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Study of acute toxicity of Chemotherapeutant in Freshwater Mussel, *Lamellidens corrianus* (Lea) and *Parreysia cylindrica* (Annandale and Prashad)

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KEYWORDS

Tetracycline, Acute toxicity, *Lamellidens corrianus*, *Parreysia cylindrica*, Finney's Probit analysis

A B S T R A C T

In freshwater pearl culture the bead of metal or shell is embedded inside the mantle or gonad epithelium to stimulate nacre formation. A little surgery is required to insert beads or nuclei and after that the freshwater mussels are kept in the water mixed with antibiotics. Implanted mussels are kept in post-operative care unit in nylon bags with antibiotic treatment. The addition of antibiotics in water heals the wound earlier and decreases bivalve's mortality. The other source of antibiotics entry in the aquatic environment via wastewater and effluents where they may affect the aquatic life. The concentration of a broad spectrum antibiotic tetracycline is tested to reduce the mortality. For that the acute toxicity of tetracycline to *Lamellidens corrianus* had not yet been studied. In the present paper, it is evaluated by static bioassays and calculation of the LC_{10} & LC_{50} by Finney's Probit analysis (1971). The bioassay is performed to find out the effect of tetracycline on *L. corrianus.* Ten mussels are exposed to increasing concentrations of tetracycline for four days. By using regression equation LC_{10} and LC_{50} values are calculated. The LC_{10} values after 24, 48, 72 and 96 hrs exposure to tetracycline for *L. corrianus* are 804.86, 714.89, 641.02 and 519.76 PPM and that of LC_{50} values are 1076.62, 950.28, 842.87 and 738.20 PPM respectively.

Introduction

Tetracyclines were discovered in 1940s by Lloyd H. Conover. It is a broad spectrum antibiotic effective against Gram+ve and Gram –ve bacteria. It inhibits protein synthesis by preventing the attachment of aminoacyl-tRNA to the A site of ribosome. Derivatives of tetracycline are chlortetracycline, oxytetracycline, doxycycline and minocycline. It has a low order of toxicity in laboratory animals.

Nutrients in water bodies may beneficial to aquaculture production in mollusc culture. However, excessive loadings of industrial wastes due to anthropogenic activities such as siltation, pollution, reservoir construction, channelization, deforestation, altered flow regimes and introduced species (non-native) (Bogan, 1993; Williams *et al.*, 1993; NNMCC, 1998) cause the main threat to mollusc culture leading to decline of unionoids. With increasing aquatic pollution there are disease outbreaks resulting in high death rate of organisms and also acts as reservoirs of infection threatening other farmed and wild populations.

Various drugs used to treat water as prophylactic measures. The misuse of these chemicals and drugs could have serious effects such as pollution of adjacent waters as well as development of resistant strains of human pathogens (Barg, 1992, GESAMP, 1991, Pullin, 1989).

Organophosphate compounds, are used in some areas of the world to regulate pests such as shrimps in fish ponds as well as ectoparasitic infestations. Most of these chemicals are toxic to aquatic life at lower concentrations than those used to treat fish (Barg, 1992, Beveridge and Phillips, 1990).

Freshwater pearl production started in Asia and conservation aquaculture in USA while in Southern India freshwater mussels are reared for human consumption (FAO 1986; Chakraborty *et al.,* 2008).

Main problem of it is high larval mortality as like in marine bivalve culture (Nichols and Garling 2002; Jones *et al.*, 2005) and possible solutions could be transferred from marine to freshwater bivalve farming (Marshall *et al.,* 2010) as water dis-infection, antibiotics, immunostimulants and probiotics, often as a combination of these (Fitt *et al.*, 1992; Kesarcodi-Watson *et al.,* 2008; Prado *et al.,* 2010; Sicuro, 2015).

During ciliary action of epithelial cells of the gills the water spaces harbours most of the pathogenic attack.

To nullify this impact high doses of antibiotics are used to mix in culture water, but it may be toxic and reduce the life span of culture. (Gardiner *et al.,* 1991). In cell line cultures of bivalve tissues, many times failure is due to fungal or bacterial contamination (Sengel, 1964)

Bacteria found in freshwater mussels are *Bacillus sp.*(Chittick *et al.,* 2001), *Streptococcus* (Chittick *et al.,* 2001), *Aeromonas hydrophila* (Chittick *et al.*, 2001; Starliper *et al.*, 2008) *Aeromonas sp.* (Starliper *et al.*, 2008), *Vibrio fluviatilis* (Chittick *et al.*, 2001) *Vibrio alginolyctus* (Chittick *et al.,* 2001), *Pseudomonas fluorescens* (Starliper *et al.,* 2008), *Flavobacterium columnare* (Starliper *et al.,* 2008).

The use of antibiotics in a large hatchery is uneconomical and a diseased batch is usually discarded. However, antibiotics are useful in smaller facilities or in a research environment. Sulmet (sulfamerazine), Combistrep, Chlortetracycline HCl and aureomycin. and for severe infections Chloromycetin are proved effective. Along with this benefit of survival, the other side of this antibiotic use on bivalve is to be studied through this present work.

Pteria penguin is commercially important bivalve species for production of mabe. To reduce mortality during egg incubation, application of antibiotics is usually done.

Wassnig and Southgate (2011) studied 3 egg densities (10, 50, and 100/mL) and 3 antibiotic treatments streptomycin sulphate, tetracycline and erythromycin and found improved survival rate.

Materials and Methods

Freshwater bivalves *Lamellidens corrianus* were collected from Girna Dam Dist: Nasik (M.S.) situated at 20^o 28'58" N latitude and 74^o 43'13"E longitude. Before subjecting them to the experiments they were cleaned and acclimatized to the laboratory conditions for 5 days

In the present study, tetracycline is used for acute toxicity evaluation. Static bioassay tests of tetracycline were conducted for 96 hours by using *Lamellidens corrianus* and *Parreysia cylindrica*.

For every experiment ten bivalves in each batch were exposed to different concentrations of tetracycline in troughs containing five liters of water.

The water of appropriate concentration of the antibiotic from each trough was changed after every 24 hours. Simultaneously, control was maintained along with each set. Mortality was recorded after every 24 hours and data was analyzed so as to compute 24, 48, 72 and 96 hrs. LC50 values for tetracycline by probit analysis (Finney, 1971).

The LC50 value for each time period was estimated by a regression analysis determined for the log of concentrations and percent survival of the bivalve. The percentage mortality in various concentrations at particular period were converted into probit values and plotted against the log of concentrations (Ghosh and Konar, 1973). The toxicity tests were taken in triplicate and $LC_1$0 and LC50 values were determined. The regression equation between the log of concentration (X) and probit mortality (Y) were determined statistically for acute toxicity using the formula Y= a + b logx (Finney, 1971)

Results and Discussion

During the bio-assay, the bivalves showed response to tetracycline treatment. At higher concentration, the animal secreted copious mucus and responds accordingly.

The LC_{10} and LC_{50} values of tetracycline to *L. Corrianus* and *Parreysia cylindrica* were calculated for 24, 48, 72 and 96 hours by Finney's method (1971). The LC_{10} values after 24, 48, 72 and 96 hrs exposure to tetracycline for *L. corrianus* are 804.86, 714.89, 641.02 and 519.76 PPM and for *P. Cylindrica* are 461.43, 328.75, 223.66 and 162.75 PPM. LC_{50} values for *L. corrianus* are 1076.62, 950.28, 842.87 and 738.20 PPM for *P. Cylindrica* are 645.76, 545.65, 429.64 and 333.09 PPM. The results of toxicity evaluation are given in Fig. 1 and Tables 1-4 of *L. corrianus* and Fig. 2 and Tables 5-8 of *P. cylindrica*

The problem of pollution of the water where the wastes are usually discharged has increased day by day. Most of the industries discharge their effluents without treatment in the water bodies which is very hazardous to aquatic life (Selvanathan *et al.*, 2011).

There are ample reports dealing with mortality and pollution (Barak, 1955). Disease in bivalve larvae, as in other cultured aquatic organism, results from stresses like poor water quality, under or overfeeding, crowding and temperature extremes, bacterial toxins or by algal metabolites.

Bivalve larvae can be affected by diseases which may cause severe mortalities. (FAO, 1986**).** Most mortalities occurring in the hatchery and growth parameters are correlated with high bacteria counts. Whether those bacteria are involved after the death of bivalves or pathogenic is not important.

In commercial bivalve hatcheries Streptomycin, Combistrep, Sulmet, Chlortetracycline, Aureomycin, Chloromycetin, Teramycin, Sulfathiazole, Sulfanilamide against *Mercenaria mercenaria, Crossostrea virginica* was tested and proved fruitful at the point of view of culture in hatcheries but seems to retard the growth of the larvae as well as may develop a problem of resistance against antibiotics used (FAO, 1986).

In *Pteria penguin* to reduce mortality during egg incubation, application of antibiotics is usually done. Wassnig and Southgate (2011) studied 3 egg densities (10, 50, and 100/mL) and 3 antibiotic treatments streptomycin sulphate, tetracycline and erythromycin to improve survival rate. After incubation in treated culture for 24 h found 23% increase in mean survival during incubation, aquaria treated with tetracycline—erythromycin (1:1) yielded an average of only 9% more veliger larvae survival, and by streptomycin—sulfate 16% than control.

A major problem in the early rearing of marine fish and shrimp is the susceptibility of the larvae to microbial infections. It is believed that the live food can be an important source of potentially pathogenic bacteria, which are easily transferred through the food chain to the predator larvae. Most *Vibrio* are opportunistic bacteria which can cause disease/mortality outbreaks in larval rearing. The use of hypochlorite treatment, however, may not kill all germs and needs further treatment (Lavens, 1996).

The use of probiotics for disease prevention and improved nutrition in aquaculture is becoming increasingly popular. The probiotic bacteria that incorporated into functional foods for use in shellfish hatcheries, may significantly improve larval survival. (Lim *et al.,* 2011)

The evaluation of LC50 concentrations of any chemical is an important step as it provides fundamental data to design experimental work to find out different biochemical and physiological changes in chemically exposed animals.

In the aquatic system the pollutant affect the non-target organism adversely, *L. corrianus* and *P. cylindrica* are such non-target organisms. The susceptibility of animals varies from pollutant to pollutant.

A wide variety of antibiotics used in aquaculture (Primavera *et al.,* 1993; Graslund *et a1*., 2003). Antibiotics not used in molluscan aquaculture except hatcheries. The use of antibiotics in mollusc culture is limited, still to reduce mortality rate during postoperational care is widely used to gain ultimate improvement in pearl production but the impact of the antibiotics used on the invertebrate is needed to be studied. To expose the animals to the proper doses of the tetracycline the present study will be useful to find out LC_{10} and LC_{50} values.

Extensive studies have been carried out all over the world on toxicity studies for the effects of pesticides on aquatic organism (Cripe, 1994; Shanmugam *et al.,* 2000). Many investigations have reported the toxicity of pesticides to different species of animals. Results of pesticide toxicity also reported by Galli *et al.,* (1994); Kaiser and Devillers, (1994); Ruiz *et al.*, (1997); Amoros *et al.*, (2000). Variations in the degree of toxicity of different pesticides have been reported by other workers (Ramana Rao *et al.*, 1987).

Table.1 Calculation of regression equation for LC10 and LC50 values of *Lamellidens corrianus* exposed to tetracycline for 24 hrs

Sr. No	Conc. Tetrac PPM	Log of conc. 'x`	No exposed 'n'	Mortality for 24hrs. 'r'	% MorP	Empirical probit 'X'	Expected probit 'Y'	Weighing coefficient 'w'	Weight W=nw	Working probit 'y'	Wx	Wy	Wx^2	Wxy	Improved expected probit Y'
I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI
1	800	2.9030	10	01	10	3.7184	3.7	0.33589	3.3589	3.719	9.7511	12.4917	28.3083	36.2645	3.6915
2	900	2.9542	10	02	20	4.1584	4.2	0.50260	5.0260	4.159	14.8480	20.9031	43.8645	61.7528	4.2105
3	1000	3.0000	10	04	40	4.7467	4.7	0.61609	6.1609	4.747	18.4827	29.2457	55.4481	87.7373	4.6747
4	1100	3.0413	10	05	50	5.0000	5.1	0.63431	6.3431	5.000	19.2918	31.7155	58.6740	96.4592	5.0946
5	1200	3.0791	10	07	70	5.5244	5.5	0.58099	5.8099	5.524	17.8897	32.0938	55.0856	98.8228	5.4779
6	1300	3.1139	10	08	80	5.8416	5.8	0.50260	5.0260	5.841	15.6506	29.3568	48.7352	91.4155	5.8305
									ΣW= 31.7248		ΣWx= 95.9140	ΣWy= 155.806	ΣWx^2= 290.115	ΣWxy= 472.452	

1. $$\bar{x} = \frac{\Sigma Wx}{\Sigma W} = \frac{95.91408}{31.7248} = 3.02331$$

2. $$\bar{y} = \frac{\Sigma Wy}{\Sigma W} = \frac{155.8069}{31.7248} = 4.91120$$

3. $$Y = \bar{y} + b(x - \bar{x}) = 10.14414x - 25.7576$$

$$b = \frac{\Sigma Wxy - \bar{x}.\Sigma Wy}{\Sigma Wx^2 - \bar{x}.\Sigma Wx} = \frac{472.45234-3.02331(155.8069)}{290.11598-3.02331(95.91408)} = 10.14414$$

$$LC_{10} = X = \frac{3.7184 + 25.75768}{10.14414}$$

Antilog(2.90572) = 804.86

$$LC_{50} = X = \frac{5.0+25.75768}{10.14414}$$

Table.2 Calculation of regression equation for LC10 and LC50 values of *Lamellidens corrianus* exposed to tetracycline for 48 hrs

Sr. No	Conc. Tetrac PPM	Log of conc. ‘x`	No exposed 'n'	Mortality for 24hrs. 'r'	% MorP	Empirical probit 'X'	Expected probit 'Y'	Weighing coefficient 'w'	Weight W=nw	Working probit 'y'	Wx	Wy	Wx^2	Wxy	Improved expected probit Y'
I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI
1	700	2.8450	10	01	10	3.7184	3.7	0.33589	3.3589	3.719	9.5563	12.4917	27.1887	35.5401	3.6235
2	800	2.9030	10	02	20	4.1584	4.2	0.50260	5.0260	4.159	14.5908	20.9031	42.3584	60.6834	4.2247
3	900	2.9542	10	04	40	4.7467	4.6	0.60052	6.0052	4.750	17.7408	28.5247	52.4105	84.2688	4.7551
4	1000	3.0000	10	06	60	5.2533	5.1	0.63431	6.3431	5.252	19.0293	33.3139	57.0879	99.9418	5.2296
5	1100	3.0413	10	07	70	5.5244	5.5	0.58099	5.8099	5.524	17.6701	32.0938	53.7418	97.6100	5.6587
6	1200	3.0791	10	09	90	6.2816	5.8	0.50260	5.0260	6.186	15.4759	31.0908	47.6532	95.7342	6.0505
7	1300	3.1139	10	10	100	-	-	-	-	-	-	-	-	-	-
									ΣW= 31.5691		ΣWx= 94.0634	ΣWy= 158.418	ΣWx^2= 280.440	ΣWxy= 473.778	

1. $\bar{x} = \frac{\Sigma Wx}{\Sigma W} = \frac{94.06347}{31.5691} = 2.97960$
2. $\bar{y} = \frac{\Sigma Wy}{\Sigma W} = \frac{158.4182}{31.5691} = 5.01814$
3. $Y = \bar{y} + b\,(x - \bar{x}) = 10.36803x - 25.87446$

$$b = \frac{\Sigma Wxy - \bar{x}.\Sigma Wy}{\Sigma Wx^2 - \bar{x}.\Sigma Wx} = \frac{473.7786-2.97960(158.4182)}{280.4408-2.97960(94.06347)} = 10.36803$$

$LC_{10} = X = \frac{3.7184 + 25.87446}{10.36803}$

Antilog(2.85424) = 714..89351

$LC_{50} = X = \frac{5.0 + 25.87446}{10.36803}$

Antilog(2.97785) = 950.281

Table.3 Calculation of regression equation for LC10 and LC50 values of *Lamellidens corrianus* exposed to tetracycline for72 hrs

Sr. No	Conc. Tetrac PPM	Log of conc. ‘x`	No exposed 'n'	Mortality for 24hrs. 'r'	% MorP	Empirical probit 'X'	Expected probit 'Y'	Weighing coefficient 'w'	Weight W=nw	Working probit 'y'	Wx	Wy	Wx^2	Wxy	Improved expected probit Y'
I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI
1	700	2.8450	10	02	20	4.1584	4.1	0.47144	4.7144	4.160	13.4128	19.6119	38.1608	55.7976	4.1303
2	800	2.9030	10	04	40	4.7467	4.7	0.61609	6.1609	4.747	17.8855	29.2457	51.9232	84.9028	4.7554
3	900	2.9542	10	06	60	5.2533	5.2	0.62742	6.2742	5.253	18.5354	32.9583	54.7582	97.3669	5.3069
4	1000	3.0000	10	08	80	5.8416	5.7	0.53159	5.3159	5.834	15.9477	31.0129	47.8431	93.0388	5.8002
5	1100	3.0413	10	09	90	6.2816	6.1	0.40474	4.0474	6.264	12.3097	25.3529	37.4386	77.1080	6.2463
6	1200	3.0791	10	10	100	-	-	-	-	-	-	-	-	-	-
									ΣW = 26.5128		ΣWx = 78.0913	ΣWy = 138.181	ΣWx^2 = 230.124	ΣWxy= 408.214	

1. $\bar{x} = \frac{\Sigma Wx}{\Sigma W} = \frac{78.09138.}{26.5128} = 2.94542$
2. $\bar{y} = \frac{\Sigma Wy}{\Sigma W} = \frac{138.1819}{26.5128} = 5.21189$
3. $Y = \bar{y} + b(x - \bar{x}) = 10.77996x-26.53963$

$b = \frac{\Sigma Wxy - \bar{x}.\Sigma Wy}{\Sigma Wx^2 - \bar{x}.\Sigma Wx}$

$= \frac{408.2144-2.94542(138.18193)}{230.1242-2.94542(78.09138)}$

$= 10.77996$

$LC_{10} = X = \frac{3.7184 +26.53963}{10.77996}$

Antilog(2.80687) = 641.02912

$LC_{50} = X = \frac{5.0+0.26.53963}{10.77996}$

Antilog(2.92576) = 842.87860

Table.4 Calculation of regression equation for LC10 and LC50 values of *Lamellidens corrianus* exposed to tetracycline for 96 hrs

Sr. No	Conc. Tetrac PPM	Log of conc. ‘x`	No exposed 'n'	Mortality for 24hrs. 'r'	% MorP	Empirical probit 'X'	Expected probit 'Y'	Weighing coefficient 'w'	Weight W=nw	Working probit 'y'	Wx	Wy	Wx^2	Wxy	Improved expected probit Y'
I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI
1	500	2.6989	10	01	10	3.7184	3.7	0.33589	3.3589	3.719	09.0655	12.4917	24.4677	33.7148	3.5767
2	600	2.7781	10	02	20	4.1584	4.3	0.53159	5.3159	4.166	14.7683	22.1460	51.0287	61.5250	4.2427
3	700	2.8450	10	04	40	4.7467	4.8	0.62742	6.2742	4.747	17.8506	29.7836	50.7867	84.7371	4.8058
4	800	2.9030	10	06	60	5.2533	5.2	0.62742	6.2742	5.253	18.2145	32.9583	52.8781	95.6807	5.2935
5	900	2.9542	10	08	80	5.8416	5.6	0.55788	5.5788	5.823	16.4811	32.4853	48.6891	95.9695	5.7238
6	1000	3.0000	10	10	100	-	-	-	-	-	-	-	-	-	-
									ΣW= 26.802		ΣWx= 76.3802	ΣWy= 129.865	ΣWx^2= 217.850	ΣWxy= 371.627	

1. $\bar{x} = \frac{\Sigma Wx}{\Sigma W} = \frac{78.09138.}{26.5128} = 2.94542$
2. $\bar{y} = \frac{\Sigma Wy}{\Sigma W} = \frac{138.1819}{26.5128} = 5.21189$
3. $Y = \bar{y} + b(x - \bar{x}) = 10.77996x - 26.53963$

$b = \frac{\Sigma Wxy - \bar{x}.\Sigma Wy}{\Sigma Wx^2 - \bar{x}.\Sigma Wx}$

$= \frac{408.2144-2.94542(138.18193)}{230.1242-2.94542(78.09138)}$

$= 10.77996$

$LC_{10} = X = \frac{3.7184 + 26.53963}{10.77996}$

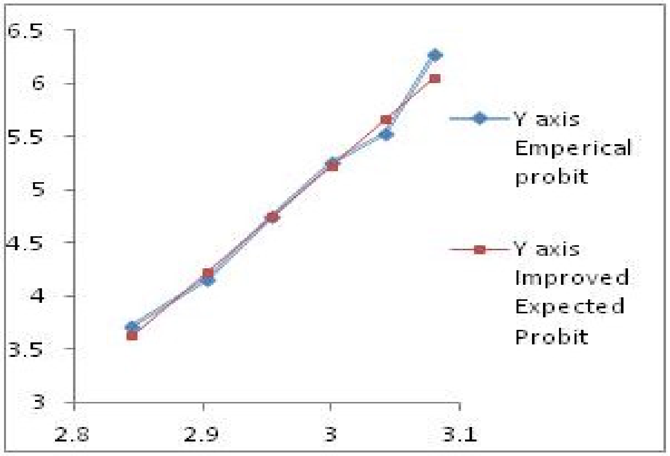
Antilog(2.80687) = 641.02912

$LC_{50} = X = \frac{5.0+0.26.53963}{10.77996}$

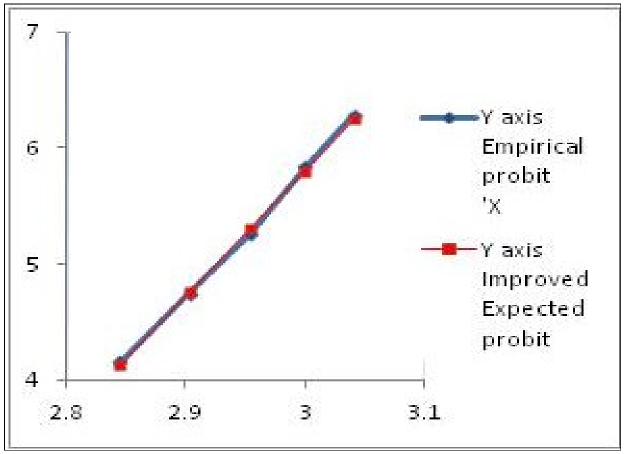
Antilog(2.92576) = 842.87860

Figure.1 Regression lines for LC10 and LC 50 values of freshwater bivalve, *Lamellidens corrianus* after acute exposure to Tetracycline

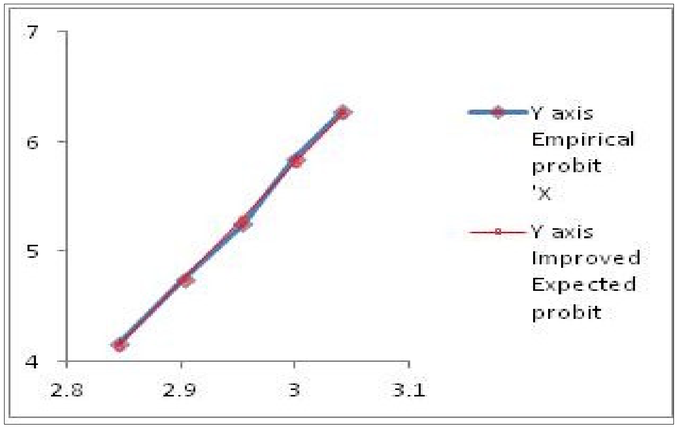
24 hrs



48 hrs



72 hrs



96 hrs

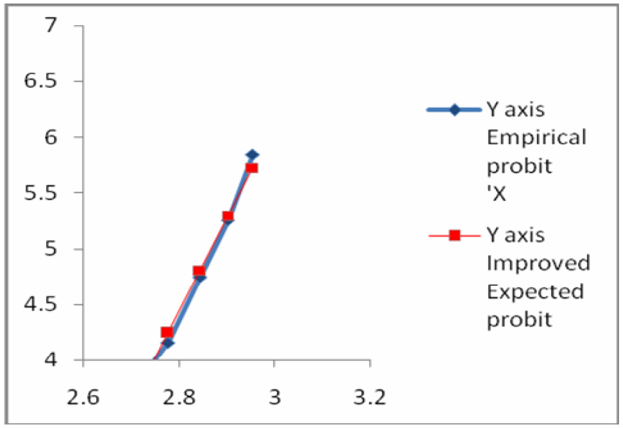


Table.5 Calculation of regression equation for LC 10 and LC50 values of *Parreysia cylindrica* exposed to tetracycline for 24 hrs

Sr. No	Conc Tetra PPM	Log of conc. ‘x`	No exposed 'n'	Mortality for 24hrs. 'r'	% MorP	Empirical probit 'X'	Expected probit 'Y'	Weighing coefficient 'w'	Weight W=nw	Working probit 'y'	Wx	Wy	Wx^2	Wxy	Improved expected probit Y'
I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI
1	600	2.7781	10	04	40	4.7464	4.7	0.61609	6.1609	4.747	17.1159	29.2457	47.5505	81.2492	4.7197
2	700	2.8450	10	06	60	5.2533	5.3	0.61609	6.1609	5.253	17.5283	32.3632	49.8696	92.0762	5.3074
3	800	2.9030	10	08	80	5.8416	5.8	0.50260	5.0260	5.841	14. 5908	29. 3568	42.3584	85.2253	5.8166
4	900	2.9542	10	09	90	6.2816	6.2	0.37031	3.7031	6.278	10.9398	23.2480	32.3189	68.6803	6.2658
5	1000	3.0000	10	10	100	-	-	-	-	-	-	-	-	-	-
									ΣW= 21.0509		ΣWx= 60.174	ΣWy= 114.213	ΣWx^2= 172.097	ΣWxy= 327.231	

1. $\bar{x} = \frac{\Sigma Wx}{\Sigma W} = \frac{60.17494}{21.0509} = 2.85854$
2. $\bar{y} = \frac{\Sigma Wy}{\Sigma W} = \frac{114.2139}{21.0509} = 5.42560$
3. $Y = \bar{y} + b(x - \bar{x}) = 8.7804704x - 19.67375$

$b = \frac{\Sigma Wxy - \bar{x}.\Sigma Wy}{\Sigma Wx^{2} - \bar{x}.\Sigma Wx}$

$= \frac{327.2311 - 2.85854(114.2139)}{172.0976 - 2.85854(60.17494)}$

$= 8.7804704$

$LC_{10} = X = \frac{3.7184 + 19.67375}{8.78047}$

Antilog(2.86488) = 461.43

$LC_{50} = X = \frac{5.0 + 19.67375}{8.78047}$

Antilog(2.81007) = 645.76

Table.6 Calculation of regression equation for LC 10 and LC50 values of *Parreysia cylindrica* exposed to tetracycline for 48 hrs

Sr. No	Conc. Tetrac PPM	Log of conc. 'x'	No exposed 'n'	Mortality for 24hrs. 'r'	% MorP	Empirical probit 'X'	Expected probit 'Y'	Weighing coefficient 'w'	Weight W=nw	Working probit 'y'	Wx	Wy	Wx^2	Wxy	Impr expe pro Y
I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XV
1	500	2.6989	10	04	40	4.7464	4.7	0.61609	6.1609	4.747	17.1159	29.2457	47.5505	81.2492	4.71
2	600	2.7781	10	06	60	5.2533	5.3	0.61609	6.1609	5.253	17.5283	32.3632	49.8696	92.0762	5.30
3	700	2.8450	10	08	80	5.8416	5.8	0.55788	5.5788	5.823	15.8721	32.4853	45.1577	92.4237	5.81
4	800	2.9030	10	09	90	6.2816	6.2	0.37031	3.7031	6.278	10.7503	23.2480	31.2092	67.4909	6.25
5	900	2.9542	10	10	100	-	-	-	-	-	-	-	-	-	-
									ΣW= 21.6037		ΣWx= 60.3665	ΣWy= 117. 342	ΣWx^2= 168.796	ΣWxy= 328.758	

1. $\bar{x} = \frac{\Sigma Wx}{\Sigma W} = \frac{60.36655}{21.6037} = 2.79426$
2. $\bar{y} = \frac{\Sigma Wy}{\Sigma W} = \frac{117.3424}{21.6037} = 5.43158$
3. $Y = \bar{y} + b(x - \bar{x}) = 7.525636798x - 15.59706683$

$$b = \frac{\Sigma Wxy - \bar{x}.\Sigma Wy}{\Sigma Wx^2 - \bar{x}.\Sigma Wx} = \frac{328.7580-2.79426(117.34240)}{168.7962-2.79426(60.36655)} = 7.525636798$$

$$LC_{10} = X = \frac{3.7184 + 15.59706}{7.52563}$$

Antilog(2.56662) = 368.65

$$LC_{50} = X = \frac{5.0+15.59706}{7.52563}$$

Antilog(2.73692) = 545.65

Table.7 Calculation of regression equation for LC 10 and LC50 values of *Parreysia cylindrica* exposed to tetracycline for 72 hrs

Sr. No	Conc. Tetrac PPM	Log of conc. ‘x`	No exposed 'n'	Mortality for 24hrs. 'r'	% MorP	Empirical probit 'X'	Expected probit 'Y'	Weighing coefficient 'w'	Weight W=nw	Working probit 'y'	Wx	Wy	Wx^2	Wxy	Improved expected probit Y'
I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI
1	200	2.3010	10	01	10	3.7184	3.7	0.33589	3.3589	3.719	7.7288	12.4917	17.7843	28.7437	3.4993
2	300	2.4771	10	02	20	4.1584	4.3	0.53159	5.3159	4.166	13.1681	22.1460	32.6190	54.8583	4.2948
3	400	2.6020	10	04	40	4.7464	4.9	0.63431	6.3431	4.748	16.5050	30.1170	42.9470	78.3660	4.8595
4	500	2.6989	10	06	60	5.2533	5.2	0.62742	6.2742	5.253	16.9338	32.9583	45.7040	88.9536	5.2982
5	600	2.7781	10	08	80	5.8416	5.6	0.55788	5.5788	5.823	15.4987	32.4853	43.0578	90.2491	5.6561
6	700	2.8450	10	10	100	-	-	-	-	-	-	-	-	-	-
									ΣW= 26.8709		ΣWx= 69.8347	ΣWy= 130. 198	ΣWx^2= 182.112	ΣWxy= 341.171	

1. $\bar{x} = \frac{\Sigma Wx}{\Sigma W} = \frac{69.83470}{26.8709} = 2.59889$
2. $\bar{y} = \frac{\Sigma Wy}{\Sigma W} = \frac{130.19855}{26.8709} = 4.84533$
3. $Y = \bar{y} + b(x - \bar{x}) = 4.52037x - 6.90264$

$b = \frac{\Sigma Wxy - \bar{x}.\Sigma Wy}{\Sigma Wx^2 - \bar{x}.\Sigma Wx}$

$= \frac{341.1710-2.59889(130.1985)}{182.1122-2.59889(69.83470)}$

$= 4.520374$

$LC_{10} = X = \frac{3.7184 + 6.90264}{4.52037}$

Antilog(2.34959) =223.66

$LC_{50} = X = \frac{5.0+6.90264}{4.52037}$

Antilog(2.63311) = 429.64

Table.8 Calculation of regression equation for LC 10 and LC50 values of *Parreysia cylindrica* exposed to tetracycline for 96 hrs

Sr. No	Conc. Tetrac PPM	Log of conc. 'x'	No exposed 'n'	Mortality for 24hrs. 'r'	% MorP	Empirical probit 'X'	Expected probit 'Y'	Weighing coefficient 'w'	Weight W=nw	Working probit 'y'	Wx	Wy	Wx^2	Wxy	Improved expected probit Y'
I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI
1	200	2.3010	10	02	20	4.1584	4.1	0.47144	4.7144	4.160	10.8479	19.6119	24.9613	28.7437	3.4993
2	300	2.4771	10	04	40	4.7464	4.8	0.62742	6.2742	4.747	15.5419	29.7836	38.4992	54.8583	4.2948
3	400	2.6020	10	06	60	5.2533	5.2	0.62742	6.2742	5.253	16.3257	32. 9583	42.4805	78.3660	4.8595
4	500	2.6989	10	08	80	5.8416	5.6	0.55788	5.5788	5.253	15.0570	32.4853	45.6384	87.6769	5.2982
5	600	2.7781	10	10	100	-	-	-	-	-	-	-	-	-	-
									ΣW= 22.8416		ΣWx= 57.7726	ΣWy= 120. 8391	ΣWx^2= 146.579	ΣWxy= 292.341	

1. $\bar{x} = \frac{\Sigma Wx}{\Sigma W} = \frac{57.77266}{22.8416} = 2.52927$
2. $\bar{y} = \frac{\Sigma Wy}{\Sigma W} = \frac{114.8392}{22.8416} = 5.02763$
3. $Y = \bar{y} + b(x - \bar{x}) = 4.12054x - 5.39436$

$b = \frac{\Sigma Wxy - \bar{x}.\Sigma Wy}{\Sigma Wx^2 - \bar{x}.\Sigma Wx}$

$= \frac{292.3413 - 2.52927(114.8392)}{146.5794 - 2.52927(57.77266)}$

$= 4.120549$

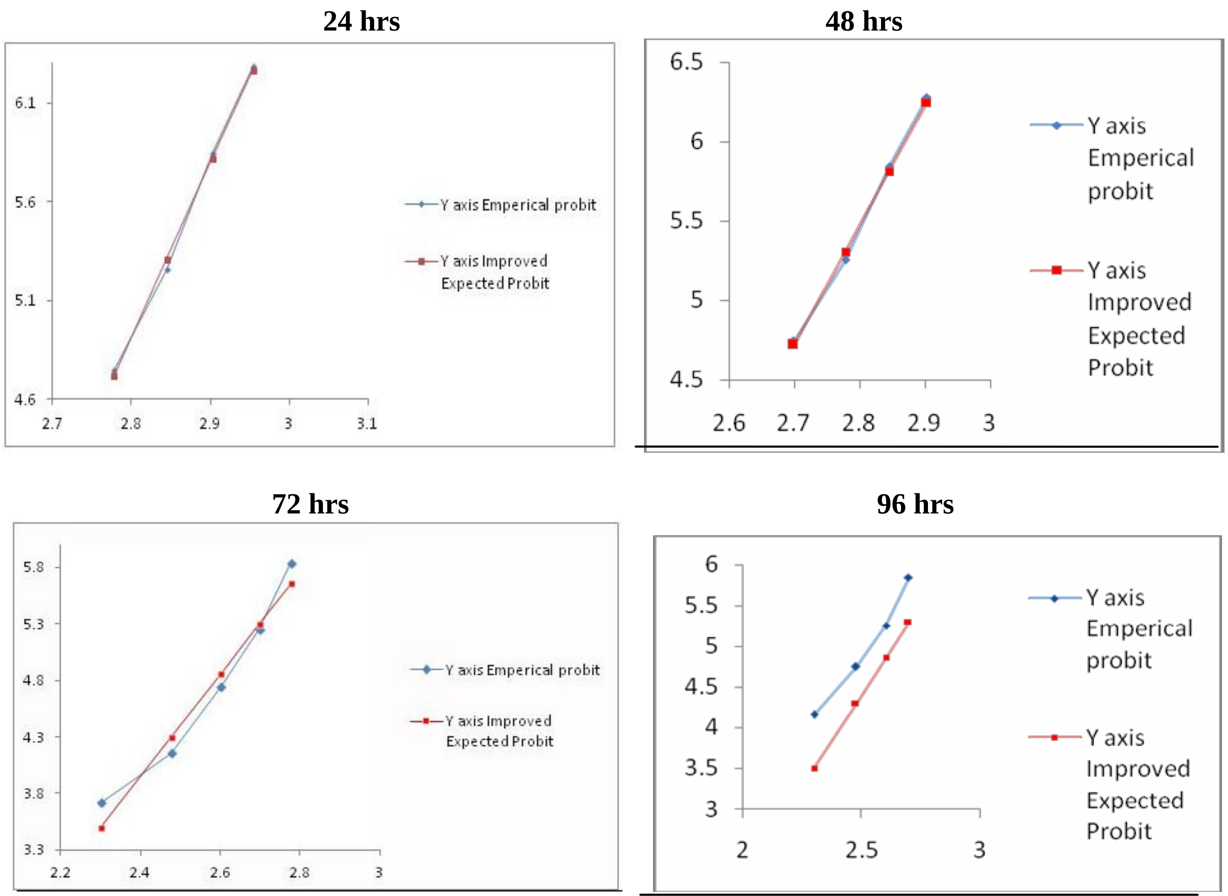
$LC_{10} = X = \frac{3.7184 + 5.39436}{4.12054}$

Antilog(2.21154) = 162.75

$LC_{50} = X = \frac{5.0 + 5.39436}{4.12054}$

Antilog(2.52256) = 333.09

Figure.2 Regression lines for LC10 and LC 50 values of freshwater bivalve, *Parreysia cylindrica* after acute exposure to Tetracycline



In the present study the rate of mortality of freshwater bivalves *L. corrianus* and *P. cylindrica* has increased with advancement in concentration and exposure time to tetracycline. Thus mortality rate is directly proportional to the time of exposure and concentration of the tetracycline in both *L. corrianus* and *P. cylindrica*. Since the LC 50 value of tetracycline for 96 hrs is higher (840.55 ppm) for *Lamellidens corrianus* than that of *P. cylindrica* (333.09 ppm), it indicates that *L. Corrianus* is highly sturdy species than *P. Cylindrica*.

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