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ORIGINAL ARTICLE

Antioxidant enzyme activity of *Macrobrachium rosenbergii* under *Vibrio harveyi* infection, supplemented with *Centella asiatica* derived active principle enriched diet

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ABSTRACT
As a good source of protein meal , fish and shell fish products demands a major share in human nutrition. These increased demands leads to the more production by intensive methods. The intensive methods yield more products from a unit area than from natural habitats. As a artificial method it will produce more worse effects than benefits like serious disease outbreaks. In this study fresh water prawn, Macrobrachium rosenbergii was taken as candidate for intensive culture. The disease can be controlled by many methods but none of them yield a better outcome. So medicinal plants can be used as feed ingredient and as medicine. In numerous cases the medicinal are using as a food material. In the present study Centella asiatica was used as a feed ingredient. Certain active principles from Centella asiatica was used as feed ingredient such as asiaticoside, madecossoside and asiatic acid were selected for feed preparation at selected concentrations. After culturing the prawns were infected with Vibrio harveyi , and the antioxidant enzyme profile of selected sites were checked and noted . The prawns fed with asiaticoside shown promising results.
Key words: *Aquaculture, Macrobrachium rosenbergii ,intensive farming, asiaticoside, madecossoside , asiatic acid.*

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INTRODUCTION

For normal growth and body activities, nutrition plays a momentous role. These homeostasis may get disrupted in many conditions like presence of stress, diseases, bacterial and viral infections, xenobiotics [20]. When the above mentioned conditions crosses the optimum level, presence of certain substances called "free radicals "will be generated. There is substantial link between free radicals and diseases such as cancer, diabetes, Alzheimer‟s disease, strokes, heart attacks and atherosclerosis [8]. When free radicals are present in excess of the defense mechanism's ability to control them is when damage may occur, and until subsequent free radicals are deactivated, thousands of free radical reactions can occur within seconds of the initial reaction [23]. Normally there is a balance between free radicals and antioxidants, known as redox balance [5]. Although ongoing oxidative damage is, thus, generally analyzed by measurement of secondary products, including derivatives of amino acids, nucleic acids, and lipid peroxidation [14]. For the protection of cells against reactive oxygen species (ROS), organisms have evolved a highly sophisticated and complex antioxidant protection system. Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPX), Glutathione S- trasferase (GST), etc are the major antioxidant enzymes and their activities are interrelated. Glutathione directly quenches ROS such as lipid peroxides, and also plays a major role in xenobiotic metabolism. In animals hydrogen peroxide is detoxified by CAT and by GPX. CAT protects cells from hydrogen peroxide generated within them. Antioxidant is a molecule that inhibits the oxidation of other molecules. It controls the free radical formation. They will provide electrons to free radicals to neutralize them [11].

Main functions of Glutathione Peroxidase(GPX) includes , removal of H_2O_2, removal of other hydroperoxides thus giving protection against lipid peroxidation, GSTs are a group of intracellular

enzymes with the main function in detoxification processes by catalyzing the conjugation of tripeptide glutathione (GSH) with some endogenous toxic metabolites and many environmental contaminants [23]. Lipid peroxidation is the process in which free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage. The final product of lipid peroxidation is reactive aldehydes, such as malondialdehyde (MDA) and 4-hydroxynonenal (HNE) [2]. AST and ALT activities are usually used as general indicators of the functioning of invertebrate Hepatopancreas. Both the ALT and AST are key enzymes for the inter conversion of amino acids and other intermediary metabolites in crustacean and are detected in the hepatopancreas, muscle and gill. AST and ALT are also biological catalyst.Lactate dehydrogenase is found in all most all organisms. In the present study we intended to check the influence of active principles from *Centella asiatica* incorporated feeds were supplemented to *Vibrio harveyi* infected *Macrobrachium rosenbergii*. The antioxidant enzyme profiles was checked.

MATERIAL AND METHODS

Post larvae of *Macrobrahium rosenbergii* (PL20) *was* purchased from ADAK (Agency for Development of Aquaculture (ADAK), Varkala, Kerala. Acclimated in the laboratory of Fisheries Biotechnology unit ,Dept.of Zoology, Fatima Mata National College, Kollam,Kerala under optimum salinity, pH and temperature(2017october–2017december).During acclimatization they were fed *ad libitum* with control diet and egg albumin. Reared the prawn until they reached a average size of ~10.00 ± 0.00. After that the prawns were reared in separate tanks. The basal feed was prepared by using the ingredients such as Fish Meal(300 g), Prawn Head(50 g), Squid Meal waste(50 g), Squilla(50 g), Soyabean Meal(250 g), Wheat Flour(250 g),Fish Oil(30 g), Vitamin Mineral Mixture(20 g) FAO.(1978).The concentrations were maintained while taking the active principles for feed incorporation is

Test Diet 1 (TD 1) : 2.9 mg of Asiaticoside per kg of basal feed.
Test Diet 2 (TD 2): 3.1 mg of Madecossoside per kg of basal feed.
Test Diet 3 (TD 3): 2.5 mg of Asiatic acid per kg of basal feed
Test Diet 4 (TD 4): Control(basal diet).

M.rosenbergii of size 10.20 ± 0.20 g was taken for experiment. 40 prawns were selected. Ten prawns in each treatment. Each treatment ran in triplicates. Proper aeration and water quality were maintained .Each treatment were fed with consent medicated feed. The experiments were conducted for a period of 60 days. After the rearing of 60 days, prawns were infected with prawn pathogen *Vibrio harveyi* . After 72 hours the prawns were sacrificed and haemolymph and tissues such as muscle, digestive gland (hepatopancresas) and gut were removed, washed thoroughly in ice-cold physiological saline, wiped and respective tissue extracts were prepared. Subsequently the corresponding tissues were prepared for antioxidant enzyme studies.

V.harveyi was grown 12 h at 37 ^{0}C in TSA broth in aerobic condition. It was cultured in 10 ml tryptic soy broth (TSB, Difco) for 12 h at 37°C as a stock culture for tests. For challenge experiments, stock cultures were centrifuged at 7155 × *g* for 15 min at 4°C. The supernatant fluid was removed and the bacterial pellet was resuspended in saline solution (0.80% NaCl) at 1x 10^7 CFU/ml–10^9 CFU/ml. 1x 10^7 CFU/ml as stock bacterial suspensions was used for challenge tests. After rearing for a period of 60 days the prawn were challenged with 30 µL of 10^7 CFU bacterial suspension between 3rd and 4th abdominal segments. Relative percentage survival (RPS) were noted and tabulated.Animals were sacrificed by removing their carapace from their abdomen with a forceps and the hepatopancreas, gills and digestive glands were dissected out quickly. Tissues were washed in ice- cold normal saline (0.67%, w/v), blotted, flash frozen in deep freezer. For haemolymph studies, Haemolymph sample was collected from each prawns by inserting a 26-guage needle attached to a 1ml syringe containing 0.1 ml tri-sodium citrate as anticoagulant into the pericardial sinus of *M.rosenbergii*.

Antioxidant assays.

Both AST and ALT activities were determined spectrophotometrically at 340 nm using a UV 2000 spectrophotometer (Hitachi) at 37 ^{0} C. 100 µl hemolymph samples were taken for each study.(G.O.T, IFCC method, Kinetic). Activities were expressed in international enzyme units (IU/L).Lactate dehydrogenase (LDH) was determined spectrophotometrically at 340 nm using a UV 2000 spectrophotometer (Hitachi) at 37 ^{0} C(LDH(P-L) kit by Mod.IFCC method). other enzyme are super oxide dismutase, SOD [13], Catalase, CAT [1], Glutathione peroxidase,GPX [6], Glutathione –S- Transferse ,GST [17], Lipid peroxidation,MDA [3]. All data were statistically analyzed ANOVA multiple comparisons (Duncan‟s) test was conducted to compare significant differences among treatments using the SPS software and differences were considered significant at $p<0.05$.

RESULTS

Table.1. Antioxidant enzyme profile of haemolymph of *M.rosenbergii* at 72 hours post infection with *V.harveyi*.

	GOT (IU/L)	GPT (IU/L)	LDH(IU/L)
Test Diet 1	0.03±0.01	0.04±0.01	0.32±0.05
Test Diet 2	0.05±0.01	0.05±0.01	0.41±0.01
Test Diet 3	0.06±0.01	0.05±0.01	0.48±0.01
Control	0.07±0.01	0.06±0.00	0.58±0.01

The Glutamate oxaloacetate transaminase (GOT) activity of all treated groups were comparatively lower than that of control. Haemolymph of prawns fed with Test Diet 1 (0.03±0.01 IU/L) reported lower GOT activity. Higher GOT activity was observed in prawns fed control diet (0.07±0.01 IU/L).Least GPT activity was in test diet 1 supplementation gone with (0.04±0.00 IU/L). Herbal active principles incorporated diets shown more promising results than control ($p<0.05$).The Lactate dehydrogenase (LDH) activity was higher in control diet (0.58±0.04 IU/L).Least activity was observed in test diet 1 fed prawns (0.32 ±0.05 IU/L).In all others the LDH activity was lower than control.

In muscle tissue highest CAT activity was observed in test diet 2 fed prawns (24.12±0.07 µmoles /mg protein), least activity was observed on control prawns (21.36±0.50 µmoles /mg protein).All the treated values are significantly higher than control treatment ($p<0.05$).The digestive gland of prawns supplemented with test diet 2(28.00±0.00 µmoles /mg protein) had higher catalase activity and least in control prawns being (24.36±0.30 µmoles /mg protein). In gut catalase activity was also higher in test diet 2 fed prawns (23.14±0.11 µmoles /mg protein), least was observed in control prawns (20.00±0.58 µmoles /mg protein).All values are statistically significant($p<0.05$).

Test diet 1 fed prawns (9.68.60±0.04 µmol H_2O_2 /min/mg protein) expressed higher SOD activity at the muscle. Lower activity was obtained in the control prawns (7.36 ±0.19 µmol H2O2 /min/mg protein). Digestive gland SOD activity of Test diet 2 fed prawns (14.60 ±0.31 µmol H_2O_2 /min/mg protein) had maximum SOD activity. Least were observed in control prawns (10.00 ±0.69 µmol H_2O_2 /min/mg protein).SOD activity at the gut tissue when analyzed, revealed Test diet 2 fed prawns had SOD activities of 10.6900±0.12 µmol H_2O_2/min/mg protein and least activity is in the group fed with control diet 6.39±0.08 µmol H2O2 /min/mg protein.

The test diet 2 fed prawns (9.23±0.05 µmol H_2O_2 /min/mg protein) expressed the highest muscle glutathione peroxidase activity. The control fed prawns having values of 8.36±0.03 µmol H_2O_2 /min/mg protein. In the three selective sites, digestive gland showed enhanced enzyme activity. The digestive gland of test diet 2 fed prawns 9.56±0.11 µmol H2O2 /min/mg protein expressed maximum enzyme activity. Control prawns recorded least enzyme activity 9.23±0.04 µmol H_2O_2 /min/mg protein. Test diet 1 fed prawns 9.56±0.03 µmol H_2O_2 /min/mg protein had higher gut GPX activity .Least were observed in control, 9.00±0.01 µmol H_2O_2 /min/mg protein.

Test diet 1 fed prawns 10.23±0.01 µmoles/mg protein had higher muscle GST activity. Least was observed in control prawns 6.23±0.02 µmoles/mg protein.On digestive gland maximum GST activity was observed in the test diet 1 fed prawns (12.65±0.03 µmoles/mg protein), followed by test diet 2 (10.23±0.02 µmoles/mg protein). Control prawns showed least enzyme activity (8.23±0.03 µmoles/mg protein). In the case of GST enzyme higher enzyme activity was observed in test diet 2 fed prawns (12.36±0.07 µmoles/mg protein). Least was observed at control fed prawns (7.00±0.03 µmoles/mg protein).

Lower lipid peroxidation was observed in the muscle tissue of *M.rosenbergii* supplemented with test diet 2 (1.36±0.02 nmol MDA/ mg protein).Higher activity was got in *M.rosenbergii supplemented with control diet(1.86*±0.03 nmolMDA/mg protein).Lipid peroxidation values of digestive glands revealed test diets 1 2.00±0.01 nmol MDA/ mg protein supplemented *M.rosenbergii* showed least enzyme activity. Higher activity was observed in control prawns 2.58±0.06 nmol MDA/ mg protein. Test diet 1(1.00±0.01 nmol MDA/ mg protein) incorporated *M.rosenbergii* had lower enzyme activity. Control prawn had higher lipid peroxidation rate 2.15±0.01 nmol MDA/ mg protein.

Figure.1 .CAT activity of bioactive phytonutrient supplemented *M.rosenbergii* at 72 hours of post infection with *V.harveyi*.

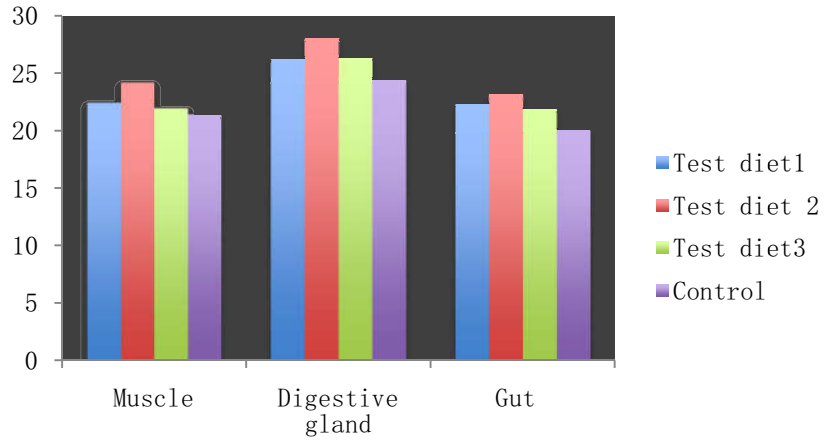


Figure.2. SOD activity of bioactive phytonutrient supplemented *M.rosenbergii* at 72 hours of post infection with *V.harveyi*.

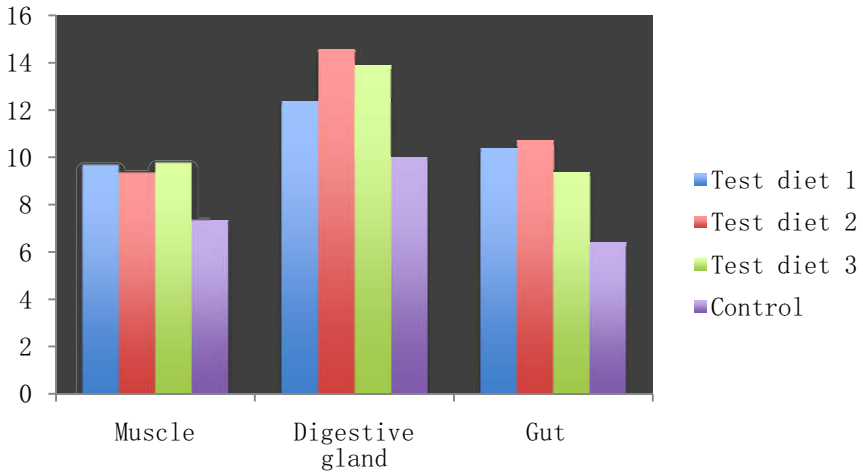


Figure.3. GPX enzyme activity of bioactive phytonutrient supplemented *M.rosenbergii* after 72 hours of post infection with *V.harveyi.*

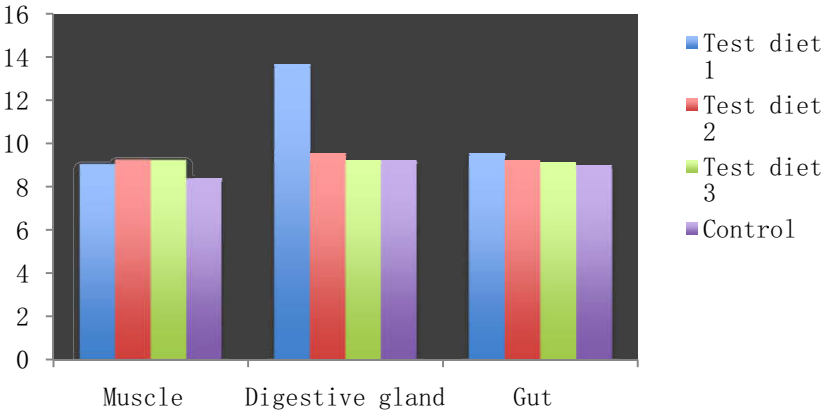


Figure.4.GST enzyme activity of bioactive phytonutrient incorporated *M.rosenbergii* after 72 hours of post infection with *V.harveyi.*

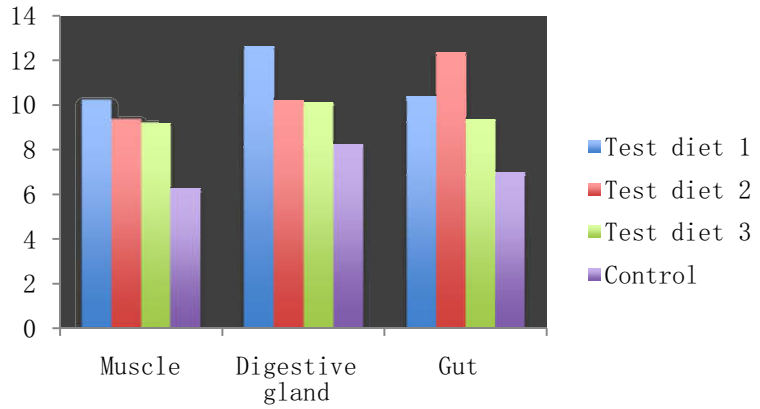
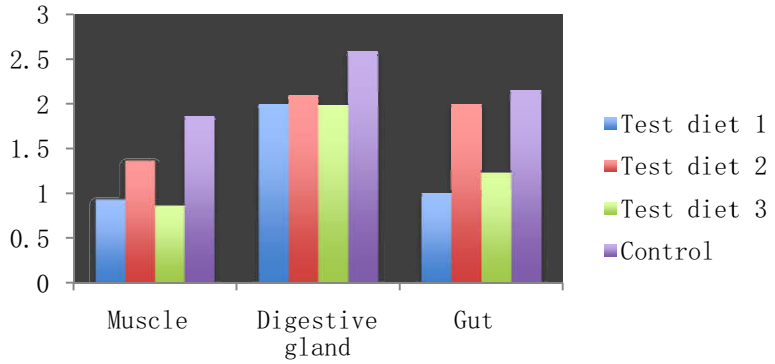


Figure.5. MDA enzyme activity of bioactive phytonutrient supplemented *M.rosenbergii* after 72 hours of infection with *V.harveyi.*



DISCUSSION

The activity of GOT, GPT and LDH in treated groups was significantly decreased than the control group after the infection. GOT activity in haemolymph of prawns fed with Asiaticoside was promising compared with the control diet. In the case of GOT and GPT activity also the Asiaticoside fed prawn obtained similar activity. In prawns fed with Medecososside the activity was not so promising. SOD activity was higher in muscles of prawns fed with all other test diets were compared to the control prawns. CAT activity was higher in Medecossoside . fed prawns. Lower values were obtained were in control fed prawns.In Asiaticoside and Medecossoside fed prawns the GPX activity was higher. Control prawns expressed least GPX activity. In Asiaticoside and Medecossoside fed prawns the GST activity was significantly improved. In control prawns the activity was least with respect to other. In lipid peroxidation, the least activity was observed in Asiaticoside fed prawns, followed by Medecossoside fed prawns. All antioxidant enzymes of digestive glands were higher. Asiaticcosside fed prawns showed higher activity with respect to GPX and GST compared to control prawns. Gut tissue of Medecossoside showed higher SOD activity with respect control prawns. GPX activity of all treatment were more less similar, but Asiaticoside fed prawns showed higher GPX activity. Medecossoside fed prawns showed comparatively highest GST activity, compared to control prawns. Gut tissue of Asiaticoside fed prawns showed least lipid peroxidation. Control prawns showed higher lipid peroxidation activity. There are many studies which reveals the effectiveness of medicinal plants in *M. rosenbergii.* The addition of antioxidant rich feed additives will increase the total antioxidant enzyme activity of the prawns such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) [12, 7, 21, 15]. In the present study the bioactive phytonutrient supplemented *M.rosenbergii* were found to have increased antioxidant enzyme activity. But in the case of toxicity makers such as GOT.GPT and LDH was found to have decreased during the treatments. The phytonutrient supplemented *M.rosenbergii* showed increased antioxidant activity when challenged with *V.harveyi. Centella asiatica* is the cocktail of antioxidant triterpenoids [22]. *Centella asiatica* is reported to improve the age-related neurological antioxidant status of mice [22]. Muthukumar *et.al.,* [19] studied the enzymatic and nonenzymatic activities of *Oreochromis mossambicus* larvae fed with commercial herbal enriched *Artemia nauplii*. The level of aspartate aminotransferase (AST) and alanine aminotransferase

(ALT) decreased when the *Penaeus monodon* juvenile supplemented with astaxanthin followed by *Vibrio damsela* challenge could alter the shrimp‟s antioxidant defense capability and hepatopancreatic enzymes. Form the present study, in most of the case hepatopancreas expressed better enzymatic profile than gut and muscle. This may due to the fact that hepatopancreas acts as key site with high metabolic rate and key site for xenobiotic detoxification .Lactate dehydrogenase(LDH), Glutamate oxaloacetate transaminase (GOT), Glutamate pyruvate transaminase (GPT) and lipid peroxidation (MDA) is known as toxicity markers . Their decrease in concentration and the increase in antioxidant status denote the effects of medicinal plants. In a study by Madhumathi, and Rengasamy [16] investigated the resistance of *Penaeus monodon* against white spot syndrome virus (WSSV) using *Dunaliella salina* algal cells. Anti-WSSV activity of *D. salina* incorporated diet by in vivo methods showed strong antioxidant activity and the immunological parameters such as proPO, SOD, catalase were higher in the WSSV infected shrimp treated with *D. salina* incorporated diet when compared to control groups. The seeds of medicinal plants, *Syzygium cumini, Phylanthus emblica, Azadirachta indica* and *Ricinus communis* on growth promotion in *Macrobrachium Malcolmsonii* early Juveniles was examined by Bhavan *et. al.,* [4]. The activities of enzymatic antioxidants, super oxide dismutase and catalase, and lipid peroxidation were not altered significantly. These states indicate the fact that these medicinal seeds are non-toxic at the tested concentrations..In many studies medicinal application through feed have decreased the lipid peroxidation and rapid increase of GST and GPX was noticed [18, 10].

On conclusion, it can be stated that presence of Asiatocosside in *C.asiatica* may be the fact for effective growth promotion of the two plants.Thus these secondary metabolites with the effective credentials can be used in aquaculture especially for a commercially important species *Macrobrachium rosenbergii.*

REFERENCES

1. Aebi, H. (1984).Catalase in vitro Meth.Enzymology.,105:121-126.
2. Aikaterini, T., Vasilaki., Donald, C., McMillan. (2012). Lipid peroxidation. Encyclopedia Of Cancer*:* 2054-2055.
3. Bar-Or, D., Rael, L. T., Lau, E. P., Rao, N. K., Thomas, G. W., Winkler, J. V., Yukl, R. L., Kingston, R. G. and Curtis, C. G. (2001). An analog of the human albumin N-terminus (Asp-AlaHis-Lys) prevents formation of copper-induced reactive oxygen species*.* Biochem. Biophys. Res. Commun.,284: 856-862.
4. Bhavan Saravana,S., Mohammedsiddiq, V., Srinivasan, T.,Muralisankar., Manickam, M.(2014). Effects of Seeds of Medicinal Plants, *Syzygium Cumini, Phylanthus Emblica, Azadirachta Indica* and *Ricinus Communis* on Growth Promotion in *Macrobrachium Malcolmsonii* Early Juveniles. International Journal of Research Studies in Biosciences (IJRSB).,2(11):95-106.
5. Bouayed Jaouad and Bohn Torsten_. (2010). Exogenous antioxidants—double-edged swords in cellular redox state Health beneficial effects at physiologic doses versus deleterious effects at high doses*.* Oxid Med Cell Longev.,3(4):228–237.
6. Brain, O and Little, C. (1969).Can.J.Biol.Chem.,47.413.
7. Cheng,W, Tung., Liu, C.H., Chen, J.C.(2006). Molecular cloning and characterization of cytosolic manganese superoxide dismutase (cytMn-SOD) from the giant freshwater prawn *Macrobrachium rosenbergii*. Fish Shellfish Immunol.,4:438-49.
8. El-Missiry Mohammed Amr. (2012). Antioxidant Enzyme. In Tech Prepress, Novi Sad. Croatia. Janeza Trdine 9, 51000 Rijeka, Croatia.
9. FAO.(1978). Fish feed technology. In aquaculture development and coordination programme. ADCP/REP/80/11.,289-325.
10. Fernandez-Dıaz,C.,Kopecka,J., Can˜avate,B.P.C.,Sarasquete,C.,Sole,M.(2006). Variations on development and stress defences in *Solea senegalensis* larvae fed on live and microencapsulated diets*.* Aquaculture.,251.573– 584.
11. Hamid, A., Aiyelaagbe. O.O., Usman1, L.M.,Ameen.O.M and Lawal.A. (2010). Antioxidants: Its medicinal and pharmacological applications. African Journal of Pure and Applied Chemistry.,4(8):142-151.
12. Hong Wang –Wei., Duan-Bo Cai.,Guo-Hua Xiao.,Chun-Long Zha., Zi-Hui Wang.,Hai-Ming Xu.,Yue-Qiang Guan.(2009).Effects of selenium on the activity of antioxidant enzymes in the Shrimp, *Neocaridina heteropoda.* The Israeli Journal of Aquaculture – Bamidgeh.,61(4):322-329.
13. Kakkar,P.,Das,B.,Viswan Anathan,P.N.(1984). A modified spectrophometric assay of superoxide dismutase.Ind.J.Biochem. Biophysics.,21:130-132.
14. Kohen, R and Nyska,A.(2002).Oxidation of biological systems:oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. Toxicol Pathol.,30: 620-650.
15. Liu Chun-Hung and Winton Cheng.(2007). Identification and cloning of the antioxidant enzyme, glutathione peroxidase, of white shrimp, *Litopenaeus vannamei*, and its expression following *Vibrio alginolyticus* infection. Fish & Shellfish Immunology.,23:34- 45.
16. Madhumathi, M.and Rengasamy.,R. (2000). Antioxidant status of *Penaeus monodon* fed with *Dunaliella salina* supplemented diet and resistance against WSSV.Aquaculture., 19(1):89–107.
17. Mannervik, B.(1985). The isozymes of Glutatione Transferase. Adv. Enzymol. Relat. Areas Mol. Biol.,57, 357-417.

18. Mathew Suseela, K.,Ashok Kumar, R., Anandan, P.G., Viswanathan Nair, K., Devadasan.(2011). Changes in tissue defence system in white spot syndrome virus (WSSV) infected Penaeus monodon. Comparative Biochemistry and Physiology. *C*(145).,315-320.
19. Muthukumar,S.,Ramasubramanian, P.,Uthayakumar, P.,, Munirasu, P.(2014). Effects of commercial herbal extracts enriched *Artemia nauplii* on enzymatic and nonenzymatic antioxidents activities of *Oreochromis mossambicus.* International Journal of Recent Scientific Research Research.,5(9):1717-1728.
20. Noori, S. (2012). An overview of oxidative stress and antioxidant defensive system., 1:413.
21. Pacheco Rosario.,Felipe Ascencio.,Martha Zarain.,Gracia Góme and Ángel Campa.(2011).Enhancement of superoxide dismutase and catalase activity in juvenile brown shrimp, *Farfantepenaeus californiensis* (Holmes, 1900), fed β-1.3 glucan vitamin E, and β-carotene and infected with white spot syndrome virus. Lat. Am. J. Aquat. Res.39(3).,534-543.
22. Subathra, M., Shila, S.,Devi, M.A.,Panneerselvam, C.(2008). Emerging role of *Centella asiatica* in improving age-related neurological antioxidant status. Exp. Gerontol.,40:707-715.
23. Valko Marian.,Dieter Leibfritz.,Jan Moncola.,Mark,T.D., Cronin.,Milan Mazura.,Joshua Telser.(2007). Free radicals and antioxidants in normal physiological functions and human disease. The International Journal of Biochemistry & Cell Biology.,39:44–84.