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# TOXIC EFFECTS OF *CURCUMA LONGA* DIFFERENT EXTRACTS ON BIOCHEMICAL AND ENZYMATIC PARAMETERS OF *TRIBOLIUM CASTANEUM*

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**A R T I C L E I N F O**

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**A B S T R A C T**

The Evolution of resistance in the red flour beetle *Tribolium castaneum* against several insecticides threatens sound storage of stored grain products. Different extracts of *C. longa* was assessed to demonstrate their toxic effects on biomolecules and certain metabolic enzymes of *T. castaneum*. After 12 hrs of extract exposure of *C. longa* different extracts the body contents of Protein, DNA and RNA content were depleted compared to control. However, it also suppressed the level of metabolically significant enzymes (GOT (66 %), GPT (77 %), ALP (53 %), LDH (76 %) and Amylase (63 %)). Among the different extracts, methanol extract of *C. longa* is proved to have strong insecticidal activity against *T. castaneum* and can be used for control of stored grain insects.



## INTRODUCTION

Stored product insects cause serious losses in weight and quality of the stored products during storage (Evans, 1987). Among the stored-product beetles, *Tribolium castaneum* (Herbst) can be a major pest in storage of grain-based products (Campbell and Runnion, 2003) and *Sitophilus oryzae* (L.) cause severe damage to cereal grains throughout the world (Agarwal *et al.,* 1979; Daglish *et al.,* 1996). This insect found wherever grains or other dried foods are stored, has a highly evolved kidney-like cryptonephridial organ to survive such extremely dry environments. It has demonstrated resistance to all classes of insecticides used against it (Tribolium Genome Sequencing Consortium, 2008). Annual post-harvest losses resulting from insect damages, microbial deterioration and other factors are estimated to be 10-25 % of worldwide production (Mathews, 1993). The development of environmental friendly insecticides, having specificity to insects along with low toxicity to vertebrates, has captured worldwide attention of scientists (Ishaaya and Degheele, 1998). However, the continuous application and excessive reliance on chemical pesticides have resulted in serious drawbacks on the environment and toxicity hazards on non target organisms including users (Isman, 2006). These hazards pushed the research toward investigating environmentally friendlier alternatives for combating insect pests.

One possible approach is the use of phytochemicals and plant-derived materials as pest control agents with no or minimal side effects, especially against those of stored grains (Cox, 2004; Rajendran and Sriranjini, 2008; Cosimi *et al.,* 2009).

Therefore, there is an urgent need to develop safe, convenient, environmental and low-cost alternatives. Considerable efforts have been focused on plant derived materials for potentially useful products as bioinsecticides (Regnault- Roger *et al.,* 2002). Bioinsecticides may be more rapidly degraded in the environment than synthetic compounds (Koul and Dhawiwal, 2001). Traditionally, plant materials have played an important role in the protection of grains against insect infestation in Africa since ancient times. Many plant-derived materials do not cause resistance development in insects, have broad spectrum activity, are safe to natural enemies, compatible with biological control agents for integrated pest management (IPM), and are non-toxic to the environment (Anonymous, 1991; Talukder and Miyata, 2002). The main advantage of plant products is that they are easily produced by farmers and small-scale industries and are less expensive (Talukder and Howse, 1995). However, in the present study, insecticidal effects of *C. longa* were observed on biochemical and enzymatic parameters of *T. castaneum.*

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## MATERIALS AND METHODS

### *Insect Culture*

The adult *Tribolium castaneum* was collected from infested grains purchased from local market and brought to the laboratory. The culture was established using rava in a plastic container of 25 X 10 cm and maintained at room temperature 30±2°C and relative humidity of 70-75 %. Sieving the culture separated the adult insects and the adults were used for subsequent experiment. The culture was continuously maintained in the containers throughout the study period.

### *Collection of plant material*

The fresh plant *Curcuma longa* was collected from Kolli hills, Tamil Nadu, India. The taxonomical identification of the plants were confirmed by Department of Botany, Government Arts College, Dharmapuri, Tamil Nadu.

### *Preparation of plant material and solvent extraction*

The fresh plant leaf was harvested, rinsed with tap water and air dried under shadow for 14 days and reduced to coarse powder using mixer blender. After defatted by petroleum ether, the samples (15 g) were extracted by stirring with 105 ml 70 % acetone at 25$^{o}$C for 48 h and filtering through Whatman No. 4 filter paper. The residues were re extracted with an additional 75 ml of acetone, as described above, for 3 h. The solvent of the combined extract was evaporated under low temperature at 40$^{o}$C in incubator respectively. The remaining residues, after acetone extraction and air drying, were extracted by stirring with 105 ml 80 % methanol (v/v) at 25$^{o}$C for 48 h and filtering through Whatman No. 4 filter paper. Finally the residues were extracted with chloroform at 25$^{o}$C for 48 h and filtering through Whatman No. 4 filter paper. The solvent of extract was evaporated under low temperature at 40$^{o}$C in incubator respectively. The extracts were stored at 4$^{o}$C until further analysis.

### *Toxicity bio-assays*

Adults of *T. castaneum* were exposed with 5 mg/ml a concentration of each plant extracts separately. For this purpose, separate filter paper strips (1 cm$^{2}$) were coated with 5 mg/ml 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. Mortality in *T. castaneum* was recorded after 24 hrs in presence and absence of various plants extracts separately.

### *Determination of nucleic acids*

Level of nucleic acids in the whole body extracts of *T. castaneum* was estimated according to method of Scheidner (1957). Insects were scarified and homogenized in 5 % TCA with glass homogenizer at 15, 000 × 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 g of diphenylamine, 100 ml 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 colour was developed in the solution which is measured at 595 nm.

### *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 g 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 colour was developed, which was measured at 660 nm.

### *Determination of total protein*

Total proteins of *T. castaneum* were estimated according to Lowry *et al.* (1951). These treated *T. castaneum* were homogenized in 4.0 ml of 10 % TCA with the help of 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 - 100 µg of Bovine serum albumin.

### *In vivo Determination of enzymatic parameters*

To observe the effect on enzymatic parameters 500 mg of *T. castaneum* were provided 5 mg/ml concentration of different solvent extract of *C. longa* was provided. Insects were sacrificed at the 12 hrs for measurement of various enzyme levels. Insects were homogenized in phosphate saline buffer (pH 6.9) in a 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 glutamate pyruvate transaminase and glutamic oxaloacetic transaminase*

GPT and GOT activity was measured according to the method of Reitman and Frankel (1957). 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/hrs/mg protein.

### *Determination of lactic dehydrogenase*

Activity of lactic dehydrogenase was measured according to the method of Annon (1984). 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 moles of pyruvate reduced/45min/mg protein.

***Determination of alkaline phosphatase***

Level of alkaline phophatase level was determined according to the method of Bergmeyer (1967). For this purpose 500 mg of *T. castaneum* were homogenized in 1 ml of PBS buffer at 4℃ and centrifuged at 15,000 × g for 15 min. A 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℃. 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 $\mu$ moles of p-nitrophenol formed /30min/mg protein.

***Amylase assay***

Enzyme dilutions were prepared using 50 mM sodium phosphate buffer at pH 7.0 for each strain separately and kept in ice-box. Dinitrosalicylic cid was mixed in 2M NaOH and slowly added sodium potassium tartarate tetrahydrate as colour reagent. 1 % starch was dissolved in 0.05 M sodium phosphate buffer by boiling gently. Enzyme dilutions were mixed with dinitrosalicylic acid and starch solution in test tubes and incubated at 25°C for 5 minutes. Optical density was measured at 540 nm. The specific activity was determined as under: Specific activity (IU/mg) = (micromoles maltose librated/ (mg enzyme used × 5 minutes)).

One unit is the amount of enzyme which under defined assay conditions will catalyze the conversion of 1 µmoles of substrate per min (Awan *et al.,* 2012).

***Statistical analysis***

The data were subjected to one-way analysis of variance (ANOVA), and the significance of the difference between means was determined by Duncan's multiple-range test ($P < 0.05$) using SPSS 13. Values expressed are means of triplicate determinations ± standard deviation. The $LD_{50}$ for each extract was determined by using probit analysis.

## RESULTS AND DISCUSSION

***Determination of biomolecules***

*T. castaneum* exposed with sub-lethal concentrations of methanol, acetone and chloroform of *C. longa* have shown significant depleted in protein, DNA and RNA content compared to control (Table - 1). The protein, DNA and RNA content was depleted ranged from (39-54 %), (29-57 %) and (29-57 %). Among the various samples, methanol extract showed high reduction of biomolecules followed by acetone and chloroform. Similarly active compounds isolated from *Piper nigrum* have shown strong insecticidal activity against *T. castaneum* (Upadhyay and Jaiswal, 2007). Our results are similar to the Upadhyay (2011) reported that fractions of *C. decidua* have shown strong significant decrease in protein (40.27 %), DNA (37.14 %) and RNA (30.31 %) contents after 16 hrs of exposure. Similarly protein and nucleic acids synthesis may also block at cellular level and catabolism get increased which results into low availability of proteins and nucleic acids.

***Determination of enzymes***

*T. castaneum* exposed with sub-lethal concentrations of methanol, acetone and chloroform of *C. longa* have shown significant depleted in GOT, GPT, ALP, LDH and Amylase content compared to control (Table - 2). Among the various samples, methanol extract showed high reduction of enzyme activities followed by acetone and chloroform. More specifically, *C. longa* methanol extract have shown significant inhibition in certain enzymes GOT (66 %), GPT (77 %), ALP (53 %), LDH (76 %) and Amylase (63 %). This reduction indicates the obstruction in their chemical pathways. This led to the formation of abnormal state in the insects and make insects unable to survive. Similarly solvent and aqueous extracts of *Gloriosa superba* (Khan *et al.,* 2007), *Cassia obtusifolia* (Kim *et al.,* 2007), *Artemisia annua* (Shekari *et al.,* 2008), *Teucrium royleanum* significantly inhibit certain enzymes activities of insects (Sigurdsson and Gudbjarnason, 2007). Therefore, the use of plant products (crude extracts, oil, powders, etc.) as insecticides in stored product protection might benefit the farmers by a reduction of protection costs, insecticide resistance development and environmental impact in terms of insecticidal hazard. Our results have shown that *C. longa* methanol extract possesses high insecticidal activity against adult *Tribolium castaneum* as compared with other two extracts. Our next approach will be targeted to concentrate efforts in a few more promising extracts to fractionate them and isolate possible active compounds.

**Table1 Effect** of $LD_{50}$ of *C. longa* different extracts on Protein, DNA and RNA of *T. castaneum*

| Parameters | Control | CLM | CLA | CLC |
|---|---|---|---|---|
| Protein | 10.6±0.2 | $4.8^{c}$ ±0.1 | $5.6^{b}$ ±0.3 | $6.5^{a}$ ±0.3 |
| DNA | 0.7±0.1 | $0.3^{b}$ ±0.2 | $0.5^{a}$ ±0.1 | $0.5^{a}$ ±0.2 |
| RNA | 0.7±0.3 | $0.3^{c}$ ±0.2 | $0.4^{b}$ ±0.1 | $0.5^{a}$ ±0.1 |

Values are mean of triplicate determinations ± standard deviation. Mean values followed by different superscript in the same row are significantly ($P < 0.05$) different. CLM; *Curcuma longa* methanol extract; CLA; *Curcuma longa* acetone extract; CLC; *Curcuma longa* chloroform extract.

**Table 2** Effect of $LD_{50}$ of *C. longa* different extracts on GOT, GPT, ALP, LDH and Amylase of *T. castaneum*

| Parameters | Control | CLM | CLA | CLC |
|---|---|---|---|---|
| GOT | 4.4±0.02 | $1.5^{c}$ ±0.01 | $2.8^{b}$ ±0.03 | $3.2^{a}$±0.02 |
| GPT | 5.2 ±0.03 | $1.2^{c}$ ±0.04 | $3.1^{b}$ ±0.03 | $3.5^{a}$ ±0.03 |
| ALP | 3.2 ±0.05 | $1.5^{b}$±0.02 | $1.8^{a}$±0.01 | $1.8^{a}$ ±0.01 |
| LDH | 7.8 ±0.22 | $1.9^{c}$ ±0.1 | $4.7^{b}$ ±0.1 | $5.2^{a}$ ±0.2 |
| Amylase | 40 ±0.54 | $15^{b}$±0.45 | $25^{a}$ ±0.54 | $27^{a}$±0.32 |

Values are mean of triplicate determinations ± standard deviation. Mean values followed by different superscript in

the same row are significantly ($P < 0.05$) different. CLM; *Curcuma longa* methanol extract; CLA; *Curcuma longa* acetone extract; CLC; *Curcuma longa* chloroform extract.

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