STUDIES ON THE EFFECT OF MS-222 ON BASAL METABOLISM OF *PENAEUS INDICUS* H. MILNE EDWARDS SEEDS

DISSERTATION SUBMITTED BY

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CERTIFICATE

This is to certify that this Dissertation is a bonafide record of work carried out by Miss **Chitra Das** under my supervision and that no part thereof has been presented before for any other degree.

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PREFACE

Aquaculture has been on the focus in recent years the worldover to augment food production. It's success involves techniques on handling, crowding, confinement, transport, nutrition and ecological management. A flaw in any one of these will adversely affect the physiology of fish leading to pathological conditions and mortality. Seed and feed are the 2 major constraints in most of the culture systems, which hatcheries and factories can take care of. But problems on handling and transport still loome large; since causes that adversely affect the animals are difficult to ascertain with certainity in most cases even today.

Some important parameters, causing such adverse effects, however, are the dissolved oxygen, carbondioxide, acidity, waste products, temperature and excess activity of fish. Anaesthetics are known to reduce activity metabolism, directly controlling the oxygen consumption and indirectly controlling the release of carbondioxide, acidity, waste products and excess activity; implicating mortality in fish during handling and transport (McFarland 1960).

Several of the anaesthetics have proved to tranquilize fish and markedly reduce metabolism and excretion of wastes, enabling to keep the chemistry of water under control for longer period.

Bearing in mind the beneficial effects of anaesthetics, the present work has been carried out on the effects of MS-222 on the seeds of Penaeus indicus, one of the most popular prawn species cultivated in India. The work involves measurement of metabolism in terms of the rate of oxygen consumption, rate of ammonia excretion, ammonia quotient, changes in pH and time taken for 50 and 100% mortality at various concentrations of MS-222, and study of stress in terms of changes in levels of protein, lipid and carbohydrate contents in the animal.

I have deep sense of gratitude for my supervising guide Dr.A.Noble, Principal Scientist, for his guidance, advise and patience without which this work would be incomplete. I am thankful to Dr.P.S.B.R.James, Director, Central Marine Fisheries Research Institute, for providing the necessary facilities for this work. I also thank Semester-in-charge, Mr. D.S.Rao, Principal Scientist, for encouragement and Mr.K.N.Kurup, Senior Scientist, for his help in computer analysis. I express my deep sense of appreciation to Mr.A.Nandakumar, Technical Assistant, for his timely help. I also take this opportunity to thank all my classmates, seniors and juniors for their assistance. Lastly, I thank the Indian Council of Agricultural Research for offering a Junior Research Fellowship during the course of studies.

INTRODUCTION

Handling of fish in live condition for various purposes such as transporting, stocking, stripping, treating for diseases, tagging, etc. without causing stress or mortality is often very difficult. But this can be overcome by proper anaesthetization.

To start with, application of anaesthetics on fish was made for experimental surgery. Their use for fisheries work has been in vogue ever since anaesthetizing effects were discovered.

Both physical and chemical methods have been used to produce anaesthesia. But whether the 'sleep' induced by them is similar or not, is still unknown; nevertheless, the severe stress caused by physical means is recognised.

The first record on the potential use of anaesthetics for transport was by Aitken (1932). Following his lead, several others have worked on the use of various anaesthetics in fish. Abramowitz (1937) produced anaesthesia in fish for experimental purpose by immersing in cracked ice. Osborn (1938) stunned them with a mild hit. Haskell (1940) produced a paralysis lasting about 2 minutes with electric shock. Pickford and Atz (1957), however, have found such methods to bring about drastic changes in the

body system causing stress.

Chemical agents capable of inducing anaesthesis have therefore been found useful in lowering the metabolic rate and mortality during handling and transport of fish. Anaesthetics hence, find a widespread application in aquaculture practices in the present days.

Chemicals including Ether, Urethane, Chlorobutanol, Sodium Amytal, Chloral hydrate, Tertiary Amyl Alcohol, Tertiary Butyl Alcohol, Reserpine, Chloretone, Thiouracil, MS-222, Veronal, Thiourea, Quinaldine and Methyl-thiouracil besides several others are being used to anaesthetics on fish at various parts of the world and a lot of literature is also available. Notwithstanding this, in India it is confined to a few like that of Sreenivasan (1962), Durve and Dharmaraja (1965), Durve (1966), Gupta and Sharma (1975) and Chakraborti et al. (1977).

The present study is done on the most worked anaesthetic, MS-222 Sandoz, also known as 'Tricaine-Sandoz, Tricaine Methanesulphonate, Metacaine and Metacaine Methanesulphonate' which is a methanesulphonate of meta-aminobenzoic acid ethylester.

The efficacy of MS-222 was discovered as early as 1932 by Baudin for narcotizing cold blooded animals (Anon 1960). MS-222 has been compared with other anaesthetics by Koppanyi and Karczmar (1948), McErlean and Kennedy (1968), Dinns and Bessa (1978) and Ferraire et al. (1979). It's

anaesthetic property is studied by Nelson (1953), Pickford (1953), Pickford and Atz (1957), Meister and Ritzi (1958), Bove (1965), Schoettget et al.(1967) Hinton and Loyacano (1978) and Gonzales et al. (1986). Gilbert and Wood (1957), McFarland (1959 and 1960), Merck Index (1960), Allison (1961), Randall (1962), Eisler and Backiel (1960), Bell (1964), Bove (1965), Durve (1966), Houston and Corlett (1976), Soivio et al. (1977), Jirasek et al. (1978), Smit and Hattingh (1979), Dazboeck and Holeton (1980), Williamson and Roberts (1981), Silveira et al. (1986), Cornish and Moon (1986), Flos et al. (1987), Takeda et al. (1987) and Quinn et al. (1988) have worked on the behavioural and physiological effects caused by MS-222. Transportation of live fish anaesthetized by MS-222 has been dealt by Martin and Scott (1957), McFarland (1960), Durve (1966), Gupta and Sharma (1975) and Rothbord (1988). While Schoettger et al. (1967), Wedmeyer (1969), Sharma et al.(1978) and Murai et al. (1979) have studied its toxic and stress effects; Webb (1958) and Taylor (1988) have worked on the distribution and orientation of fishes treated with MS-222..

Contrary to the voluminous work on fish very little has been done on crustacea even outside our country. Oswald (1977) has tested 14 drugs on immobilization of the crabs, Cancer and Carcinus, for experimental procedures. Chakraborti et al. (1977) used Tertiary Amyl Alcohol to anaesthetize prawn seeds for segregating them before selective stocking. Kulkarni and Fingerman (1986) worked on the effects of two tranquilizers, Reserpine and Chlorpromazine on neurosecretory cells and the ovary of

the fiddler crab. In view of no work done on MS-222, the present study was taken up on the seeds of the Indian white prawn whose fast growth rate and size make it an ideal candidate species for commercial culture.

The physiological state of an animal is reflected on the metabolism and the rate of oxygen consumption is an index of it. Loft (1956), Subramanyam (1962), Reeve (1969), Kutty et al. (1971), Kuttyamma (1980), Laxminarayana (1980) and Laxminarayana and Kutty (1982) have studied oxygen uptake in prawns.

Ammonia forms the major portion of the total nitrogen excreted (Parry 1960, Needham 1957, Binns and Peterson 1969, Gerhardt 1980 and Spaargaren 1982). Ammonia excretion is important as a measure on protein degradation and is of value as an index in aquatic organisms under stress (Kutty, 1971, 1972 and 1978; Kutty and Peer Mohamed, 1975; Laxminarayana, 1980; Laxminarayana and Kutty, 1982; Peer Mohamed, 1974 and Prosser and Brown, 1961). Relative changes in ammonia excretion and ammonia quotient - a ratio of rate of ammonia excreted to the rate of oxygen consumption, were therefore investigated in different concentrations following Laxminarayana (1980).

The negative logarithm of hydrogen-ion-concentration, as an important parameter that controls the survival and biological process of aquatic organisms, has also been considered. The percentage change of the pH from the initial level after the test period has been investigated

for each of the concentrations and control, to have a record on the acid-base electrolytic changes in the medium caused by metabolism.

The anaesthetic property of MS-222 on the prawn seeds has been assessed by the duration to attain 100% mortality and the differences in the metabolic rates in various concentrations by the time for 50% mortality following McFarland's (1960) method.

The stress response of rainbow trout by MS-222 has been evaluated by Black and Connor (1964) and Wedmeyer (1969). Stress in a prawn is studied by measuring the levels of major metabolites like protein, carbohydrate and lipid concents in the animals.

On this background, the present study has the following objectives:

(i) Find out the tolerable range of MS-222.

(ii) Estimate the rate of oxygen consumption, rate of ammonia excretion, ammonia quotient and changes in pH in different concentrations within the range.

(iii) Evaluate the changes in protein, lipid and carbohydrate contents in the animal on exposure to these concentrations.

MATERIALS AND METHOD

1. Test animals - Collection, transportation and acclimation.

Seeds of Penaeus indicus H. Milne Edwards of 15 to 40 mm sizes were the test animals (Plate I). About 2000 seeds required for the present study were collected using velon screen from the canal systems and perennial ponds of Edavanakkad and Puduvypu; and the Prawn Hatchery of Central Institute of Brackishwater Aquaculture, Narakkal.

The seeds were transported from the collection sites to the laboratory in 30 litre jerry cans. During transportation, the water in the cans was stirred manually for aeration.

In the laboratory, the seeds were acclimated to 4 days and fed on formulated pellet feed once in 12 hours approximately at the rate of 5% to 10% of the body weight. The seeds were starved for the last 25 hours prior to the test following Fromme (1963). Leftover feed and the faecal matter were removed daily and the medium was replenished every alternate day.

2. Acclimation medium and test medium

Seawater of 33 to 35 ppt, collected off Cochin was filtered, allowed

to settle to make it sediment free and stored in 100 litre plastic bins. This, diluted with freshwater to 15 ppt, formed the medium for acclimation and tests.

MS-222 Sandoz was dissolved in the 15 ppt water to get concentrations ranging from 1 ppm to 5000 ppm as the test media.

NaOH of 1 normalcy was used following Wedemeyer (1970) to rectify the reduction of pH caused by MS-222 and maintained between 7.8 and 8.1. Throughout the experiment the temperature ranged from 22°C to 26°C, initial content of dissolved oxygen levelled through aeration to around 4.33 ml/L and ammonia at 0.15 ppm.

3. **Experiment set up.**

3.1. For acclimation:

Six circular plastic pools of 40 litre capacity filled with 35 litres each of preaerated medium of 15 ppt were used for acclimation. Each pool was stocked with 250 seeds providing continuous aeration.

3.2. For test:

Test on 1, 5, 10, 15, 20, 25, 50, 100, 150, 200, 250, 500, 1000, 2500 and 5000 ppm MS-222 were conducted to range out the suitable concentrations for the experiment. Simultaneously a control without MS-222 was also kept. One litre conical flasks containing a litre each of the above

media with 50 prawn seeds, sealed off by liquid paraffin to prevent diffusion of oxygen were used. The time of setting was recorded. The seeds were considered dead when no response was shown to the stimulus of mild knock to them with a glass rod. The test period lasted till 100% mortality occurred. Time for 50% mortality was also noted down.

Concentrations below 50 ppm equalled the control in the time for 100% mortality. Above 250 ppm reduction in time was observed. Between 50 and 250 ppm the period for 100% mortality was more than the control, and hence considered as suitable range for the experiment.

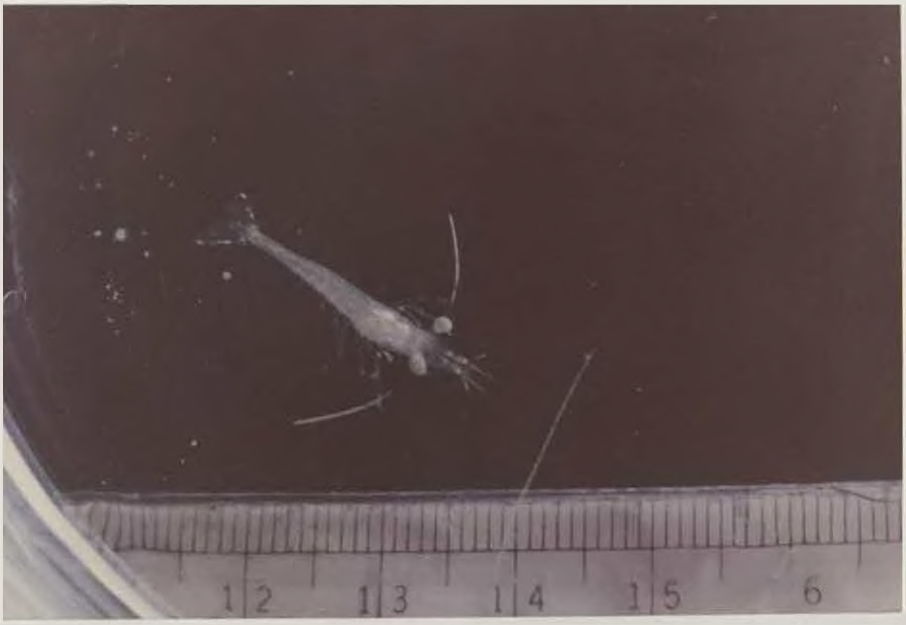
Six conical flasks of one litre capacity formed a set and three such sets were used in an experiment. Five flasks in a set were filled with a litre each of 50, 100, 150, 200 and 250 ppm of the test media. One flask was kept as control with one litre of the medium without MS-222. A record on the initial oxygen, ammonia and pH was noted, and 50 prawn seeds were introduced into each of the containers and sealed (Plate II and III). The period of 50% and 100% mortality was recorded. After the test period, the prawn seeds were used for biochemical analysis and the medium to estimate the final content of oxygen, ammonia and pH.

4. **Chemical analysis**

Salinity was estimated by Mohr's method (Strickland and Parsons 1968) and the pH was measured by a Toshniwal pH-meter. Oxygen content

Plate I. Seed of Penaeus indicus with a scale showing size.

Plate II. Experimental set up.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was measured by Winkler's method (Strickland and Parsons, 1968). Measurement of total ammonia content was done following Solarzano's (1969) method. As Spotte (1979) reported changes in the ammonia level in water preserved in glass or plastic container, the total ammonia content were analysed immediately.

5. Biochemical analysis

Quantitative analyses of protein, lipid and carbohydrate were conducted for 5 of the seeds taken at random from each test containers. Each of the seeds was weighed and homogenised individually in an electrical homogenizer. The following flow-chart gives the sequence of the analysis.

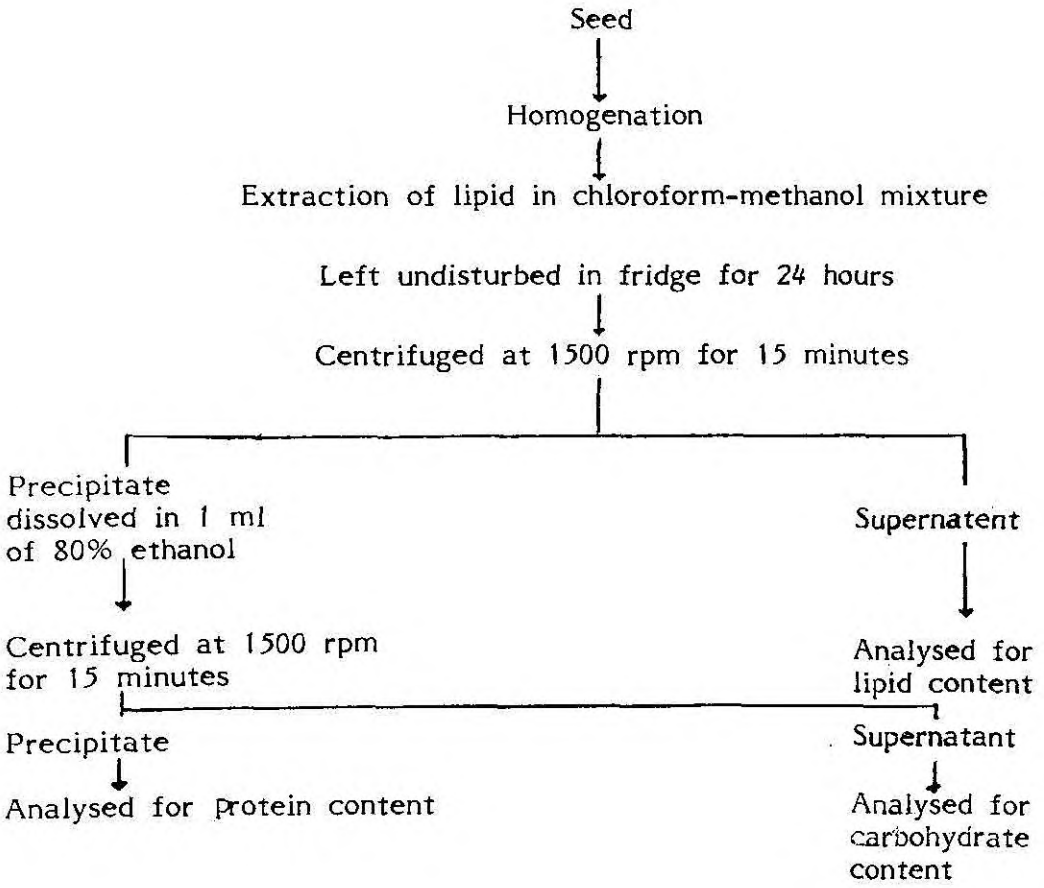
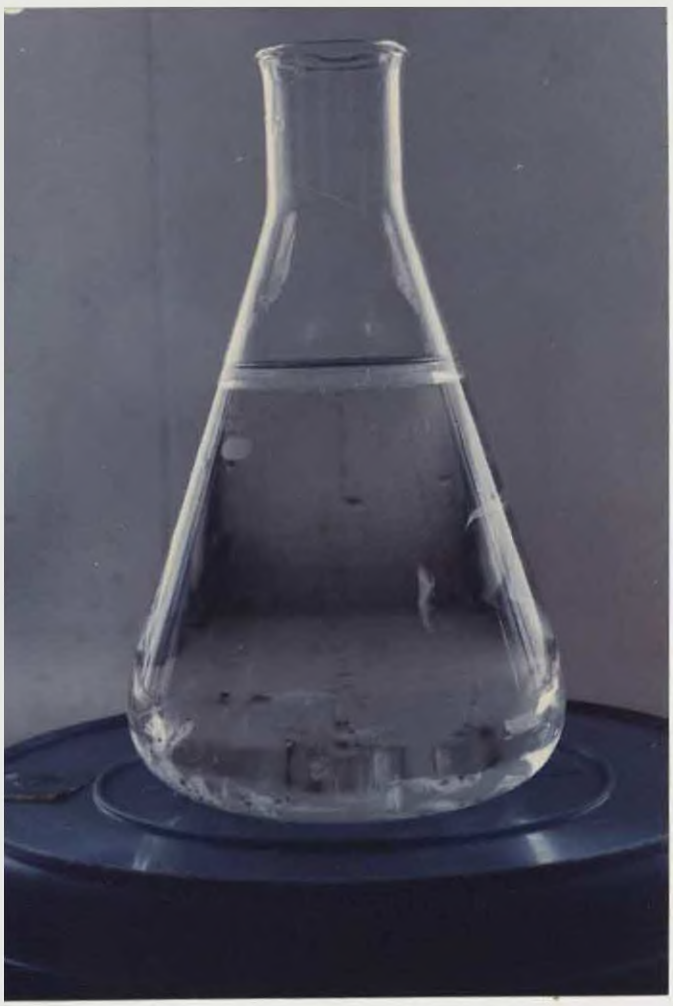


Plate III. A conical flask containing 50 test prawn seeds.



Spectrophotometric methods were used for the analyses. Lowry's method (Lowry et al. 1951) was followed to estimate the protein content, Sulphovanillin method (Blackstork and Barnes 1973) for lipid and Phenol-Sulphuric acid method (Dubois et al. 1956) for carbohydrate.

For all the spectrophotometric analysis GS 866D ECIL Senior Spectrophotometer was used

6. Statistical analysis

Computer analyses of data for ANOVA, linear regression and correlation coefficient were conducted following Snedecor and Cochran (1967).

OBSERVATIONS AND RESULTS

1. Tolerable range

The time for 50% and 100% mortality in 1, 5, 10, 15, 20, 25, 50, 100, 150, 200, 250, 500, 1000, 2500 and 5000 ppm concentrations of MS-222 and control is shown in Table-1. It can be seen that below 50 ppm there is no significant difference in time from that of the control for 50% and 100% mortality while between 50 and 250ppm there is an increase, and above 250 ppm a reduction. The time for 100% mortality was lowest at 5000ppm (1 minute) and the highest at 150 ppm (1035 minutes).

The range of 50 to 250ppm was thus considered suitable.

2. Rate of oxygen consumption

The rate of oxygen consumption of the test concentration together with control and the percentage variation of oxygen consumption from control are given in Table-2a. It is seen that rate of oxygen consumption ranged between 0.000385 ml/mg/Hr at 150ppm and 0.008590 ml/mg/Hr in control. The percentage variation of oxygen consumption from control was observed least at 150ppm (-55.18). Fig. 1 shows the linear regression expression for the rate of oxygen consumption with a slope value of -0.00001231 ± 0.00000749 and an intercept of 0.00774857 ± 0.00113451. The correlation

coefficient 'r' was found to be -0.6346. Table-2b gives the results of ANOVA. The F-value in it was 19.62 and it is highly significant at 1% level.

3. Rate of ammonia excretion

The rate of ammonia excretion of the test concentrations and control and the percentage variation of ammonia excretion from control is presented in Table-2a. The highest rate of excretion observed was 0.000170 ppm/mg/Hr in control and the lowest 0.000022 ppm/mg/Hr occurred in 150ppm. The percentage variation from control ranged between -20.0 (250ppm) and -87.059 (150ppm). Fig.2 shows the regression relation with a slope of -0.00000031 ± 0.00000027 and an intercept of 0.00013281 ± 0.00004072. The correlation coefficient 'r' was found to be -0.4934. The results of ANOVA is shown in Table-2c, giving an F-value of 25.95 which is highly significant at 1%.

4. Ammonia quotient

Table-2a shows the ammonia quotient and the percentage variation from control. The quotient was found to decrease with increase in concentration. The highest quotient was 0.01979 in control and the lowest 0.01070 at 250ppm. The percentage variation of each quotient from that of control ranged between -71.147 (150ppm) and -14.098 (250ppm). Fig. 3 shows the linear regression observed with a slope of **0.00018436 ± 0.00022734** and intercept of 0.00910610 ± 0.03441466 and an 'r' of 0.3785. The result of ANOVA given in Table-2d shows the F-value of 6.34 which is significant at 1%.

6. Time of 50% and 100% mortality

Table-4 gives the time of 50% and 100% mortality of the 5 concentrations and control and the percentage variation from control. The highest time of 50% and 100% mortality was observed at 150ppm with 823 and 1035 minutes respectively, and the least time was recorded for control with 293 and 420 minutes respectively. The percentage variation of time for 50% mortality from control ranged from 21.84 at 50ppm to 180.89 at 150ppm and that of 100% mortality ranged between 19.76 at 250ppm and 146.43 at 150ppm. Fig. 5 gives the linear regression with a slope of 0.8674286 ± 0.958991 and an intercept of 406.5714 ± 145.1881 for 50% mortality. The Fig. 6 gives the linear regression of 100% mortality with a slope of 1.18 ± 1.07 and an intercept of 558.00 ± 161.54. The correlation coefficient 'r' was 0.4121 for 50% mortality and 0.4389 for 100%.

7. Biochemical studies

7.1. Protein content:

Table-5a and Fig. 7 show the changes in protein content due to the difference in concentrations of MS-222. The protein content ranged from 17.209 mg in control to 19.280 mg at 150ppm per 100 mg body wet-weight. Fig. 10 shows the percentage variation of protein level from control. According to this, the protein content was more than that of control at all concentrations, with the highest being at 150ppm (12.049%) and the lowest at 250ppm (0.320%). Table-5b shows the results of ANOVA. The F-value in it being 51.49 the changes in protein level are highly significant at 1%.

7.3. Lipid content:

Table-5a and Fig. 8 show the changes in lipid content due to the difference in concentrations of MS-222. a range between 4.079 mg in control and 3.904 mg at 150ppm per 100 mg of body wet-weight of lipid level was observed in the study. In Fig.10 where the percentage variations from control are given a depletion ranging between - 4.29% at 150ppm and -1.882% 50ppm was observed. The ANOVA results in Table-5c gives an F-value of 1.25 which is insignificant. The depletion is caused by lipolysis.

7.3. Carbohydrate content:

Table-5a and fig. 9 depicting the changes in carbohydrate contents show the maximum of 0.8216 mg at control and minimum 0.7840 mg at 150ppm per 100 mg of body wet-weight. Fig. 10 giving the percentage variation of carbohydrate level from control also as in the case of lipid, shows a depletion caused by breakdown of carbohydrate with values ranging between -1.339% at 250ppm and -4.576% at 150ppm. The results of ANOVA given in Table-5d provides an F-value of 0.38 which is insignificant.

Table-1. Time (in minutes) for 50% and 100% mortality of seeds of Penaeus indicus exposed to different concentrations of MS-222.

	Control	Concentrations (in ppm)														
		1	5	10	15	20	25	50	100	150	200	250	500	1000	2500	5000
Time for 50% mortality	293	289	295	290	310	298	300	357	613	823	588	416	156	2	2	-
Time for 100% mortality	420	420	398	416	422	409	428	515	840	1035	800	503	178	3	3	1

Table-2a. Rate of oxygen consumption, rate of ammonia excretion, ammonia quotient and their percentage variation from control.

Parameter	Control	Concentrations (in ppm)				
		50	100	150	200	250
Rate of oxygen consumption (ml/mg/Hr)	0.00859	0.00825	0.00536	0.00385	0.00517	0.00800
% variation from control		-3.958	-37.601	-5.180	-39.814	-6.868
Rate of ammonia excretion (ppm/mg/Hr)	0.00170	0.000114	0.000051	0.000022	0.000064	0.000136
% variation from control		-32.941	-70.000	-87.059	-62.353	-20.000
Ammonia quotient	0.01979	0.01421	0.00951	0.00571	0.01232	0.01070
% variation from control		-28.196	-51.945	-71.147	-37.494	-14.098

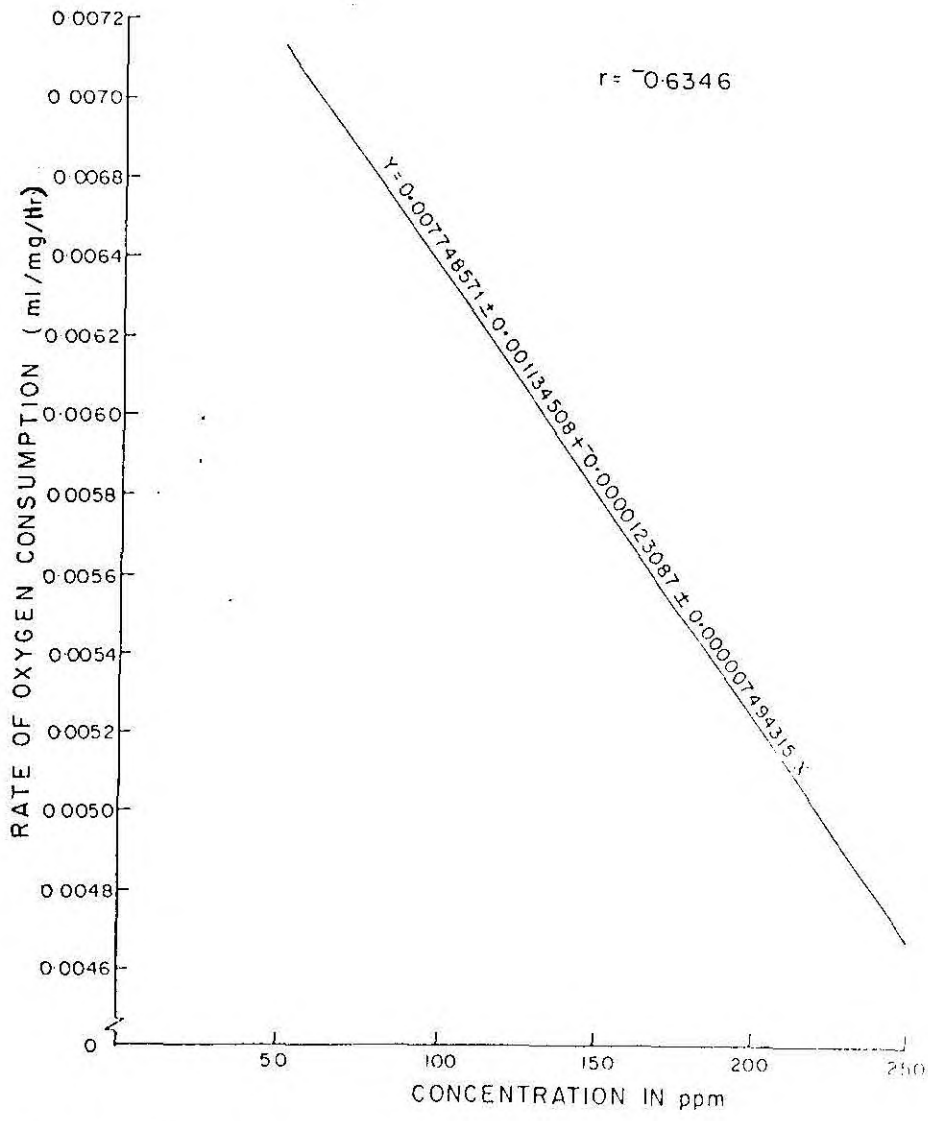


Fig.1. Regression line for the rate of oxygen consumption (ml/mg/Hr) of P. indicus seeds exposed to the different concentrations.

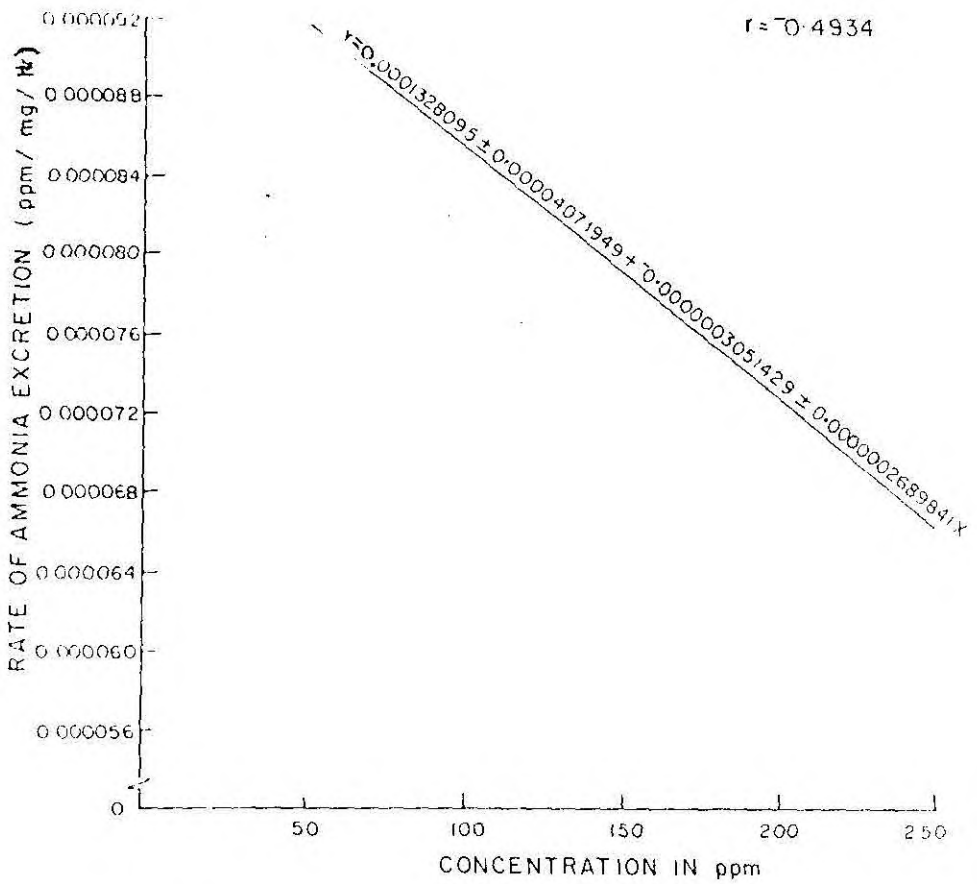


Fig.2. Regression line for the rate of ammonia excretion (ppm/mg/Hr) of *P. indicus* seeds exposed to the different concentrations

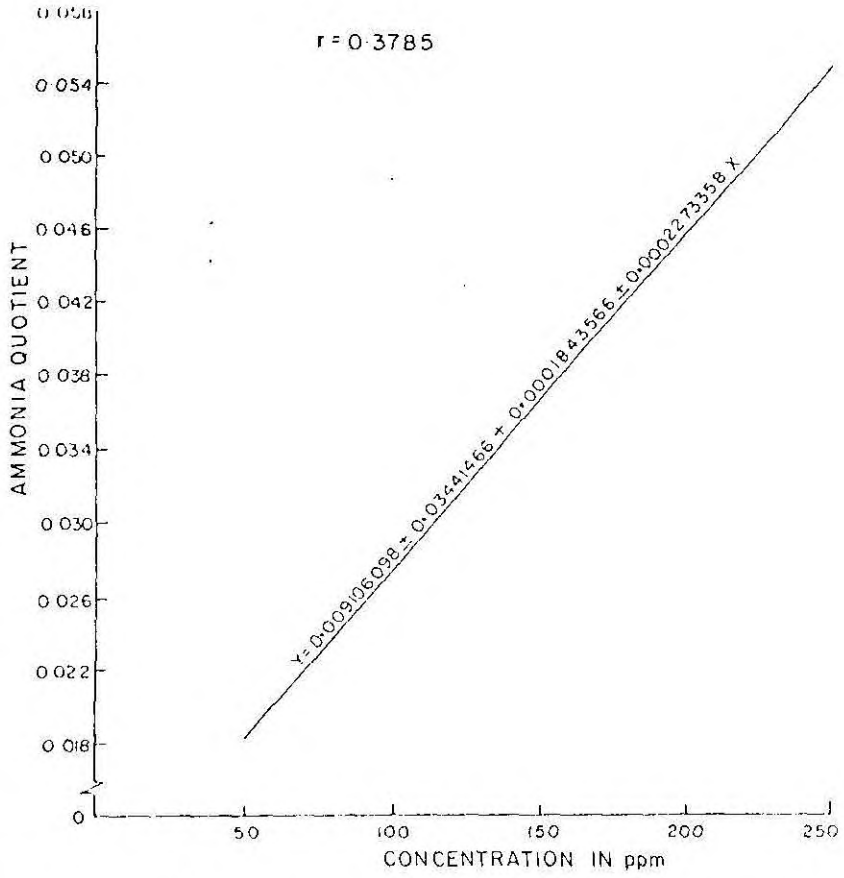


Fig.3. Regression line for ammonia quotient of P. indicus seeds exposed to the different concentrations

Table-2b. ANOVA of oxygen consumption.

Source	Degree of Freedom	Sum of Squares	Mean of Sum of Squares	F-Value	Remarks
Treatment	5	0.00025	0.0004905	19.62	Highly significant at 1% level
Error	12	0.00030	0.0000251		

Table-2c. ANOVA of ammonia excretion

Source	Degree of Freedom	Sum of Squares	Mean of Sum of Squares	F-value	Remarks
Treatment	5	0.000467	0.0000934	25.95	Highly significant at 1% level
Error	12	0.0000432	0.0000036		

Table-2d. ANOVA of ammonia quotient

Source	Degree of Freedom	Sum of Squares	Mean of Sum of Squares	F-value	Remarks
Treatment	5	0.000259	0.00005199	6.34	Highly significant at 1% level
Error	12	0.0000984	0.00000820		

Table-3. Changes in pH levels in medium and percentage variation from the initial level.

Concentrations (in ppm)	pH		
	Initial level	Final level	Percentage change from initial level
Control	7.8	6.9	12.24
50	8.0	7.5	7.46
100	8.0	7.2	10.787
150	8.1	7.1	11.568
200	8.0	7.4	7.88
250	8.0	7.3	8.75

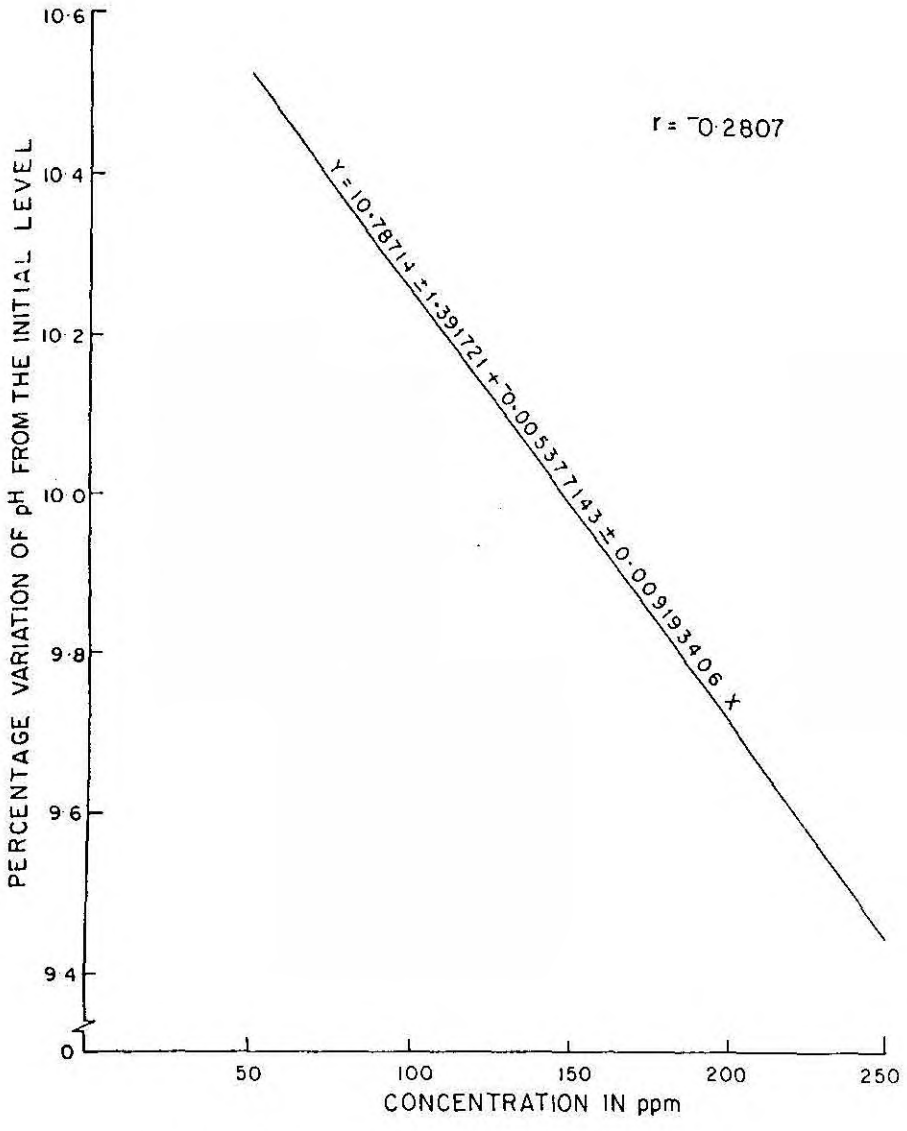


Fig.4. Regression line for the percentage variation of pH from the initial level in medium of P. indicus seeds exposed to the different concentrations.

Table-4. Time (in minutes) of 50%, 100% mortality and percentage variation of time from that of control

Concentrations (in ppm)	Time (in minutes)			
	50% mortality	% variation from control	100% mortality	% variation from control
Control	293		420	
50	357	21.84	515	22.62
100	613	109.22	840	100.00
150	823	180.89	1035	146.43
200	588	100.68	800	90.48
250	416	41.98	503	19.76

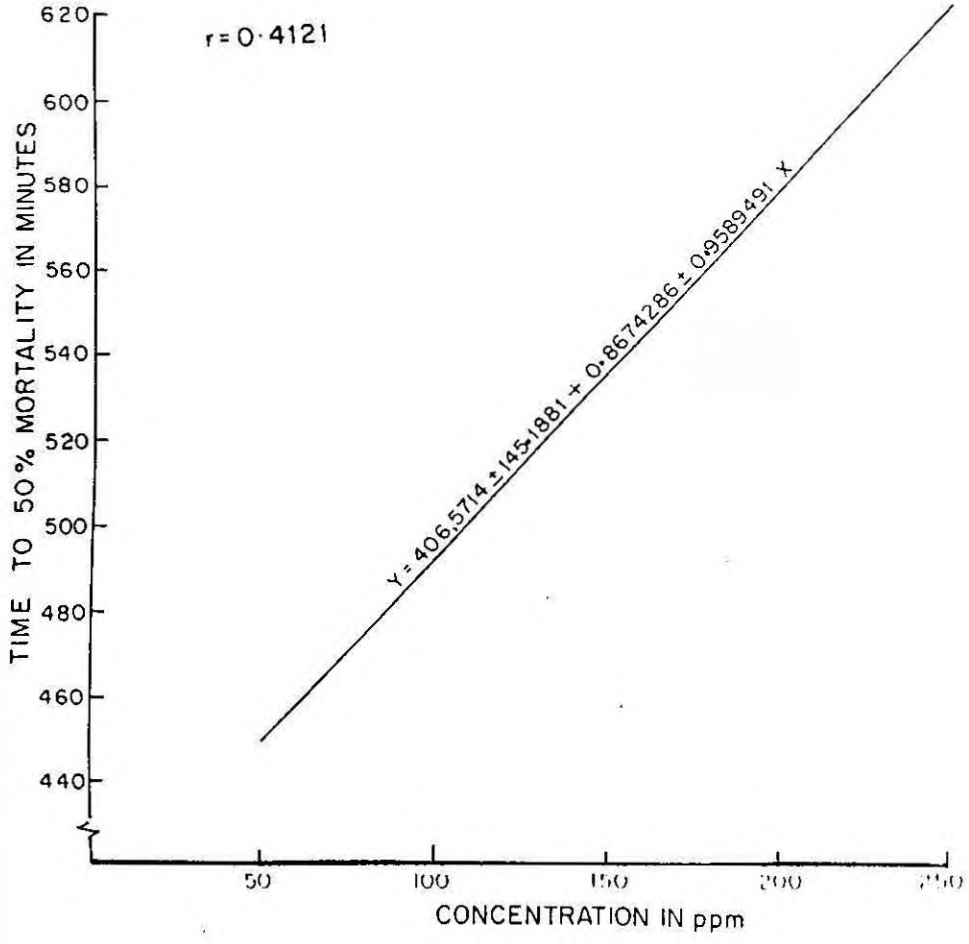


Fig.5. Regression line for the time for 50% mortality (in minutes) of P. indicus seeds exposed to the different concentrations.

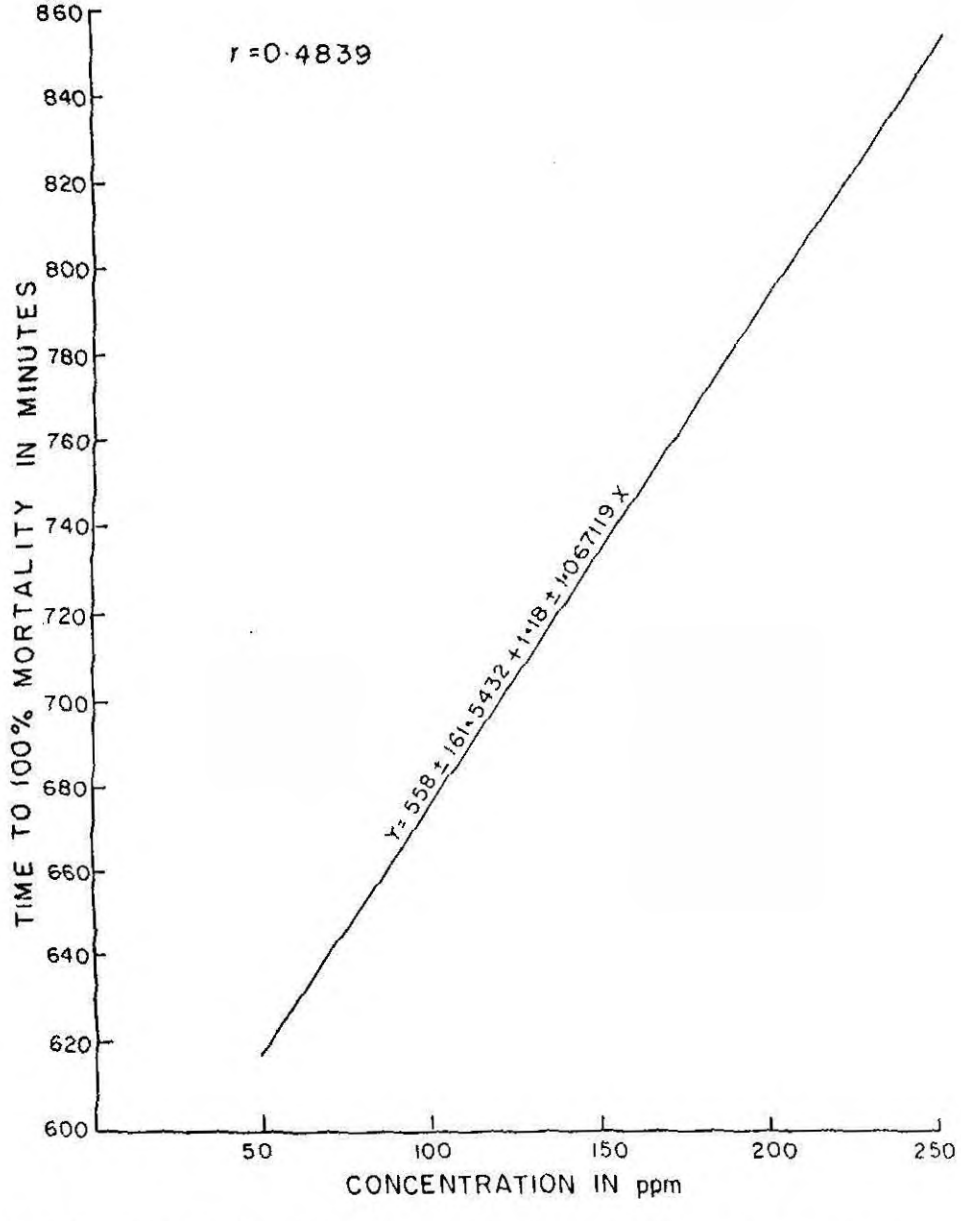


Fig.6. Regression line for the time for 100% mortality (in minutes) of P. indicus seeds exposed to the different concentrations.

Table-5a. Levels of protein, lipid and carbohydrate contents (in mg/100mg body wet-weight)

Concentration (in ppm)	Protein content		Lipid content		Carbohydrate content	
	Mean	SD*	Mean	SD*	Mean	SD*
Control	17.207	0.375	4.079	0.131	0.8216	0.0008
50	18.218	0.095	4.043	0.017	0.8029	0.0012
100	19.000	0.156	3.927	0.240	0.7914	0.00005
150	19.280	0.559	3.904	0.164	0.7840	0.439·0
200	18.246	0.166	3.948	0.285	0.8073	0.7350
250	17.262	0.190	4.02	0.353	0.8106	0.0297

*SD ± Standard deviation.

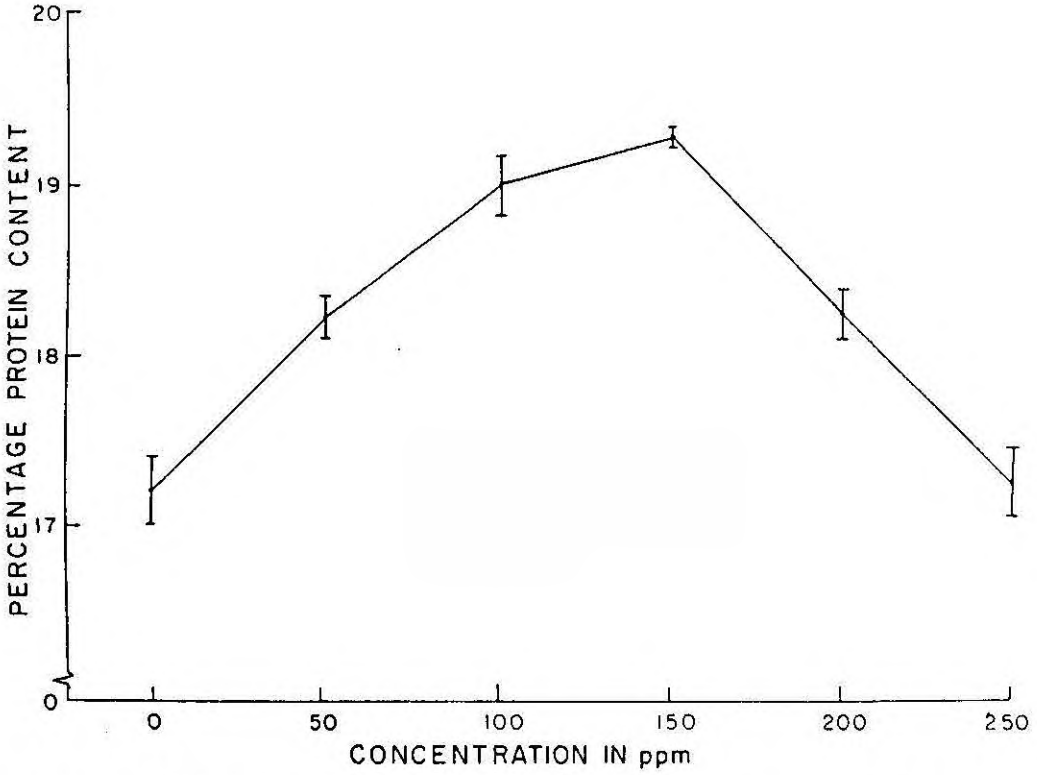


Fig.7. Percentage protein content of the P. indicus seeds in control and different concentrations after the test period.

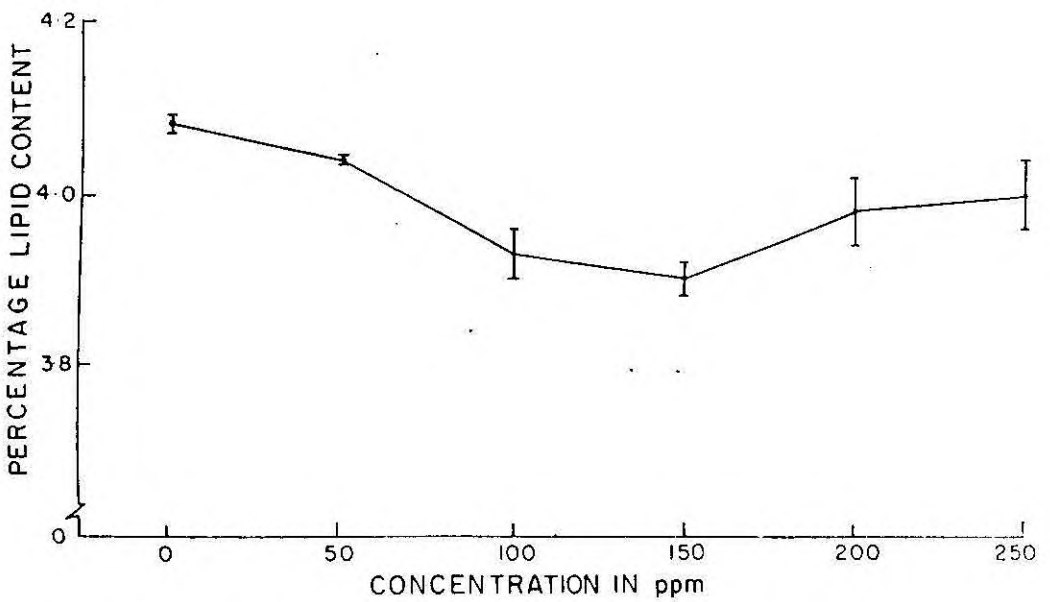


Fig.8. Percentage lipid content of the P. indicus seeds in control and fifferent concentrations after the test period.

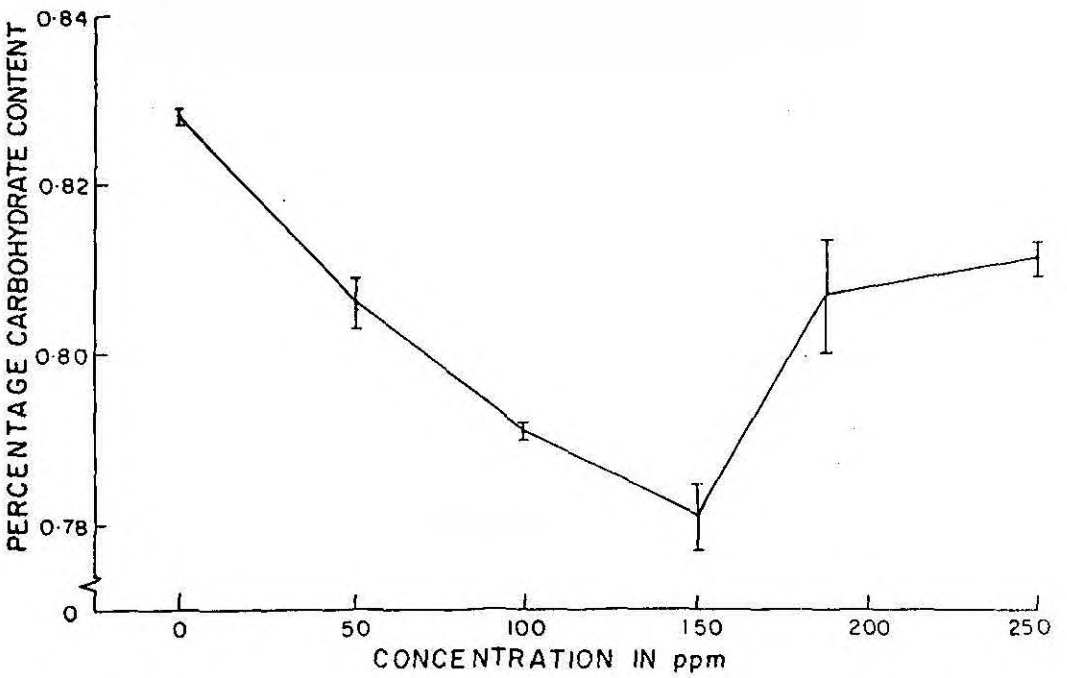


Fig.9. Percentage carbohydrate content of the P. indicus seeds in control and different concentrations after the test period.

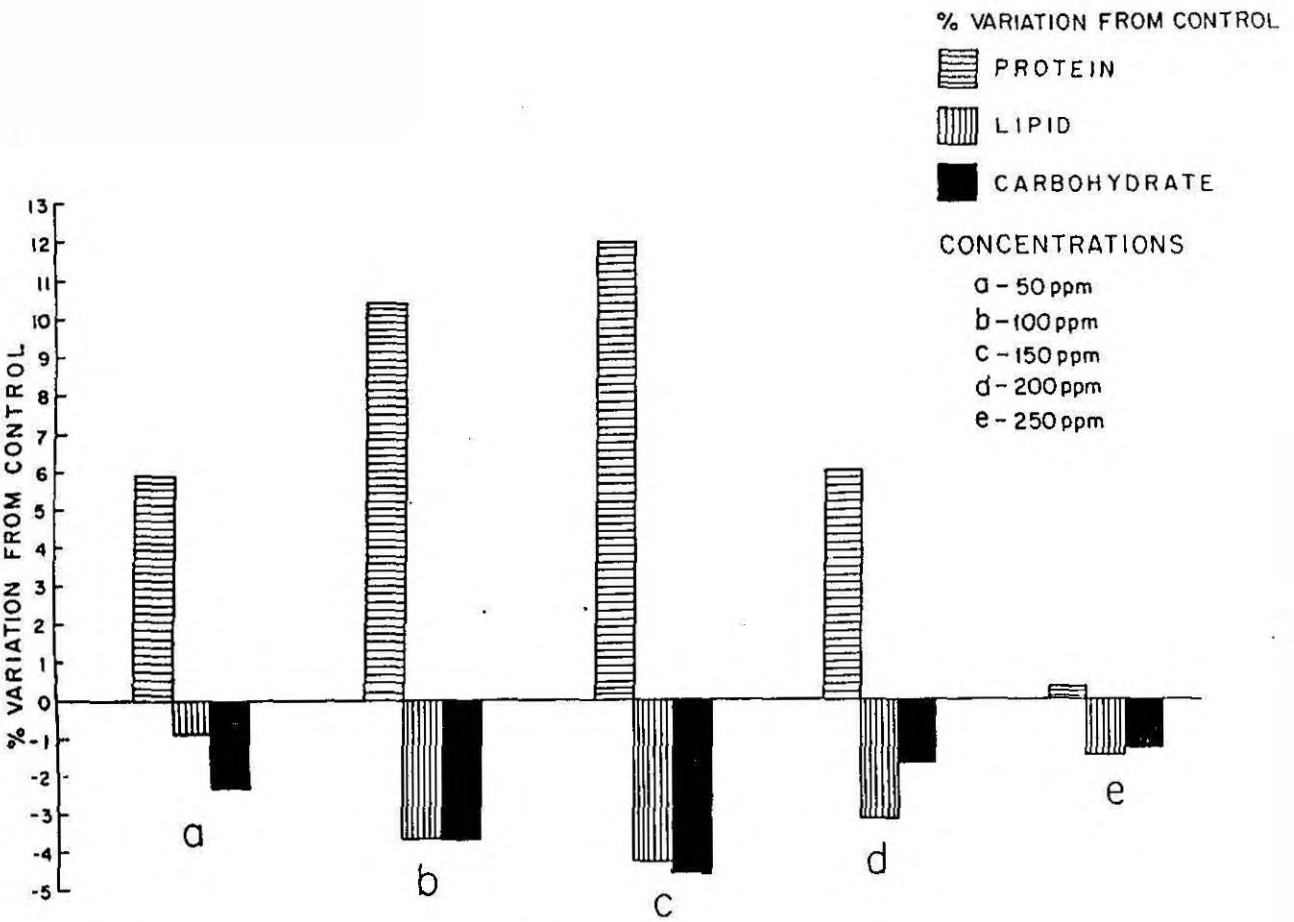


Fig.10. Percentage variation of protein, lipid and carbohydrate levels in the different concentrations of MS-222 from *control.*

Table-5b. ANOVA of protein content.

Source	Degree of Freedom	Sum of Squares	Mean of Sum of Squares	F-value	Remarks
Treatment	5	59.449	11.890	51.49	Highly significant at 1% level
Error	84	19.396	0.231		

Table-5c. ANOVA of lipid content.

Source	Degree of Freedom	Sum of Squares	Mean of Sum of Squares	F-value	Remarks
Treatment	5	4.150	0.830	1.23	Not significant
Error	84	56.498	0.673		

Table-5d. ANOVA of carbohydrate content.

Source	Degree of Freedom	Sum of Squares	Mean of Sum of Squares	F-value	Remarks
Treatment	5	0.026	0.005	0.38	Not significant
Error	84	1.152	0.014		

DISCUSSION

An evaluation of the results of the foregoing experiments highlights that anaesthetized prawn seeds in the tolerable range of MS-222 tended to live longer than in control.

Nelson (1953) found the MS-222 to rapidly induce complete anaesthesia in fish at a concentration of 0.34 mM/L but it caused 100% mortality at a slightly higher concentration of 0.4 mM/L. Pickford (1953) employed 0.13 mM/L only to completely anaesthetise Fundulus. Gilbert and wood (1957) used a concentration of 4 mM/L for quieting elasmobranchs as large as 400 pounds. McFarland (1960) suggested the MS-222 to rapidly induce a deep anaesthesia from which recovery was extremely fast, and recommended a range of 0.02 to 0.025 g/U.S. gal. for total loss of equilibrium and 0.025 to 0.030 g/U.S. gal. for total loss of reflex activity. Eisler and Backiel (1960) found that 20 ppm was ineffective, while 100ppm was fatal to fingerlings of Chinook salmon. Sreenivasan (1962) found 40ppm to be effective but harmless. Bove (1965) recommended a range of concentrations from 0.25 to 1.0 g/U.S. gal. or 1:3,800 to 15,000 water for anaesthetising salmon, trout and bass. For transport of tropical ornamental fish, the range suggested was between 1:12,000 and 24,000 water depending upon the time of transport, species and density. Durve (1966) suggested a concentration of 0.01 g/100 ml suitable to induce loss of equilibrium in mullet

fingerlings for 30 minutes and a range from 0.003 to 0.005 g/100 ml for transport. Gupta and Sharma (1975) have effectively transported Chinese carp at 1 to 6ppm without mortality. Gonzales et al. (1986) have recommended doses from 150 to 200ppm for short duration handling and from 250 to 300ppm for more time for Ictiobus cyprinellus. In the present investigation, the concentration of 50ppm to 250ppm was found suitable to anaesthetise the prawn seeds (Table-1).

With regard to metabolism, McFarland (1959) reported an initial period of excitment in Girella and Fundulus at high concentrations of all anaesthetics tested. He found that anaesthetized Fundulus had a rate of oxygen consumption lower than that in control. Guerin-Ancey (1970) reported the metabolism to be lowered in MS-222 treated fish. Baudin(1932) observed 50ppm MS-222 to half oxygen consumption of Carassius auratus. Jirasek et al.(1978) found it to lower oxygen consumption in Tinca tinca. Takeda et al. (1987) have suggested a solution of 85 to 90 /ug/L to safely force branchial irrigation in Cyprinus carpio. The results on the rate of oxygen consumption (Table-2a) in the present work show the oxygen consumption to be lower than in control at all concentrations and at 150ppm the oxygen consumption was almost halved.

The rate of ammonia excretion as already given in results, was found to be lower at all concentrations than in the control (Table-2a). Similar results have been achieved by Guerin-Ancey in 1970. Kutty (1972) explained the energy value of ammonia excretion in Tilapia mossambica

under the assumption that ammonia is the only end product of protein metabolism and the substrates (for protein metabolism) are whole protein and not intermediates. Following this assumption, the greater ammonia production can indicate a higher rate of protein metabolism, attributing to higher utilization of protein. The results of ammonia production (Table-5a) support this. According to Krishnakumar (1982) the lethal ammonia level for seed of P. indicus is 80ppm. The level of ammonia during the course of this experiment has increased to a maximum of 75ppm which is well below the lethal level, indicating the cause of mortality not due to ammonia toxicity.

The observations made on the ammonia quotient value are also comparable with previous literature. The ammonia quotient of T. mossambica during aerobic phase remained at about 0.2 but shot up to a value of 1.0 at low concentration (Kutty 1972). In Rhinomugil corsula, the routine ammonia quotient value increased from 0.109 to 0.384 (Kutty and Peer Mohamed 1975). In Carassius auratus and Tilapia the increased was from 0.104 to 0.219 (Peer Mohamed, 1974). In case of Macrobrachium malcolmsoni and Paradelphusa hydrodromus the routine ammonia quotient values increased respectively from 0.078 to 0.339 and 0.2612 to 0.534, clearly indicating extra release of ammonia anaerobically as observed by Kutty (1972 and 1978) and Peer Mohamed (1975). Similar results have been found in the present study as the ammonia quotient increased from the initial level to the end of the test period. This increase in ammonia quotient has been suggested by Kutty (1972) to be a coupling effect of the increased ammonia

excretion and increased carbondioxide production under low oxygen tension. But in the present study carbondioxide measurements were not done as measurement of oxygen consumption alone could give the results. It is possible that the relative increase in ammonia production during anaerobiosis helps to prevent acidosis as suggested by Kutty (1967 and 1972). Kripa (1984) found that the ammonia quotient value increase with decrease in ambient oxygen, and it is comparable to the results obtained in the present study. The ammonia quotient in the study decreased with increase in concentrations of MS-222. In control it was the highest. The MS-222 decreased the metabolism and reduced the rate of ammonia excretion resulting in lower ammonia quotient in the tests than in control.

In the present study, the pH level ranged from 6.9 to 8.1 during the course of the experiments. Ellis (1937) reported a good fish population at pH 6.3-9.0 under natural condition. In establishing the water quality criteria, Oransco (1955) pointed out that though fishes were found at pH values of 4-10, the safe range was between 5 and 9. Bishai (1960) reported mortality in young Atlantic salmon and trout exposed to 5.8-6.2 pH for 2 days. Results of Sarada (1984) suggested the pH range 6-9 not to affect the postlarvae of P. indicus. The results in the present study indicate that pH has not been the cause for mortality.

Since the time for 50% mortality is related to available dissolved oxygen the differences in the time could be accounted for their metabolic rates (McFarland, 1960). The increase in the time of 50% mortality (Table-4)

of the anaesthetized prawn seeds can be due to decreased metabolism. The time for 100% mortality (Table-4) also suggest that the anaesthetized prawn seeds showed decreased metabolism consequently on account of reduction in oxygen consumption and ammonia excretion. Indirectly controlling the pH, it enabled the prawn to live long before 100% mortality.

Since, the values for pH appear well above the reported lethal levels for P. indicus seeds, and the rate of ammonia excretion lowered; the only major limiting factor causing mortality is oxygen deficiency. The supposition that anaesthetics cause delay in mortality by decreasing metabolism is supported by the results.

It is well known that stress changes the physiology of the animal. Bayne (1986) has attributed the changes in biochemistry of body to be fundamentally adaptive to maximise the individual's fitness to changes in the environment. Stress caused by MS-222 has been worked by a few on fish. Wedmeyer (1969) indicated, that MS-222 cause pituitary activation in rainbow trout (Salmo gairdneri) and coho salmon (Onchorhynchus kisutch). While, Black and connor (1964) found no changes in blood and muscle lactate, haemoglobin, or muscle glycogen in rapidly anaesthetized rainbow trout; Randal (1962) showed the MS-222 to slowly increase the heart and respiratory rate of teleost fishes, probably via the parasympathetic innervation.

These are, however, conflicting inferences in the literature on possible biochemical changes in fish anaesthetized with MS-222. Wedmeyer

(1970) found rainbow trout anaesthetized with MS-222 for periods up to 12 minutes experiencing interrenal ascorbate depletion, uremia, and moderate hypercholesterolemia. Anaesthesia with neutralized MS-222 (pH-7) prevented these changes and significantly reduced the variability in plasma glucose, cholesterol and cortisol, indicating that the stress of anaesthesia with MS-222 was due to the low pK of the sulphonic moiety. In the present study, the stress due to the sulphonic moiety of MS-222 has been avoided by neutralising MS-222 with sodium hydroxide of 1 normalcy as has been mentioned in materials and methods. The levels of protein show that its metabolism is reduced. Whereas, the depletion in lipid content found could be attributed to lipolysis. A similar depletion (glycolysis) has also been indicated for carbohydrate content (Table-5a). for energy. Rao and Rao (1981) having found similar results in fish, reported a decrease in lipid content of liver along with decreased carbohydrate content indicating induced glyconeogenesis, which also supports the findings of the present study.

The findings in the present work suggest that MS-222 of 150ppm concentration can be used for handling and transport of the P. indicus seeds for 17 hours without aeration at 4.33 ml/l oxygen level, 0.15ppm ammonia level and 8.1 pH.

SUMMARY

1. The present study was done on the effect of MS-222 on the basal metabolism of seeds of Penaeus indicus. Rate of oxygen consumption, rate of ammonia excretion, ammonia quotient, changes in pH levels, the time for 50% and 100% mortality and the changes in the levels of protein, lipid and carbohydrate contents were studied in the tolerant range.

2. The seeds tolerated a concentration between 50 and 250ppm. Up to 25ppm, MS-222 was ineffective and above 250ppm it was harmful.

3. The seeds in 50 to 250ppm MS-222 lived longer than in the control.

4. The rate of oxygen consumption was lower in all concentrations in the range on account of reduced metabolism. The lowest rate observed as at 150ppm.

5. The rate of ammonia excretion also had the same trend as that in the rate of oxygen consumption, suggesting reduced protein metabolism. The ammonia content in the medium of all concentrations remained well below the reported lethal level.

6. The measurements of ammonia quotient showed decreased rate of oxygen consumption and ammonia excretion due to reduced metabolism;

the quotient was the highest in control.

7. The changes in the pH were within the reported safe level. The pH change was highest in control.

8. The time for 50% and 100% mortality was higher than that for the control in all concentrations. The maximum time was recorded in 150ppm.

9. The biochemical analyses showed reduced protein metabolism in combination with lipolysis and glycolysis.

10. The above results can be useful in the application of MS-222 for practical purpose of safely handling and transporting of prawn seeds at a concentration of 150ppm for 17 hours.

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