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Research Paper

Methomyl and lambdacyalothrininduced alterations in the protein content and recovery due to L-ascorbic acid in different tissues of the freshwater bivalve, *Lamellidensmarginalis* (lamarck).

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Abstract: The freshwater bivalve *Lamellidensmarginalis* were exposed to acute and chronic dose of lambda cyalothrin (0.75 PPM $LC_{50/2}$ valuesof 96 hours) and methomyl (35 PPM $LC_{50/2}$ valuesof 96 hours) upto 96hours and 21 days alone and in combination with 50mg/L and 100mg/L L-ascorbic acid for 21 daysrespectively. Percent protein contents in the mantle, foot, gills, digestive glands, gonad and whole body of bivalve, *Lamellidensmarginalis* on methomyl and lambda cyalothrin intoxication and in combination with 50mg/L and 100mg/L L-ascorbic acid were observed. Protein contents in all soft body tissue of methomyl and lambda cyalothrin exposed bivalve, *Lamellidensmarginalis* showed remarkable decreasein protein content as compared to control.The higher depletion of protein was observed in digestive glands as compared to other tissues. Animal exposed to methomyl and lambda cyalothrin intoxication in combination with 50 mg/L of L-ascorbic acid showed considerable reduction in the depletion of protein levels which further improved on treatment with methomyl and lambda cyalothrin intoxication in combination with 100 mg/L of L-ascorbic acid. Fast recovery of percent protein contents was observed in presence of L-ascorbic acid than the recovery in the normal freshwater. This study indicates the protective and curative property of the L-ascorbic acid against methomyl and lambda cyalothrininduced damage.

Keywords:
Lamellidensmarginalis,methomyl, lambda cyalothrin, protein, L-ascorbic acid

INTRODUCTION:

The biochemical changes occurring in the body gives a first indication of stress. During the stress, to overcome the altered situation extra energy is needed. Bivalves are potential biomonitoring organisms for toxicity evaluation by being sedentary organisms, reflect local condition, an efficient filter feeder(Ullven 1993; Huang *et al.*, 2007). They have a long-life span (Farrington *et al.*, 1983). In addition, their ability to bio-transform accumulated toxicants is generally lower than many

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other aquatic organisms (Borchert et al. 1997). They are sturdy enough to survive in laboratory and field studies, provides a time integrated indication of environmental contamination hence fulfilling the criteria as good bioindicators (Regoli, 1998; Olivier *et al.*, 2002; Huang *et al.*, 2007). Bivalves have a very low level of activity of enzyme systems capable of metabolizing persistent pollutants. Therefore, contaminants concentration in the tissues of bivalves more accurately reflects the magnitude of environmental contamination (Phillips, 1977, Phillips, 1980, Phillips, 1990).For all these reasons, mussels are very widely used in programs monitoring the pollution of the aquatic environment (Goldberg 1975, Amiard et al. 1986, Kljakovic-Gaspic et al. 2006).The bivalve resists against such unwanted conditions by its own way and try to minimize the effect of the altered situation by removing the toxicants or by its bio-transformation. The toxicants interact with certain receptors and proteins in the organisms and cause the harmful effect. The toxic chemicals bring about the damage to different organs or disturb the physiological and biochemical processes within the organism, since different environmental pollutants are likely to affect biological system in different ways according to the mode of action of the pollutants. Recent understanding of different biochemical processes has proved useful in determining the mechanism of toxicity of different toxicant as also in unfolding the adaptive protective mechanism of the body to fight the toxic effect of the pollutant (Thateyush *et. al.*, 1987). Besides it is also now felt that some of the biochemical alternation occurring in the body gives the first indication of the stress in the organism and hence effect on the part of the pollution (Ruparelia*et. al.*, 1992).Therefore, the study of metabolic changes in the organisms induced under stress of pesticides assumes the importance.

MATERIALS AND METHODS:

The adult fresh water bivalve molluscs *Lamellidensmarginalis* were collected from the banks of Darna river at Chehedi water works (pumping station, Latitude 19 ^{0} 55.873'and Longitude 73 ^{0} 51.429'), village Chehedi near Nashik (M.S.) during summer season. After collection the animals were brought to the laboratory, the shells of the bivalves were brushed and washed with water to remove the mud and fouling algal and fungal biomass. The bivalves were acclimatized in the laboratory condition at room temperature for 4-5 days in dechlorinated tap water. The active acclimatized bivalves of approximately same size were selected for experiment.

Experimental Design: -

Set –I For the experimental studies the animals were divided into four groups.

A) Group 'A' was maintained as control.
B) Group 'B' animals were exposed to acute dose of lambda cyalothrin(0.75 PPM $LC_{50/2}$ valuesof 96 hours) and methomyl (35 PPM $LC_{50/2}$ valuesof 96 hours) upto 96hours.
C) Group 'C' animals were exposed to acute dose of lambda cyalothrin(0.75 PPM $LC_{50/2}$ valuesof 96 hours) and methomyl (35 PPM $LC_{50/2}$ valuesof 96 hours) along with 50mg /1 of L-ascorbic acid upto 96hours.
D) Group 'D' animals were exposed to acute dose of lambda cyalothrin(0.75 PPM $LC_{50/2}$ valuesof 96 hours) and methomyl (35 PPM $LC_{50/2}$ valuesof 96 hours) along with 100mg /1 of L-ascorbic acid upto 96hours.

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Acute exposure was carried over upto four days. Every day the solutions were changed.

Set- II- Experimental Design for Recovery Studies:

1) Group 'A' animals were maintained as control.
2) Group 'B' animals from set –I were divided into three groups for recovery study.

I. Animals pre-treated to lambda cyalothrinand methomyl were allowed to self – cure normally in untreated fresh water up to 21days.

II. Animals pre-treated to lambda cyalothrinand methomyl were allowed to cure in 50mg /l of L-ascorbic acid in fresh water upto 21days.

III. Animals pre-treated to lambda cyalothrinand methomyl were allowed to cure in 100mg /l of L-ascorbic acid in fresh water upto 21days.

After 24 and 96 hours of interval animals from set-I were taken out, dissected and part of their mantle, foot, gills, digestive glands and whole body were taken out and after 4, 7,14 and 21 days, animals from control and set-II were taken out, dissected and their gills, gonads and digestive glands were taken out and dried at 80^{o} Cin an oven till constant weight was obtained. The dried powders of different tissues of control and experimental animals were used for estimation of proteins.The total proteins were estimated by (Lowry *etal.*, 1951).All the values expressed in terms of mg/100 mg of dry weight of tissue powder. Qualitative and quantitative study of changes in protein components of organisms is useful to know different toxicants and defensive mechanism of the body against toxic effects of pesticides.

RESULTS AND DISCUSSION:

The results obtained for the protein contents in differentsoft body tissues after acute and chronic exposure to methomyl and lambdacyalothrinwithout and with L-ascorbic acid and during recovery are summarizedin the table no. 1 to 4. The obtained results revealed that, after acute and chronic exposure to pesticides, a marked decrease in the protein contents in the mantle, foot, gills, gonad, digestiveglands and whole soft body tissues of the experimental freshwaterbivalve, *Lamellidensmarginalis*were observed and comparedto bivalves maintained as control. The results obtained showedthat, there wasprogressive decrease in the protein content with the increase in the exposure period.

The results recorded inthe present study are in harmony with the results of previousinvestigators (Waykar and Pulate, 2012; Pardeshi and Gapat, 2012; Waykar and Tambe 2014). Proteins are among the most abundant biological macromolecules and are extremely versatile in the function. Proteins provide structure, catalyze cellular reactions and carry out a myriad of other functions. Proteins are important biomolecules involved in a wide spectrum on cellular functions. Proteins are one of the main targets of free radicals' attack. Over produced radicals can react with protein and amino acids to oxidize and cross-link them. Radical-protein reactions can impair the function of important cellular and extracellular proteins like enzymes and connective tissue proteins permanently. Any alteration in the environment affects the level of protein content by changing the physiology of organism (Young, 1970). All enzymes are protein in nature and they control sub-cellular functions. In the metabolism of proteins, many enzymes, coenzymes, intermediate proteins and amino acids involved are studied in many animals.

In the present investigation the total protein content was found to be depleted in digestive gland, gill and whole-body respectively. This might be due to highly metabolic potency and efficiency of the

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digestive gland as compared to other tissues like gill and whole-body. The digestive gland may be the site of action of pollutant in the body of bivalve or digestive gland seems to be the main site of degradation and detoxification of pesticides and hence has the largest demand of energy for the metabolic processes resulting into increasing utilization of protein to meet energy demand. In the presence of reactive oxygen species (ROS), proteins can be damaged by oxidative attack, results in site-specific amino acid modifications, fragmentation of the peptide chain and aggregation of cross-linked reaction products, altered electrical charge and increased susceptibility to proteolysis (Grune, 2000; Requena*et al.*, 2003).The observed low level of protein contents in different tissues indicate that environmental stress reduces the rate of protein synthesis or increase the proteolysis to cope with the high energy demands under toxicants stress (Vincent *et al.*, 1995; Waykar and Lomte, 2001a). The low protein content might be due to the destruction/necrosis of cells and consequent impairment in protein synthetic machinery (Umminger, 1977; Bradbury *et al.*, 1987). It is known that structural proteins are used as energy source under stressful conditions (Claybrook, 1983). Pottinger *et al.*, (2002) reported that at high pollution stress, protein synthesis can be suppressed representing disturbance of regular metabolic processes. Waykar and Pulate (2012) reported decreased protein contents in different soft tissues of freshwater bivalve, *Lamellidens marginals* (L) after exposure to pollutants.Deshmukh (2013) reported that bivalve species inhabiting at higher level of polluted site showed low level of protein content, while bivalve species inhabiting at low level of polluted site showed higher level of protein content. Waykar and Tambe (2014) reported that freshwater bivalve *Parreysiacylindrica* exposed to pesticide decreased the protein content in soft body tissues.

CONCLUSION:

It may be concluded from the given results that the physiological disturbances observed in freshwater bivalves after exposure to pesticides methomyl and lambdacyalothrin reveals trends towards normalization and the rate of recovery from pesticide induced damage improved on exposure to L-ascorbic acid showed the preventive and curative property of the L-ascorbic acid against the pesticide induced damage. Thus, it became evident that vitamin C not only confirms protection against pesticide toxicity but also performs therapeutic role against pesticide toxicity in freshwater bivalves.

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Table 1: Impact of pesticides on protein content in different tissues of *Lamellidensmarginallis* after acute exposure.

Sr. No.	Tissue	Control	methomyl				lambda Cyalothrin			
			24 hrs.	48 hrs.	72 hrs.	96 hrs.	24 hrs.	48 hrs.	72 hrs.	96 hrs.
1	Mantle	48.9579 ±1.7310	44.7737 ±1.976 -7.57 P<0.01	40.5563 ±0.2652 -15.62 P<0.01	37.5694 ±1.6679 -21.83 P<0.001	33.5324 ±1.8924 -30.97 P<0.001	42.5740 ±1.5965 -11.19 P<0.001	37.4678 ±1.6686 -21.64 P<0.001	34.3324 ±0.8344 -28.86	30.869 ±1.7578 -35.50 P<0.001
2	Foot	70.9322 ±1.6529	67.4761 ±1.9549 -4.87 P<0.01	65.7669 ±1.9572 -7.40 P<0.05	61.7963 ±1.2762 -13.34 P<0.001	55.6505 ±1.2938 -21.67 P<0.001	66.5764 ±1.0965 -6.04 P<0.01	63.4613 ±1.0676 -10.44 P<0.01	59.2242 ±1.9720 -16.57 P<0.001	54.6734 ±1.7764 -23.66 P<0.001
3	Gill	60.5157 ±3.9734	53.3549 ±3.6863 -12.44 P<0.01	46.4971 ±3.1386 -22.08 P<0.001	43.4691 ±2.2765 -28.51 P<0.001	40.5508 ±2.1654 -32.81 P<0.001	50.5442 ±2.1870 -16.30 P<0.01	41.3829 ±1.4361 -31.63 P<0.001	38.3436 ±2.2766 -36.48 P<0.001	35.8727 ±3.1235 -41.46 P<0.001
4	Digestive Gland	54.4687 ±2.6478	44.5477 ±2.9873 -18.57 P<0.01	39.7698 ±1.3359 -26.60 P<0.001	35.8641 ±2.9451 -33.86 P<0.001	32.4709 ±2.4652 -40.41 P<0.001	43.8460 ±3.1399 -19.55 P<0.01	36.2448 *** ±2.3577 -33.73 P<0.001	31.5642 ±1.5875 -42.42 P<0.001	28.8912 ±2.189 -46.53 P<0.001
5	Whole Body	66.8755 ±1.9557	62.6792 ±1.6569 -6.35 P<0.01	58.5764 ±0.9653 -12.69 P<0.001	53.6790 ±1.2578 -19.20 P<0.001	48.5413 ±1.2135 -27.54 P<0.001	60.0454 ±0.8845 -9.77 P<0.01	55.4889 ±2.7973 -16.71 P<0.01	48.6712 ±0.9674 -26.31 P<0.001	46.6514 ±3.5678 -30.72 P<0.001

1. Values expressed as mg/100 mg dry wt. of tissue.
2. (+) or (-) indicate percent variation over control.
3. ± indicate Standard deviation of three observations.
4. Values are significant at *=P<0.05; **=P<0.01; ***=P<0.001; NS= Not Significant.

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Table 2: Impact of pesticides on protein content in different tissues of *Lamellidensmarginallis* after chronic exposure.

S.N	Tissue	Control			methomyl			lambda Cyalothrin		
		7 days	14 days	21days	7 days	14 days	21days	7 days	14 days	21days
1	Mantle	46.3214 ±1.9551	45.2492 ±1.6892	45.7567 ±0.4946	33.2577 ±0.9568 -29.57 P<0.001	28.7727 ±1.2671 -37.64 P<0.001	25.9357 ±0.8254 -43.43 P<0.001	30.9894 ±1.3446 -33.25 P<0.01	24.8766 ±1.2667 -45.97 P<0.001	22.5877 ±1.2567 -50.75 P<0.001
2	Foot	67.5068 ±0.4944	68.2157 ±0.7547	67.8989 ±0.9737	58.8553 ±1.4912 -14.31 P<0.001	48.8918 ±2.1701 -28.19 P<0.001	45.4892 ±1.2872 -33.18 P<0.001	50.3074 ±1.6396 -25.57 P<0.001	47.4665 ±0.8460 -30.43 P<0.001	43.9675 ±0.6456 -35.76 P<0.001
3	Gill	58.2927 ±0.7876	57.584 ±2.5674	57.1451 ±0.8913	40.5474 ±1.0169 -30.83 P<0.001	35.4361 ±2.2654 -38.43 P<0.001	29.6526 ±1.2685 -48.36 P<0.001	33.6977 ±0.8254 -42.29 P<0.001	29.5656 ±0.4680 -48.96 P<0.001	24.2567 ±3.2978 -57.48 P<0.001
4	Digestive Gland	52.0946 ±1.6456	51.2991 ±2.7234	50.5458 ±1.6659	32.7660 ±1.2672 -37.68 P<0.001	30.1875 ±0.4787 -41.33 P<0.001	22.9363 ±1.6459 -54.64 P<0.001	29.2662 ±2.4504 -44.10 P<0.001	20.453 ±1.6239 -59.54 P<0.001	18.783 ±1.4679 -62.66 P<0.00
5	Whole Body	64.9822 ±1.984	63.2952 ±0.9350	62.5951 ±1.0543	49.4320 ±0.4869 -23.80 P<0.001	40.3936 ±1.7478 -36.28 P<0.001	35.3766 ±0.7656 -43.34 P<0.001	43.1087 ±1.7557 -33.70 P<0.001	33.5328 ±2.4644 -47.42 P<0.001	29.2662 ±0.8164 -53.11 P<0.001

1. Values expressed as mg/100 mg dry wt. of tissue.
2. (+)or (-) indicate percent variation over control.
3. ± indicate Standard deviation of three observations.
4. Values are significant at *=P<0.05; **=P<0.01; ***=P<0.001; NS= Not Significant.

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Table No 3: Protein content in selected tissues of *Lamellidansmargianlis after* acute and chronic exposure to methomyl without and with ascorbic acid during recovery (Value represent percentage in dry weight)

Treatment		Tissue	24 hrs	96 hrs	Recovery			
					4 days	7 days	14 days	21 days
Control		**Gill**	59.5910±0.6163	59.2989±0.9763				
		Gonad	52.6986 ±1.0235	51.8567±0.9642				
		D.gland	54.5391 ±0.8972	53.8976±0.4593				
methomyl		**Gill**	46.8164±1.0828 -21.43	31.3782±0.9748 -47.08				
		Gonad	44.6789±0.9786 -15.21	29.2346±0.9502 -43.62				
		D.gland	42.4297±0.7254 -22.20	26.6789±0.7280 -50.50				
methomyl 50 mg/L A.A.		**Gill**	50.8443±0.5497 -14.67	38.0214±1.1432 -35.88				
		Gonad	47.8417±0.6975 -9.21	35.9692±0.6798 -30.63				
		D.gland	46.2094±1.5797 -15.27	32.1785±0.4970 -40.29				
methomyl 100 mg/L A.A.		**Gill**	52.6723±1.2580 -11.61	40.6840±0.7902 -31.39				
		Gonad	48.8417±0.6975 -7.31	38.2187±0.5246 -26.29				
		D.gland	47.7892±0.6789 -12.37	36.6782±0.8596 -31.94				
After 96 hrs exposure to Acutemethomyl	Normal water	**Gill**			37.4662 ±0.8674	40.9869 ±0.8469	48.4469 ±0.7945	55.4531 ±0.7652
		Gonad			33.7896 ±0.7431	36.9426 ±0.2546	43.6785 ±1.0085	48.253 ±1.2184
		D.gland			32.9472 ±0.9973	34.5682 ±0.8420	42.5673 ±1.0350	50.589 ±0.8054
	Normal water ±50mg/ L AA	**Gill**			46.4284 ±0.2105	51.8976 ±1.0539	56.9889 ±1.7024	59.5508 ±1.2114
		Gonad			42.8679 ±1.2810	47.3642 ±1.3054	49.5672 ±2.0054	52.7824 ±1.0481
		D.gland			42.7829 ±1.3052	46.9542 ±1.0341	52.4312 ±2.1531	55.6402 ±1.2861
	Normal water ±100mg/ L AA	**Gill**			51.6109 ±2.1642	56.4572 ±0.8679	59.8679 ±2.0057	60.2314 ±1.0842
		Gonad			45.9786 ±0.9875	48.6221 ±1.0039	50.2408 ±1.8642	54.9743 ±2.1538
		D.gland			48.0642 ±0.4628	51.0431 ±1.3240	52.9675 ±2.0164	58.7891 ±1.7453

1. Values expressed as mg/100 mg dry wt. of tissue.
2. (+)or (-) indicate percent variation over control.
3. ± indicate Standard deviation of three observations.
4. Values are significant at *=P<0.05; **=P<0.01; ***=P<0.001; NS= Not Significant.

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Table No. 4: Protein content in selected tissues of *Lamellidansmargianlis after* acuteand chronic exposure to lambda cyalothrin without and with ascorbic acid during recovery (Value represent percentage in dry weight)

Treatment		Tissue	24 hrs	96 hrs	Recovery			
					4 days	7 days	14 days	21 days
Control		Gill	59.5910±0.6163	59.2989±0.9763				
		Gonad	52.6986±1.0235	51.8567± 0.9642				
		D.gland	54.5391±0.8972	53.8976±0.4593				
lambda cyalothrin		Gill	50.3799±1.0764 -15.45	34.8539±0.8560 -41.22				
		Gonad	45.4563±0.6782 -13.74	32.9486±0.5472 -36.46				
		D.gland	43.7235±0.7587 -19.83	29.4965±0.8231 -45.27				
lambda cyalothrin 50 mg/L A.A.		Gill	53.6408±0.9341 -9.98	41.4243±0.6476 -30.14				
		Gonad	48.9496±0.9782 -7.11	37.1245±0.6742 -28.40				
		D.gland	46.9009±0.7289 -14.00	36.9185±0.4267 -32.09				
lambda cyalothrin 100 mg/L A.A.		Gill	55.8994±0.8966 -6.19	43.3421±0.8175 -23.90				
		Gonad	50.0862±0.6274 -4.95	39.0439±1.0135 -24.70				
		D.gland	48.6822±0.7894 -10.73	38.640±0.4236 -28.30				
After 96 hrs exposure to Acutelambda cyalothrin	Normal water	Gill			36.5182 ±0.8457 +25.02	39.3946 ±0.8618 +13.02	43.2401 ±0.7213 +24.06	49.9685 ±0.0562 +43.36
		Gonad			35.7482 ±0.8677 +23.74	37.3789 ±0.0437 +13.44	39.8475 ±0.1026 +20.93	46.0452 ±1.0942 +39.74
		D.gland			33.6754 ±0.8572+15. 09	36.4238 ±0.0256 +23.48	38.7649 ±0.1264 +31.42	45.1649 ±0.2008 +53.11
	Normal water ±50mg/ L AA	Gill			39.9573 ±0.9364+33. 02	46.6249 ±0.1354 +33.77	50.8091 ±0.0035 +45.77	59.19891 ±1.0586 +69.84
		Gonad			40.3875 ±0.7854+45. 05	42.7986 ±1.0281 +36.57	48.4352 ±0.8842 +47.00	51.2046 ±1.0372 +55.40
		D.gland			43.5976 ±0.5839+53. 42	47.7985 ±1.7105 +62.04	51.0894 ±2.003 +73.20	53.7764 ±0.9835 +82.31
	Normal water ±100mg/ L AA	Gill			48.6983 ±0.6271 +48.62	54.7668 ±1.0053 +57.13	59.1846 ±2.0054 +69.66	57.5876 ±1.0028 +65.22
		Gonad			45.3966 ±0.8673 +39.85	48.3416 ±0.9473 +46.71	51.6792 ±1.0251 +56.84	52.7492 ±2.008 +60.09
		D.gland			49.4676 ±1.0576 +68.56	51.3426 ±1.0453 +74.06	52.9521 ±0.9975 +79.51	54.9785 ±0.8746 +86.38

1. Values expressed as mg/100 mg dry wt. of tissue.
2. (+)or (-) indicate percent variation over control.
3. ± indicate Standard deviation of three observations.
4. Values are significant at *=P<0.05; **=P<0.01; ***=P<0.001; NS= Not Significant.