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## ANTHELMINTIC ACTIVITY OF CYAMPOSIS TETRAGONOLOBA (L.) TAUB.

Sumitra Singh\*<sup>1</sup>, Bhagwati Devi<sup>1</sup>

<sup>1</sup>Department of Pharmaceutical Science, Guru Jambheshwar University of Science and Technology, Hisar, Haryana India

### ABSTRACT

Ethanol and aqueous extracts from leaf, stem and fruits of *Cyamposis tetragonoloba* (L.) Taub. were investigated for their anthelmintic activity by using four concentrations (12.5, 25, 50, 100 mg/ml) of each extract against *Pheretima posthuma* as test worms. Results were expressed in terms of time of paralysis and time of death of worms and the activity was compared with albendazole as reference standard. Dose dependent decreased paralyzing time and death time was observed. The ethanol and aqueous extract of fruit and leaves exhibited significant anthelmintic activity but extracts from stem part was not effective against test worms. The results thus support the use of fruits and leaves of *Cyamposis tetragonoloba* (L.) Taub. as an anthelmintic agent.

**KEYWORDS** : Anthelmintic activity, *Pheretima posthuma*, *Cyamposis tetragonoloba*

### INTRODUCTION

People living in poverty in developing countries often suffer from helminth infections, which more often physically impair their hosts than kill them. Although the majority of infections due to worms are generally limited to tropical regions, they can occur to travellers who have visited those areas and some of them can develop in temperate climate<sup>[1]</sup>. Anthelmintic drugs are used to treat infections with parasitic worms. Parasitic worms also infect livestock and crops, affecting food production with a resultant economic impact<sup>[2]</sup>. Helminthes infections are among the most common infections in man, they pose a large threat to public health and contribute to the prevalence

of malnutrition, anemia, eosinophilia and pneumonia in developing countries<sup>[3]</sup>. *Cyamposis tetragonoloba* (L.) Taub. family Fabaceae is a native plant of India. It is commonly known as cluster bean<sup>[4]</sup>. It is drought resistant and cultivated as grain, fodder or vegetable<sup>[5]</sup>. Traditionally, leaves are used in asthma and to cure night blindness where as the pods and seed are used to cure inflammation, sprains, arthritis<sup>[6]</sup>, as anti-oxidant, antibilious, laxatives, in polluting boiling, night blindness, dyspeptic complaints and constipation<sup>[7]</sup>. As per Ayurveda the plant can be used as cooling, digestive, tonic, galactagogue, useful in constipation, dyspepsia, anorexia, agalata,

### Correspondence to Author

Sumitra Singh

Department of Pharmaceutical Science, Guru Jambheshwar University of Science and Technology, Hisar, Haryana India

Email: sumitra.singh32@gmail.com

hyetalopia and vitated condition of kapha and pitta<sup>[6]</sup>.

## **MATERIAL AND METHODS:**

### **Plant material**

The plant of *Cyamopsis tetragonoloba* (L.) Taub. was collected from cultivated field at Charkhi Dadri (Haryana, India) in the month of August 2012. The plant was taxonomically identified and authenticated by Dr. Roshni Nayar, Head, Raw Materials, Herbarium and Museum Division, NISCAIR, New Delhi, vide reference no, NHCP/NBPGR/2012-34, date 20/10/12. A voucher specimen of the same has been retained in the Department for the future reference. The leaves, stem and fruits were used to carry out the experimental work.

### **Preparation of Extract**

The air-dried crude samples of leaves, stem and fruits were pulverized and extracted with ethanol using soxhlet apparatus for 24 hours. The solvent was removed under vacuum. Semi solid masses with a yield of 15%, 11% and 19% w/w respectively were obtained. The marc obtained after ethanol extraction were air dried and cold maceration was done with distilled water to get aqueous extracts (yield 18% ,16.5% and 20% respectively). The extracts were further for preliminary photochemical screening and for the evaluation of anthelmintic activity.

### **Phytochemical screening**

The ethanol extract of leaves, stem and fruit of *Cyamopsis tetragonoloba* (L.) Taub. were screened for the presence of various phytoconstituents such as carbohydrates, glycosides, phenolic, tannins, sterols, saponins, proteins, amino acids and fatty acids.

### **Collection and authentication**

Adult Indian earthworms (*Pheretima posthuma*) were used for the evaluation of *in vitro* anthelmintic activity. They were collected from Agronomy Department of Chaudhary Charan Singh Haryana Agriculture University (CCSHAU), Hisar (Haryana) and authenticated by Dr. Thakral (Senior Scientist), Agronomy Department, CCSHAU, Hisar. Then all collected worms were washed with normal saline to remove all the fecal matter and used for

the anthelmintic study. The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were used for all the experimental protocol.

### **Sample preparation**

Test samples for *in-vitro* study were prepared by dissolving and suspending 2.5 gm of ethanol and aqueous extracts of each part of herb in 1% acacia and the volume was adjusted to 25 ml with distilled water to obtain a stock solution of concentration of 100 mg/ml, from this stock solution further dilutions were prepared to obtain concentrations range of 12.5, 25 , 50 and 100 mg/ml<sup>[8,9]</sup>.

### **Anthelmintic Assay**

The anthelmintic activity was performed on adult Indian earthworm *Pheretima posthuma* as it has anatomical and physiological resemblance with the intestinal roundworm parasites of human beings<sup>[10]</sup>. These are easy available and suitable model for screening of anthelmintic drugs. The worms were divided into fourteen groups containing six earthworms of approximately equal sizes placed in petridishes for each concentrations separately. 50 ml suspension of ethanol extracts (12.5, 25, 50 and 100 mg/ml) of leaves, stem and roots respectively were used as test samples, albendazole (20 mg/ml) as reference standard while distilled water as control group were poured into the petridishes<sup>[11]</sup>. Observations were made for the time taken to paralyse or death of individual worms. Paralysis was said to occur when the worms do not move even in water on shaken vigorously. Death was concluded when the worms lose their motility followed with fading away of their body color and was ascertained by transferring it into a beaker containing hot water at 50°C<sup>[12]</sup>.

## **RESULTS AND DISCUSSION**

Preliminary phytochemical studies on *Cyamopsis tetragonoloba* (L.) Taub. revealed the presence of carbohydrates, proteins, saponins, phenolic and tannins in different extracts of leaves, stem and fruits, but sterols polyphenolic compounds<sup>[13]</sup> and alkaloids were only present in leaves and fruits extracts. Some of these phytoconstituents may be responsible for anthelmintic activity<sup>[14]</sup>. Briefly, Four different concentrations, each of crude

ethanol and aqueous extract of fruit, stem and leaf (12.5, 25, 50 and 100 mg/ml in distilled water) were prepared and six earthworms (same size). Both the test solution and standard drug solution were freshly prepared and 'time for paralysis' was noted when no movement of any sort could be observed except when the worms were vigorously shaken. The 'time for death' of worms was recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water at 50°C. A maximum time period of 120 min was ascertained for the paralyzing as well as death time of *Pheretima posthuma* worms. Albendazole (20 mg/ml) was used as reference standard with distilled water as the vehicle control. All experiments were repeated thrice. The mean and SEM were analyzed statistically by ANOVA followed by Dunnett's test,  $P < 0.05$  being considered as significant.

The test extracts showed anthelmintic activity in a dose-dependent manner but the ethanol extracts

of fruit and leaf appeared to be more effective for worms. The stem ethanol and aqueous extract showed non-significant result [Fig. 1(a), 1(b)]. The ethanol extracts (100mg/ml) of the leaf and fruit of *Cyamposis tetragonoloba* Linn. Taub. caused significant ( $p < 0.001$ ) paralysis at  $30.6 \pm 0.33$ ,  $38.8 \pm 0.6$  min. and time of death at  $54.33 \pm 0.843$ ,  $60.67 \pm 0.803$  min. for *Pheretima posthuma* worms [Fig. 2(a), 3(a)]. Result with the aqueous extracts (100mg/ml) of leaf and fruits, the significant ( $p < 0.01$ ) paralysis time of  $37 \pm 0.577$ ,  $46.6 \pm 0.6$  min while time of death was  $67.67 \pm 0.749$ ,  $67.5 \pm 0.803$  min, respectively was observed [Fig. 2(b), 3(b)]. The reference drug albendazole showed the time of paralysis and time of death as  $8.67 \pm 0.6009$  and  $16.67 \pm 0.8437$ , respectively. Considering the ethanol extract of leaf and fruit showed comparable activity (highly significant), it would be important to identify the key phytoconstituents responsible for this activity.

#### TABLES & FIGURES

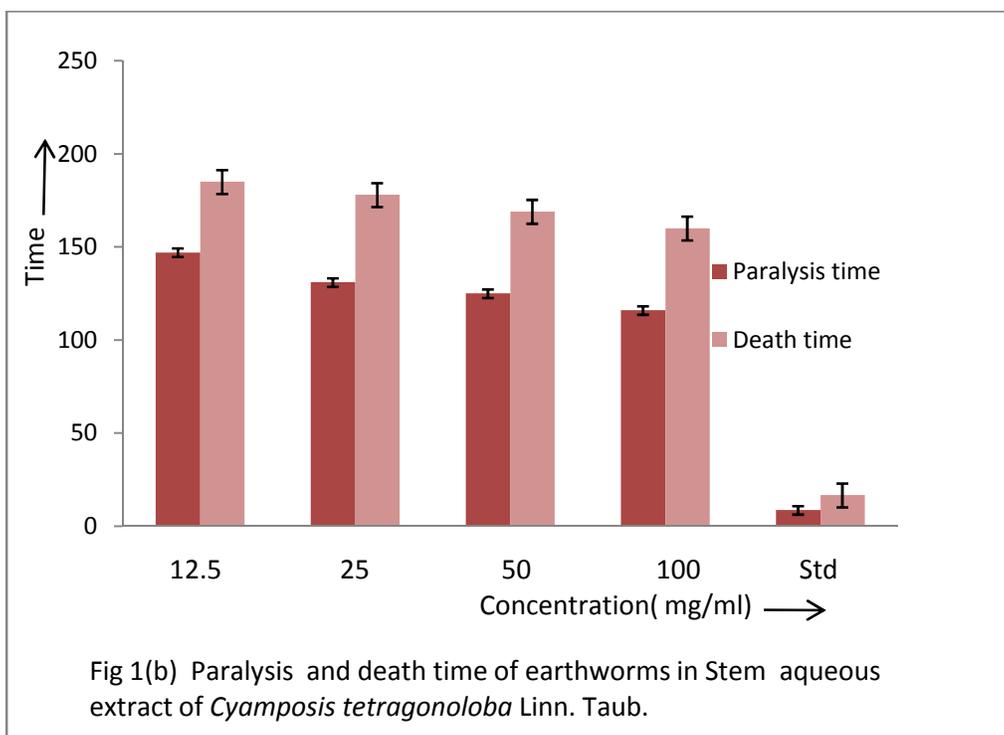
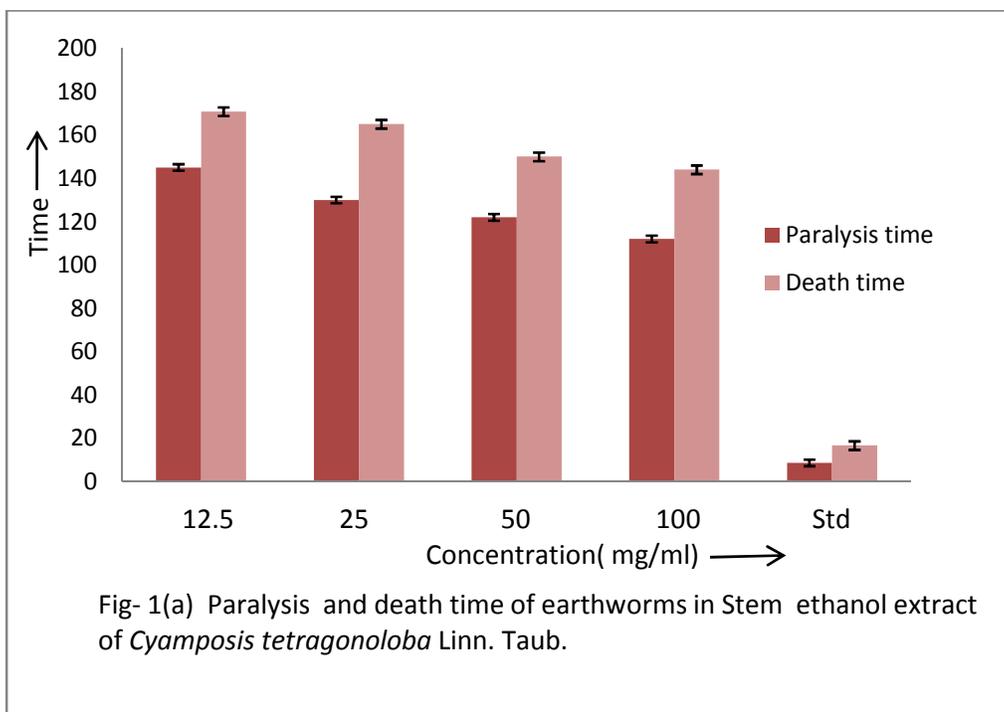
Table No: 01. Anthelmintic activity of Different solvent extracts of Fruit, Leaf and Stem of *Cyamposis Tetragonoloba* Linn. Taub.

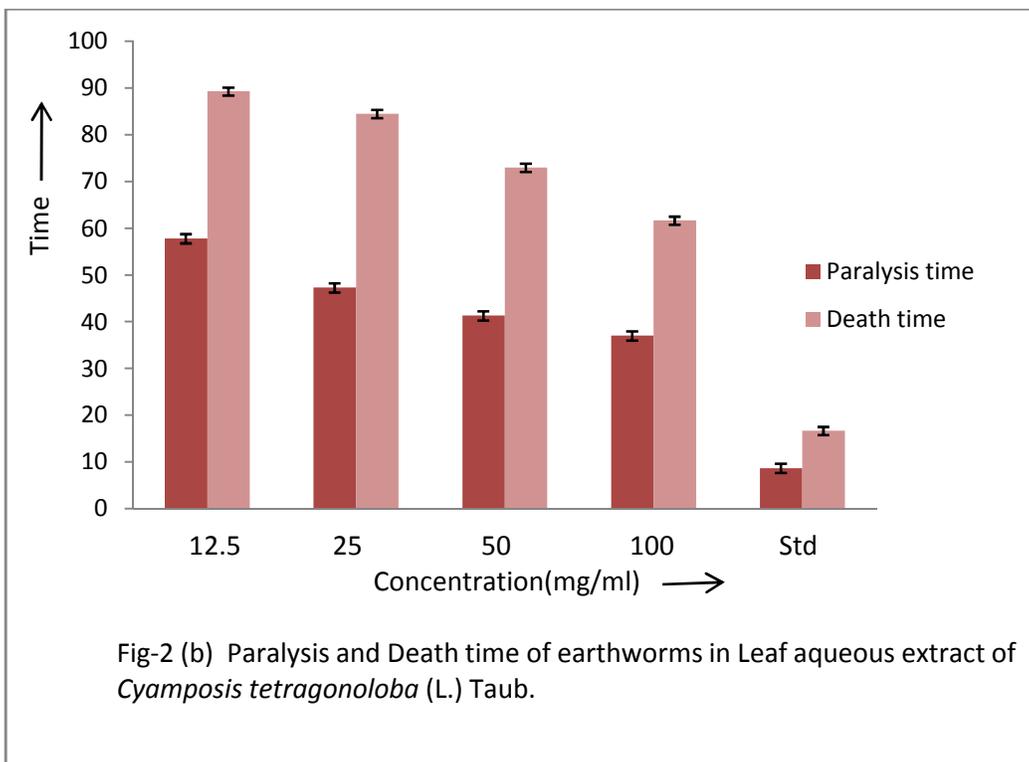
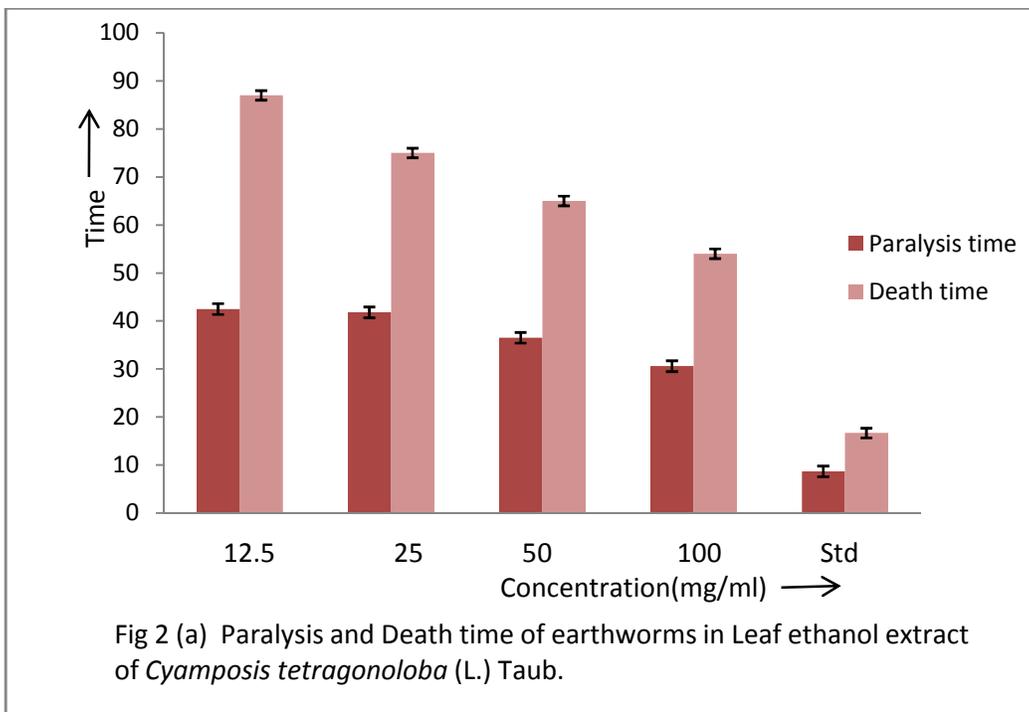
SNo	Different solvent extracts		Paralysis time				Death Time			
			12.5	25	50	100	12.5	25	50	100
1	Leaf	Ethanol	42.5±0.428*	41.83±0.65**	36.5±0.5**	30.6±0.3**	87.67±0.7*	75.83±0.60**	65.5±0.4**	54.33±0.8***
		Aqueous	57.8±0.87*	47.67±0.76*	41.3±0.99*	37±0.577**	89.33±0.7*	84.5±0.50*	73±0.73**	61.67±0.79**
2	Fruit	Ethanol	67±0.421*	60.5±0.428**	50±0.577**	38.8±0.6**	85.8±0.61	80.5±0.428**	74±0.817**	60.67±0.8***
		Aqueous	75.3±0.955*	64.5±0.764*	58.8±0.7**	46.6±0.6**	96.5±0.73	84.3±0.989*	75±0.683**	69.5±0.764**
3	Stem	Ethanol	145±1.46	130±1.138	122.8±1.13	112±1.25	170.8±0.87	162.4±1.03	152±1.003	142.1±0.749
		Aqueous	147.1±0.703	131±0.909	125.3±1.05	116±1.56	185.1±1.35	176.8±0.94	167±0.966	157.8±0.946
4	Control		-----				-----			
5	Standard(20 mg/ml)		8.67±0.6009***				16.67±0.8437***			

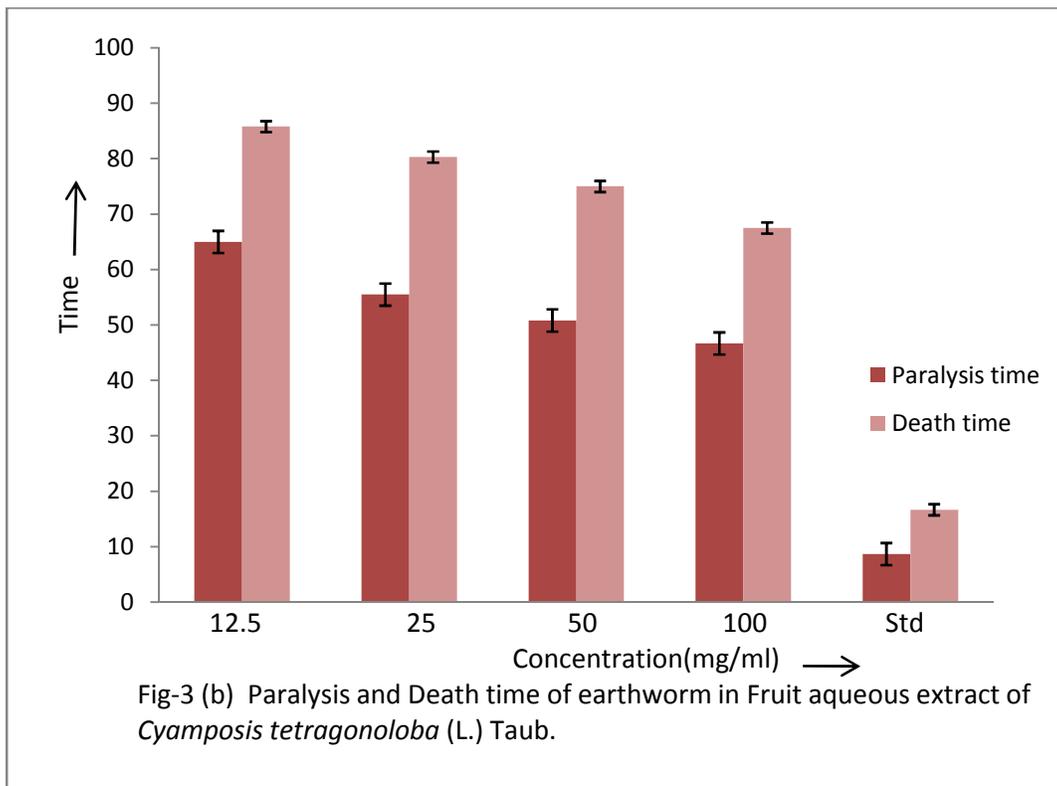
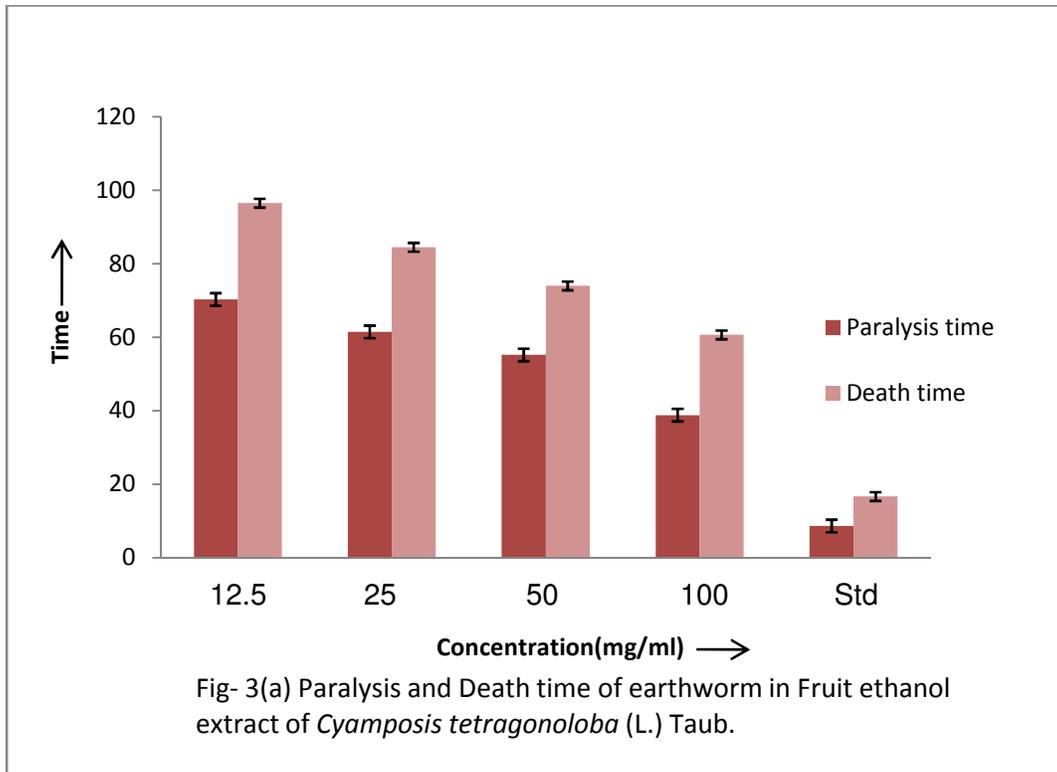
All the data are expressed as mean± SEM

\*\*\* Denotes  $p < 0.001$  extremely significant. \*\* Denotes  $p < 0.01$  and \* denotes  $p < 0.05$  as compared to standard group, n=6 Significant.

(One-way ANOVA followed by Dunnett's test)







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