.641±0.011 (92.04)	7.432±0.037 (89.53)	7.111±0.031 (85.66)
AChE	0.938±0.012 (100)	0.903±0.011 (96.26)	0.891±0.0022 (94.98)	0.885±0.031 (94.34)	0.871±0.025 (92.85)	0.857±0.0023 (91.36)	0.841±0.019 (89.65)	0.836±0.0013 (89.12)	0.811±0.008 (86.46)

Values are mean ±SE of three replicate

Table 10: Effect of 40% and 80% of LD_{50} of *Cassia alata* petroleum ether fraction on ACP, ALP,GPT, GOT, LDH and AChE of *Callosobruchus chinensis*

Parameters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	2.240±0.004 (100)	2.028±0.0017 (90.53)	1.865±0.016 (83.25)	1.856±0.0019 (82.85)	1.674±0.018 (74.73)	1.629±0.0001 (72.72)	1.612±0.0013 (71.96)	1.595±0.0012 (71.20)	1.583±0.012 (70.66)
ALP	1.792±0.01 (100)	1.511±0.05 (84.31)	1.502±0.036 (83.81)	1.459±0.0022 (81.41)	1.439±.0081 (80.30)	1.421±0.0011 (79.29)	1.401±0.036 (78.18)	1.391±0.015 (77.62)	1.365±0.03 (76.17)
GPT	4.145±0.007 (100)	3.811±0.007 (91.94)	3.711±0.01 (89.52)	3.632±0.0083 (87.62)	3.412±0.012 (82.31)	3.398±0.0021 (81.97)	3.201±0.0021 (77.22)	3.179±0.002 (76.69)	3.109±0.033 (75.00)
GOT	3.019±0.002 (100)	2.801±0.0009 (92.77)	2.792±0.03 (92.48)	2.768±0.0019 (91.68)	2.756±0.002 (91.28)	2.702±0.033 (89.49)	2.659±0.001 (88.07)	2.652±0.003 (87.84)	2.414±0.002 (79.96)
LDH	8.301±0.019 (100)	8.066±0.0019 (97.16)	8.048±0.012 (96.91)	8.035±0.0083 (96.79)	7.788±0.045 (93.82)	7.771±0.016 (93.61)	7.523±0.011 (90.62)	7.369±0.017 (88.77)	7.242±0.011 (87.24)
AChE	0.938±0.012 (100)	0.811±0.013 (86.46)	0.781±0.0011 (83.26)	0.772±0.031 (82.30)	0.765±0.045 (81.55)	0.731±0.014 (77.93)	0.712±0.019 (75.90)	0.703±0.03 (74.94)	0.687±0.011 (73.24)

Values are mean ±SE of three replicates

Table 11: Effect of 40% and 80% of LD_{50} of *Cassia alata* methanol fraction on ACP, ALP,GPT, GOT, LDH and AChE of *Callosobruchus chinensis*

Parameters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	2.240±0.004 (100)	2.118±0.002 (94.55)	2.004±0.04 (89.46)	1.898±0.009 (84.73)	1.787±0.018 (79.77)	1.766±0.0013 (78.83)	1.731±0.0023 (77.27)	1.715±0.002 (76.56)	1.703±0.011 (76.02)
ALP	1.792±0.01 (100)	1.587±0.015 (88.56)	1.546±0.016 (86.27)	1.492±0.005 (83.25)	1.468±.0009 (81.91)	1.454±0.03 (81.13)	1.447±0.016 (80.74)	1.403±0.05 (78.29)	1.391±0.0023 (77.62)
GPT	4.145±0.007 (100)	3.825±0.006 (92.27)	3.717±0.001 (89.67)	3.641±0.0081 (87.84)	3.417±0.0032 (82.43)	3.401±0.021 (82.05)	3.209±0.003 (77.41)	3.181±0.0032 (76.74)	3.116±0.0013 (75.17)
GOT	3.019±0.002 (100)	2.813±0.02 (93.17)	2.802±0.015 (92.81)	2.779±0.009 (92.05)	2.764±0.0012 (91.55)	2.711±0.0013 (89.79)	2.676±0.01 (88.63)	2.666±0.034 (88.30)	2.425±0.021 (80.32)
LDH	8.301±0.019 (100)	8.086±0.0019 (97.40)	8.059±0.032 (97.08)	8.041±0.0031 (96.86)	7.869±0.05 (94.79)	7.848±0.016 (94.54)	7.591±0.002 (91.44)	7.397±0.0009 (89.10)	7.261±0.0021 (87.47)
AChE	0.938±0.012 (100)	0.887±0.012 (94.56)	0.872±0.0011 (92.96)	0.862±0.005 (91.89)	0.853±0.01 (90.93)	0.846±0.004 (90.19)	0.839±0.009 (89.44)	0.822±0.013 (87.63)	0.801±0.001 (85.39)

Values are mean ±SE of three replicates

Table 12: Effect of 40% and 80% of LD_{50} of *Cassia alata* hexane fraction on ACP, ALP,GPT, GOT, LDH and AChE of *Callosobruchus chinensis*

Parameters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	2.240±0.004 (100)	1.877±0.001 (83.79)	1.745±0.006 (77.90)	1.564±0.009 (69.82)	1.446±0.008 (64.55)	1.421±0.0013 (63.43)	1.382±0.0031 (61.69)	1.355±0.001 (60.49)	1.311±0.05 (58.52)
ALP	1.792±0.01 (100)	1.482±0.01 (82.700)	1.442±0.02 (80.46)	1.431±0.0012 (79.85)	1.425±.0001 (79.52)	1.412±0.021 (80.13)	1.391±0.006 (77.62)	1.372±0.03 (76.56)	1.351±0.0023 (75.39)
GPT	4.145±0.007 (100)	3.802±0.007 (91.72)	3.703±0.009 (89.33)	3.522±0.008 (85.96)	3.401±0.0012 (82.05)	3.359±0.015 (81.03)	3.195±0.003 (77.08)	3.172±0.002 (76.52)	2.979±0.0013 (71.86)
GOT	3.019±0.002 (100)	2.783±0.032 (92.18)	2.751±0.009 (91.12)	2.658±0.0019 (88.04)	2.639±0.0022 (87.41)	2.621±0.013 (86.81)	2.619±0.04 (86.75)	2.602±0.001 (86.18)	2.401±0.032 (79.52)
LDH	8.301±0.019 (100)	7.969±0.0001 (96.00)	7.849±0.012 (94.55)	7.735±0.008 (93.18)	7.728±0.006 (93.09)	7.702±0.016 (92.78)	7.513±0.011 (90.50)	7.319±0.0009 (88.17)	7.214±0.0011 (86.90)
AChE	0.938±0.012 (100)	0.781±0.011 (83.26)	0.774±0.0081 (82.51)	0.766±0.01 (81.66)	0.754±0.015 (80.38)	0.711±0.004 (75.79)	0.703±0.009 (74.94)	0.685±0.0013 (7302)	0.672±0.021 (71.65)

Values are mean ±SE of three replicates

Table 13: Effect of 40% and 80% of LD_{50} of *Cassia alata* aqueous fraction on ACP, ALP,GPT, GOT, LDH and AChE of *Callosobruchus chinensis*

Parameters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	2.240±0.004 (100)	2.134±0.0061 (95.26)	2.038±0.002 (90.98)	1.941±0.003 (86.65)	1.938±0.013 (86.51)	1.931±0.023 (86.20)	1.925±0.038 (85.93)	1.913±0.019 (85.40)	1.903±0.003 (84.95)
ALP	1.792±0.01 (100)	1.786±0.006 (99.66)	1.717±0.005 (95.81)	1.686±.0001 (94.08)	1.671±0.0009 (93.24)	1.666±0.04 (92.96)	1.624±0.031 (90.62)	1.619±0.0002 (90.34)	1.594±0.0011 (88.95)
GPT	4.145±0.007 (100)	4.139±0.005 (99.85)	4.131±0.0031 (99.66)	4.127±0.0021 (99.56)	4.115±0.011 (99.27)	4.112±0.0013 (99.20)	4.109±0.002 (99.13)	4.056±0.013 (97.85)	3.991±0.02 (96.28)
GOT	3.019±0.002 (100)	3.006±0.002 (99.56)	2.949±0.003 (97.74)	2.915±0.005 (96.55)	2.911±0.031 (96.42)	2.906±0.021 (96.25)	2.897±0.0034 (95.95)	2.891±0.012 (95.76)	2.671±0.0045 (88.47)
LDH	8.301±0.019 (100)	8.297±0.012 (99.95)	8.285±0.0083 (99.80)	8.269±0.001 (99.61)	8.256±0.006 (99.45)	8.248±0.03 (99.36)	8.149±0.017 (98.16)	8.041±0.031 (96.86)	7.981±0.01 (96.14)
AChE	0.938±0.012 (100)	0.926±0.001 (98.72)	0.919±0.031 (97.97)	0.911±0.024 (97.12)	0.909±0.0023 (96.90)	0.901±0.04 (96.05)	0.896±0.0023 (95.52)	0.881±0.006 (93.92)	0.863±0.003 (92.00)

Values are mean ±SE of three replicates

DISCUSSION

Stored grain infestation is a very serious problem in South East Asia. For effective and fast control of stored grains insect pest's farmers and godown owners have massively used synthetic pesticides, which have shown very high lethality, but subsequently, these insecticides contaminated the environment and put adverse effects on non-target animals. Still after use these chemicals persist for longer periods in the medium in form of residues. However in the present investigation natural extracts isolated from *C. alata* have been used to observe its lethality in *C. chinensis.* The solvent and aqueous extracts of *C. alata* have shown potent toxicity against the *C. chinensis* as it have shown very low LD_{50} 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, for acetone, chloroform, petroleum ether, methanol, hexane and water extracts respectively (Table 1). Similarly, some other plants have been studied for its toxicity against certain insect pest such as *Artemisia princepi* and *Cinnamomum camphora* (L) have shown potent toxic activity against *Sitophilus oryzae* and *Bruchus rugimanus* [17] having low LD_{50} value. Active compounds isolated from *Foeniculum vulgare* [18] and Japanese mint (*Mentha arvensis*) [19] have successfully control *S. oryzae, T. castenum* and *Rhizopertha dominica* (F) [20]. Solvent and aqueous extracts of *C. alata have* significantly cut down the glycogen, protein DNA, RNA amino acids and lipid content in *C. chinensis*. Similarly cypermethrin treated larvae, pupae and adults of *Pimpla turionella* (wasp) have shown cut down of glycogen, lipid protein, nucleic and acids [21]. Methanol extract have reduced the glycogen content 33.00% in comparison to control (Table 11). Depletion of glycogen indicates more and more utilization of food reserves to cope up the insecticide induced stress that induces release of glucagon, corticosteroids and catecholamines stimulating glucose production to combat energy demand. Normally in the body free glycogen floats in the haemolymph 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 [22].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 [23]. It is well known that stored lipids are most important and convenient reservoirs of metabolic energy, which fulfill prolonged energy demand in insects in stress. Physiologically lipids play an important role in insect survival. Once lipid metabolism is inhibited most of the normal physiological activities of insect get obstructed that result in to death [24].

However, certain metabolic enzymes such as ACP, ALP GPT, GOT, LDH and AChE have been found to be reduced after the treatment of *C. alata* extracts. This inhibition indicates obstruction in their chemical pathways that led to the formation of abnormal state in the insects and make insects unable to survive [25].Although al the extracts have displayed toxic effects on enzyme activity of *C. chinensis,* hexane extract have shown some what higher activity against the same enzyme. Experiment insect have shown lower ACP (58.53%) and ALP (75.39%) content than that of control insects (Table 12).

Further, an increase in glycogenesis causes a significant decrease in free amino acid level and transamination of amino acids was reduced, hence the level of glutamate pyruvate transaminase (71.86%) and glutamate oxalo acetate transaminase (79.52) get decreased (Table 12). As a result of obstruction in transaminase activity, protein synthesis was retarded. Therefore, a sharp decrease or increase in the level of above enzymes effect oxygen consumption in insects. In the

present study change in the level of various enzymes in whole body extract of *C. chinensis* may be due to physiological alterations which are induced by compounds isolated from different solvent extracts of *C. alata*. However, elevation or reduction in enzyme level is associated with physiological imbalance in insects [26]. Similarly, it have been also reported the changes in the levels of certain enzyme during the course of insecticidal effect of malathion and carbaryl [27]. However, *C. alata* it can be concluded that solvent extracts of *C. alata* work as potent molecular toxicant showing very high lethality on body tissues of *C. chinensis*. A sharp decrease in lactic dehydrogenase level shows tissue necrosis in insects [28]. However, this imbalance in enzyme level indicates inhibition of important metabolic pathways [29]. Similarly Upadhyay et al., [30] have already reported the lethal effects of plant extract against bio-molecule and enzymatic parameter of Indian white termite, *Odontotermes obesus*. Hence, all significant changes in the level of ALP, ACP, GPT, GOT, LDH and AchE indicate very high insecticidal activity of the *C. alata* extracts towards the *C. chinensis* However, it can be concluded that *C. alata* 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 *C. chinensis* at a very low dose and caused significant inhibition of metabolic enzymes. Therefore, it is recommended that *C.alata* active ingredients could be used for preparation of herbal insecticidal formulation to control stored grain insects.

Hence, significant alteration in body content of bio-molecules and vital enzymes are the sign of very high insecticidal activity of the *C. alata* extracts against the *C. chinensis* However, it can be concluded that *C. alata* 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 *C. chinensis* at a very low dose and caused significant inhibition of metabolic enzymes. Therefore, it is recommended that *C.alata* active ingredients could be used for preparation of herbal insecticidal formulation to control stored grain insects.

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