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Kotiya Anil S
Asstt. Research Scientist, College of Fisheries Science, Junagadh Agricultural University, Veraval, Gujarat, India

Vadher KH
Associate Professor, College of Fisheries Science, Junagadh Agricultural University, Veraval, Gujarat, India

Corresponding Author:
Kotiya Anil S
Asstt. Research Scientist, College of Fisheries Science, Junagadh Agricultural University, Veraval, Gujarat, India

Effect of different stocking density on *Litopenaeus vannamei* cultured during monsoon season on carcass composition at province of Gujarat states in India

Kotiya Anil S and Vadher KH

Abstract

Shrimp culture activity was conducted at commercial pond at Datardi village, Rajula (Gujarat). *Litopenaeus vannamei* shrimp sample was collected treatment wise at final harvest taken out at 120 DOC. One kg ofshrimp sample were randomly collected from chilled tank for biochemical composition. Each treatment were implemented in triplicate. The present study was conducted to evaluate the effect of stocking density on *L. vannamei* with the emphasis on proximate, nutritional, amino acids and fatty acid composition. The proximate composition of *L. vannamei* was higher protein (%) in MT1 (30 nos/m^2) treatment followed by crude fat (%), carbohydrate (%) and Ash (%). Comparing all treatment for major elements like calcium content was higher in MT6 (80 pc/m^2) with 25.22±1.97 than potassium (K) in MT2 (40 pc/m^2), sodium (Na) in MT2 (40 pc/m^2) and magnesium (Mg) in MT6 (80 pc/m^2) whereas minor elements predicted were iron (Fe) followed by copper (Cu), Zinc (Zn), Manganese (Mn) and Chromium (Cr). Totally 19 amino acids were detected, among these, arginine, histidine, isoleucine, threonine, leucine, methionine, phenylalanine, tryptophan, lysine and valine are essential amino acids and alanine, asparagine, aspartic acid, cysteine, glutamic acid, glycine, proline, serine and tyrosine are non-essential amino acids. In total essential amino acids recorded in MT4 (60 pc/m^2) followed by MT3 (50 pc/m^2), MT5 (70 pc/m^2), MT6 (80 pc/m^2), MT1 (30 pc/m^2) and MT2 (40 pc/m^2) whereas the total non essential amino acid (NEAAs) in treatment MT2 (40pc/m^2) followed by MT1 (30pc/m^2), MT6 (80 pc/m^2), MT5 (70 pc/m^2), MT3 (50 pc/m^2) and low in MT4 (60 pc/m^2).Total 16 fatty acid were detected in all treatment. The results indicated that *L. vannamei* shrimp has higher volume of SFA, PUFA, MUFA. The total unsaturated fatty acids (USFAs) was higher in treatment MT3 with (59.3 µg/g of FAME) followed by MT1 (58.67µg/g of FAME), MT4 (57.57 µg/g of FAME), MT2 (56.11 µg/g of FAME), MT6 (55.93µg/g of FAME) and MT5 (55.37µg/g of FAME). Comparing all the treatment there is clear evident that stocking density directly affect the proximate, nutritive, amino acid and fatty acid level in *L. vannamei* cultured shrimp, may be due to over crowding of shrimp stock or Alang ship breaking yard on one side and commercial port of pipava on another.

Keywords: *Litopenaeus vannamei*, fatty acid profile, amino acid profile, shrimp, nutritional value, summer crop

Introduction

Shrimp farming business is fastest growing segment among all aquaculture industry [1] because of popular aroma, taste and palatability and preference for healthier foods for human beings [2]. Stocking density of shrimp directly affects the proximate, nutritive, amino acid and fatty acid level in *L. vannamei* cultured shrimp [3]. The Decapoda order of crustacean group like shrimps, lobsters and crabs has achieved the special market demand worldwide because it provides high-quality rich protein, lipids, calcium, various extractable compounds and major and minor minerals and vitamins [4, 5] while low in calorie and fat [6] and excellent source for growth and nourishment of consumers health and prevents several nutritional deficiency diseases [7]

Fish and shellfishes protein are most essential for the sustenance of life and its exist in large quantity of all nutrients as a component of human body [8] and shrimps are capable of extracting some of the elements from water, they do respond to dietary sources [9-11]. The importance of these micronutrients is very essential, as their absence in the diet may lead to deficiency disease and hence it is still a supplemental diet to a large section of Indian population.

The annual per capita fish consumption in India is very low i.e., 8 kg against the world average of 10 kg. At the same time, according to the WHO standards a person needs 11kg of fish to fulfill the minimum nutritional requirement [12]. In India, millions of people are suffering from malnutrition disease.

The biochemical composition of the edible tissues of marine invertebrates is affected by their surrounding environment they adapt such as nutritional habits, age, sex, season and other ecological factors [13]. Crustaceans are rich in protein, minerals and vitamins A & D, and excellent sources of high quality proteins that is low in saturated fat and excellent source of polyunsaturated fats especially the ω-3 fatty acids namely Eicosapentaenoic acid (EPA,C20:5n-3) and Docosahexaenoic acid (DHA, C22:6n-3) that are available is superior to the meat obtain from goat and poultry meat. It also severs as a good source for iodine, phosphorus, magnesium, iron, copper, sulphur and calcium which are essential to keep up the health and stamina [14]. Shrimps are known to be a excellent source of protein rich in essential amino acids (lysine, methionine, cystiene, threonine and tryptophan) [15] and shellfishes fats are best sources of essential fatty acids that cannot be synthesized in the human body and they are required for the maintenance of growth, reproduction and synthesis of vitamins.

Lipids are the organic resources of the crustaceans and it's contain omega-3 polyunsaturated fatty acids (PUFA), especially EPA and DHA has both anti-atherogenic and anti-thrombotic effects as well as an important role in the control of hypertension, reducing the risk of coronary heart diseases, diabetes and cancer [16-18]. PUFAs play an important role in the development of the nervous (brain), photoreception (vision) and reproductive systems [19].

Carbohydrates are major energy sources in human diet. The ratio of carbohydrates was meagre with compared to other nutrients like proteins and lipids in animal tissues of aquatic species. For physiological and biochemical process, minerals are necessary materials, acquires assimilates as utilized food to maintain health and activity and below normal level of mineral causes some health problem. So studies on minerals in chief source like shrimps are essential. Therefore, the present study was under taken with following objectives effect of different stocking densities on cultured *Litopenaeus vannamei* (Boone, 1931) carcass composition during monsoon crop.

Materials and Methods

Experiment was conducted in commercial shrimp pond at Kavya Aqua Farm, Datardi village, Taluka: Rajula, Dist: Amreli, Gujarat, India. (Latitude 20º 57'35.38" N and 71º 32'35.60" E and Longitude 20º 57'35.93" N and 71º 32'32.03" E)**.** Experiment was taken up in total 22 ponds, from total 18 were culture ponds and 2 were sedimentation ponds and 2 were reservoirs. Each culture pond was size of 0.5 ha with 1.8 m depth. The experiment was conducted over shrimp stocking density m^{-1} with completely randomized design (CRD) over 6 treatments with 3 replications. Monsoon crop treatment was represented as MT1 with stocking density of 30 nos/$m^2$was denoted has MT1 30 nos/m^2 as on for 2nd treatment MT2 with 40 nos/m^2, treatment MT3 50 nos/m^2, treatment MT4 60 nos/m^2, treatment MT5 70 nos/m^2, and in treatment MT6 80 nos/m^2 was maintained. The experiment was carried out for total 120 DOC (days of culture). The culture ponds were prepared as per standard procedures [20].

The shrimp, *Litopenaeus vannamei* post larvae (PL 09) were procured from commercial shrimp hatchery West Coast Hatchery Pvt. Ltd. Kotda. Post larvae average weight of 0.06±0.02g were PCR tested and transferred in oxygenated polythene bags to commercial pond at Kavya Aqua Farm at Datardi. The PL were acclimatized before pond stocking. The experimental culture activity was carried out for 120 days. At final harvest, *L. vannamei* shrimp weighing 1kg sample were randomly collected from the final chilling storage basket, treatment wise. The sample were packed in polythene bags and marked, placed in thermocoel box filled with crushed ice and transferred to the laboratory at Fisheries Research and Training Centre, J.A.U., Mahuva. The shrimp samples were washed with deionized water, cleaned with filter paper, placed in marked petridish as per treatment, placed in dry oven at 45 ºC for three days. After completely drying, shrimp sample were crushed and powdered, which was marked and packed and sent to Food Technology Laboratory, J.A.U., Junagadh for amino acid and fatty acid profiling and proximate composition analysis.

Whole Body Proximate and Mineral Composition Analysis

Moisture, crude protein, crude lipid, carbohydrate and ash content of dried and powdered shrimp tissue were analyzed according to the established AOAC, 1990 [21]. Moisture were dried in an oven at 105 ºC until constant weight; crude protein (N x 6.25) by Kjeldahl method after acid digestion; lipid by ether extraction using Soxhlet; ash by combustion at 550 ºC for 5h. Quintuplicate sample reading was taken and the formula is as mentioned below.

Crude protein (CP)

Crude protein (%) = Nitrogen (%) X 6.25

Crude lipid

$$\text{Crude lipid (\%)} = \frac{\text{Weight of the ether extract}}{\text{Weight of sample}} \times 100$$

Moisture

$$\text{Moisture (\%)} = \frac{\text{Wet weight of sample-Dried weight of sample}}{\text{Wet weight of sample}} \times 100$$

Ash

$$\text{Ash (\%)} = \frac{\text{Weight of Ash}}{\text{Weight of sample}} \times 100$$

Total Carbohydrate

Total carbohydrate = 100 - (CP + CL+ Moisture+ Ash)

Mineral

Mineral composition of cultured *L. vannamei* shrimp was determined using MPAES 4200 (Microwave Plasma Atomic Emission Spectrophotometer). 1.0 g dry shrimp powder sample was weighed, 30 mL of Di acid (HNO3 : HCLO4), predigest for 1 hr and further digested by heating the sample in a hot plate up to it remain just 4-5 mL. Cool and makeup 100 mL by Di Water by several wash to flask. The mineral composition was determined in triplicate using a spectrophotometer.

Sr. no	Element	Wavelength (nm)	Method
1	Calcium	445.478	MP AES
2	Magnesium	383.829	
3	Copper	324.754	
4	Iron	259.940	
5	Manganese	257.610	
6	Zinc	213.857	
7	Cobalt	340.512	
8	Sodium	-	Flame photometric method
9	Potassium	-	Flame photometric method

Amino acid profiling

The process for amino acid profiling was taken up by:
(a) Hydrolysis
(b) Derivatization
(c) UHPLC Analysis

Fatty acid profiling

Fatty acids of samples were identified and quantified as methyl esters using
GC-MS (Gas Chromatography Mass Spectrophotometer) unit.
The process of fatty acid profiling was initiated with
(1) Lipid extraction
(2) Preparation of Fatty Acid Methyl Esters (FAME)
(3) GC-MS Analysis

Results and Discussion

Biochemical composition is the baseline to measure and assess the nutritional quality of food items. Any living organisms, body composition directly correlate with dietary nutrients. The proximate body composition including moisture, fat, protein and ash are good indicators of physiological condition of any living organism. The most preferred and health friendly, nutritive food and flavor for human being are shrimp, lobsters and crabs.

Proximate composition of *L. vannamei* shrimp

The proximate composition detected from the flesh of the *L. vannamei* stocked with different stocking density during monsoon crop is shown in (Table 1. Fig.1). Crude protein content in the flesh of *L. vannamei* shrimp varied from 16.86% to 17.60%. The highest protein level was recorded in MT1 (17.6±0.42) followed by MT2 (17.48±0.31), MT3, (17.25±0.14), MT4 (17.02±0.32), MT6 (16.96±0.37) and MT5 (16.86±0.07). Crude fat content in *L. vannamei* shrimp flesh varied from 3.03 to 3.51%, with high concentration of crude fat noted was in MT3 (3.51±0.05) followed by MT4 (3.47±0.06) and lowest in MT2 (3.03±0.02) whereas carbohydrate content varied from 3.03%to 3.47% with highest concentration in treatment MT3 (3.51±0.05) followed by MT4 (3.47±0.06) and lowest in MT2 (3.03±0.0) and moisture content ranged between 75.62 to 75.76% where higher concentration was recorded in treatment MT1 (75.76±0.24) followed by MT2 (75.66±0.07) and lowest MT3 (75.56±0.10) and Ash content varied from 2.63%to 3.23% with higher concentration of ash content varies in treatment MT5 (2.75±0.14) and MT6 (2.74±0.30) followed by MT4 (2.49±0.35) and lowest in MT1 (2.07±0.55) during monsoon crop.

Table 1: Proximate composition (%) in the flesh of *L. vannamei* shrimp during monsoon crop

SD (nos/m^2)	Proximate composition (%)				(n= 5)
	Moisture	Crude protein	Crude Fat	Carbohydrate	Ash
MT1	75.77±0.24	17.60±0.42	3.11±0.04	2.08±0.56	1.45±0.05
MT2	75.67±0.08	17.48±0.31	3.03±0.02	2.42±0.37	1.42±0.03
MT3	75.57±0.10	17.25±0.14	3.51±0.05	2.30±0.20	1.38±0.05
MT4	75.62±0.11	17.02±0.32	3.47±0.06	2.49±0.35	1.40±0.07
MT5	75.64±0.16	16.86±0.07	3.32±0.06	2.76±0.15	1.42±0.05
MT6	75.63±0.09	16.96±0.37	3.25±0.02	2.74±0.30	1.42±0.02

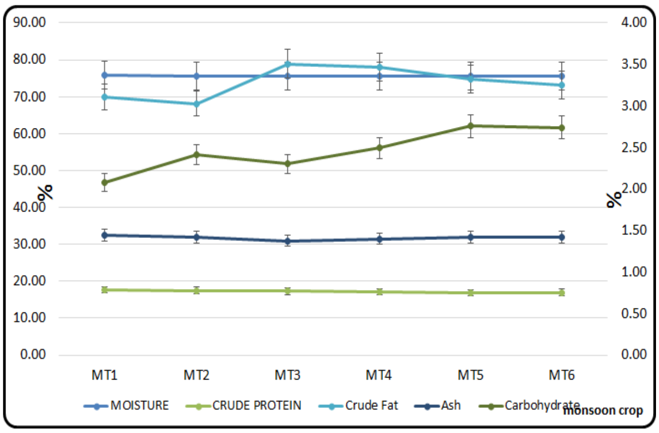

Fig 1: Proximate composition (%) in the flesh of *L. vannamei* shrimp during monsoon crop.

The chemical composition, especially the protein, lipids in cultured fish and shellfish can vary between interspecies and intera-species, being influenced by several factors including diet, overcrowding, growth stage, quality and salinity of the water, and variations attributed to the time of year [6]. Gunalan *et al.* noted that shellfish provides high-quality rich protein, calcium and various extractable compounds and minerals for the human body, low in calorie and fat. Nutrients, is the bond between food for normal growth and development. Comparing meat and poultry, shrimps is one of the highly nutritious foods [22]. Achuthankutty and Parulekar reported that penaeid prawns muscle tissues are buildup by maximum protein. The proximate composition of shrimp muscles is dependent on the factors like species, growth stage, feed and season [23, 24]. [25, 26] Azevedo *et al.* and Lupatsch *et al.* suggested that nutrient and energy deposition, and thus carcass composition, follow rational patterns [27]. Ravichandran *et al.* reported that proximate composition shows that the percentage of protein in the flesh was higher (41.3%). According to [28] Silva and Chamul state that the protein content of crustaceans and molluscs were around 20%. Protein was found as the major constituent in the muscle of shrimps. The same difference in the proximate composition in the edible muscle was reported for *F. Pennicillatus* and *F. merguiensis* [29] (Rosa and Nunes, (2003), black tiger shrimp and white shrimp [30, 31]. The protein content was found to vary between 44-54% of the dry weight of the three species [32, 33] Gopakumar reported that protein in meat varied from 13.6-15.4%, which is lower to the obtained result and the observed varying moisture content in shrimps depends on the species and size. [34] Priyadarshini *et al.* stated that nutritional qualities of Penaeidean shrimps protein content observed during monsoon season was highest in *M. stridulans* (3.09 mg/g) followed by *M. mogiensis* and *S. crassicornis* (2.93 and 1.7 mg/g) respectively. The decrease in water content resulted in the relative increase in protein, fat and ash content that is highly correlating the present study.

Carbohydrates serve as booster for the synthesis of dispensable amino acids and certain nutrients, which are free and bound state along with proteins as protein-bound sugars and glycogen. Carbohydrate content in all penaeid prawns ranges between 3% of the body weight. In the present investigation it was around 2.08 to 2.74%. Carbohydrates in fishery products contain no dietary fiber but only glucides, the majority of which consist of glycogen, containing traces of glucose, fructose, sucrose and other mono and disaccharides [8]. In the present study, carbohydrate ranged between 2.08 to 2.74%. The results obtained in the present investigation clearly demonstrate that, the proportion of protein content was dominating over carbohydrates and lipid contents in muscle tissue. Penaeid shrimp may not have a definite lipid requirement but are provide high energy source, containing more than twice the energy of carbohydrates and proteins [8]. Dietary lipids helps to reduce osmotic shock in aquatic animals [35] Chen *et al*., stated lipid play an important role in maintaining structural and physiological integrity of the cellular membranes. [36] Akiyama *et al*. recommended maximum 6% to 7.5% lipid levels [37]. Pillay and Nair marked an inverse relationship between lipids and moisture content. [38] Shaikhmahmud and Magar stated matured female obtained higher lipid content, when compared to immature ones [39]. Gopakumar and Nair did not find any variation in the lipid content of muscle tissue of shrimp's species [22]. Achuthankutty and Parulekar suggested that maturity condition influences the lipid composition of muscle tissue. In the present study also there is no regularity in fat values among the size groups between 3.03 to 3.51 % of the total body. Generally lipids act as major food reserves and known to play an important role in the production of energy at cellular level also acts as vehicles for the transport of lipid soluble vitamins A, D, E and K.

Ash is one of the least studied biochemical constituents in crustaceans. Total ash quantities obtain from the non-edible portions of the shrimps. The ash content was high in the samples containing the exoskeleton ranging from (6.66 – 9.03% wet basis). Marginal increases in ash during growth were reported by [22] Achuthankutty and Parulekar in *P. stylifera* and [40] Ajithkumar in *M. idella* [41]. Nair and Prabhu reported that ash composition in *M. dobsoni* was 15.79% and in Jawla prawn (*Acetes* sp.) was 17.11%. Almost similar values were obtained by [42, 43] Tiwary (2009); Chandra (2009) in *M. scabriculum* and *M. idea* respectively. The present study reflected that increased ash content was noticed in increased size groups, regardless of sex. Ash is left out after complete combustion of fish meat and gives total mineral content. The ash content of fish varies 0.5-2% [44]. In the present investigation, ash content ranged between 1.38 to 1.45% of the total body.

The decrease in water content resulted in the relative increase in protein, fat and ash content that is highly correlating the present study.

Minerals composition of *L. vannamei* shrimp during monsoon crop

Mineral composition of shrimp is in low proportion but very important for nutritional point of view [7]. Agusa *et al*. reported that minerals are excellent for growth and booster of metabolic activity for human body and prevents several nutritional deficiency diseases [45]. Belitz *et al*. stated that minerals constitute important mechanisms of enzymes, hormones and are enzyme activators.

Major elements of *L. vannamei* shrimp during monsoon crop

The minerals of the *L. vannamei* flesh are shown in (Table 2. Fig. 2 and 3). Comparing major elements (gkg^{-1}) availability in all treatment, Calcium (Ca) content was higher in MT6 (25.22±1.97) followed by MT5 (23.68±1.57) than all other treatment. Potassium (K) elements was recorded in MT2 (9.48±0.41) followed by MT1 (9.22±0.43). Sodium (Na) noted in MT2 (9.13±0.93) followed by MT6 (9.08 ± 0.92) whereas Magnesium (Mg) in MT6 (3.57±0.09) followed by MT1 (3.36±0.07).

Minerals such as calcium, magnesium, potassium and sodium increased gradually with increase in salinity and its serve as essential components for enzymes, vitamins, hormones, pigments, and co-factor in metabolism, catalysts, and enzyme activators. The ratio of Mg:Ca and Na:K is directly proportional to the rearing medium and ionic changes on growth rate and body compositions. Fish and shellfish contain significant amounts of minerals such as, calcium, magnesium, phosphorus, potassium and sodium [46, 47]. In the present study during investigation the mineral like Ca, K, Mg, Na, Zn, Cu, Fe, Mn and Cr were detected in the edible part of *L. vannamei.* Calcium varies from 14.24 to 25.22 gKg^{-1}, which is higher (59.5 mg) than green tiger shrimp [48], sea bass (63.6 mg) and sea bream (19.2 mg) [49]. The calcium and phosphorus together account for 70 to 80% of the minerals in the skeleton of fish [50, 51]. Dincer and Aydin stated that Ca content of female shrimp samples was lower compared to the male samples [52]. Adeyeye *et al.* reported that higher Ca content in *Penaeus notobulis* shrimp. *L. vannamei* strictly regulated almost 30gkg^{-1} of calcium in the body between 3 and 30‰ salinity for the various physiological processes. Calcium content gradually gets reduce by 24-48% between 40 and 60‰ as compared to the optimum treatment. *L. vannamei* excretes body calcium in hyper saline conditions to maintain homeostasis [24]. Karakoltsidis *et al*. reported that *Aristeus antennatus* body contain nearly one-fifth part of the Ca. These results indicate that calcium supplementation in water is not required in low saline water, because its level (147mg^{-1}) was far above the reported value in an earlier trial (63 mgl-1) with *L. vannamei* [53]. In present investigation, calcium ranged between 14.24 to 25.22 g Kg^{-1}, so it highly correlating the present study. The shrimp waste is well-known in high calcium contain [54, 55]. Davis *et al.* stated maintenance of sodium, potassium and magnesium is necessary for proper physiological functioning of body, osmoregulation, building of body and also as activities for many enzymes which play role in carbohydrate metabolism and protein synthesis. Sodium is the principal cation of the extra cellular fluid and regulator of its volume [48]. Yanar and Celik investigated the Ca, K, P, and Na mineral contents of the speckled shrimp (*Metapenaeus monoceros*) in different seasons whereas [56] Hagashi *et al*. stated that in the boiled crabs, sodium and potassium comprised the major part of minerals [57]. Whithney reported that sodium helps to retain acid-base balance and is essential for nerve system, additionally he noted that level of Na in flesh of *P. longirostris* and *P. martia* was found as 876 and 574 mg g^{-1} respectively, whereas [6] Gunalan *et al*. stated that 67.7mg g^{-1} in *L. vannamei* shrimp. In present investigation, Na in flesh of *L. vannamei* was noted between 6.73 to 9.08g Kg^1.which is higher may due to higher saline soil in this area.

Potassium plays a major role in maintaining fluid and electrolyte balance and cell integrity. During the nerve transmission and muscle contraction, potassium and calcium briefly exchange places across the cell membrane [51]. Dincer and Aydin stated that higher K and Mg values were found in female samples, as all microelement levels were compared, only the K level was found to be higher compared to [48] study. For penaeid and pandalid shrimps, these values were lower compared to the study by [58]. Potassium requirement for human is about $2gday^{-1}$ [47]. Abdullah *et al*. reported that deep seawater rose shrimp and golden shrimp 996 and 644 mg/100 g respectively [6], Gunalan *et al*. stated in farm shrimp *L. vannamei* 56.7mg/g. In present investigation, K contents of *L. vannamei* were found in between 8.55 to 9.48g kg^{-1}, which is higher than but lesser than reported by [48] Yanar and Celik for green tiger shrimp and [49] Erkan and Ozden stated for sea bass and sea bream.

Magnesium content of *L. vannamei* was about 2.56 to 3.57mg g^{-1}. Magnesium is essential for human nutrition and it is required for body's enzyme system and maintains bone health, whereas [59] Furriel *et al.* reported that magnesium act as cofactor in many enzymatic reactions, osmoregulation, protein synthesis and growth [11]. Davis *et al*. added that a lack of dietary Mg2+ has been shown to depress K^+ concentrations of the carapace in juvenile, *L. vannamei*, indicating a possible interaction between K^+ and Mg^{2+} [11]. Similarly, this present study clearly indicates that Mg (2.83%) was deposited in *L. vannamei* shrimp harvest.

Minor elements of *L. vannamei* shrimp during summer crop

Comparing minor elements (mg g^{-1}) (Table 2. Fig 3), Iron (Fe) concentration was higher in MT3 (941.94±0.21) followed by MT5 (846.12±0.11) than all other treatment whereas copper (Cu) high concentration in treatment MT1 (128.6±0.40) followed by MT3 (117.8±0.14). Zinc (Zn) higher conc. in treatment MT6 (87.79±0.12) followed by MT5 (83.72±0.60). Manganese (Mn) in treatment MT5 (26.13±0.06) followed by MT6 (24.33±0.14). Chromium (Cr) high in MT1 (9.34±0.16) followed by MT3 (8.82±0.09) and all other treatment.

Vital and most essential trace elements is iron (Fe) in human system. It serves as a carrier of oxygen to tissues from the lungs by red blood cell. Adequate Fe in diet is very important for avoiding some major health problems [45, 60]. Iron is one of the very important essential trace elements since it has several vital functions in human system. Iron levels predominate in gills, contributing 91-96% of the total metal present [61, 62]. Simpson *et al*.; Nash *et al.* stated that, the pond soil having high iron conc. lead to brown gill syndrome. The solubility of iron oxides commonly found in acid sulphate soil environments deficiency in Iron cause hypochromic microcytic anaemia, reduced growth and feed efficiency etc. As per [63] Wheaton and Lawson Iron concentration in different shrimps species ranged between 9.5-135mg kg^{-1} this statement was supported by [64] Gokoglu *et al* stated that Fe concentrations in *P. semisulcatus* was (33.89 mg kg^{-1}) in the present investigation, Fe concentration was highest than all studies which range between 538.2 to 941.9mg kg^{-1}.

In the other essential elements such as copper, manganese and zinc, plays important roles in many physiological functions. Manganese (Mn) is essential biological function where it present in enzymes like oxido-reductases, transferases, hydrolases, lyases and isomerases. High consumption of manganese food can cause dermatitis, glucose metabolism issue, bad formation of bones and the nervous system is the most vulnerable to it [65]. Lee *et al.* reported that conc. of manganese in prawn head, flesh and shell are 2.81 mgl^{-1}, 0.239 mgl^{-1} and 0.832 mgl^{-1} respectively. In the present study, Mn concentration in shrimp sample was between 15.52 to 26.13$mgKg^{-1}$, which are above the permissible level permitted by WHO. Chromium (Cr) is an important trace element which plays a vital role in animal physiology [66] and enzyme glycogen synthetase [67]. Copper has been known one of the major catalysts for oxidation [67, 68] Oehlenschlager stated that despite the high copper concentrations in aquatic food present no problem for human health. Copper (Cu) and iron are important minerals found in fish as respiratory pigment, the most commonly occurring heavy metals in industrial wastewaters [69]. In aquaculture, for eradication of filamentous algae copper sulfate is commonly applied to shrimp ponds ultimately cause Cu pollution [70, 71, 64] Gokoglu *et al*. reported levels of Cu and Zn for *P. longirostris* as 1.33 and 14, 57 mg g^{-1}, in the present study, the range of Cu in *L. vannamei* shrimp was (82.89 to 128.6 $mgKg^{-1}$), which is higher than reported earlier. Concentrations values of chloride, sulfate, calcium, magnesium, potassium, and sodium were lower than values recorded in deep seawater [72, 73]. In the present investigation the results were similar. Shellfish are usually higher in minerals Ca, Mg, K, Na, Zn, Cu, Fe, Mn and Cr than fish. Heavy metals, like Zn, Cu and Cr may be present in seafood depending on how they feed and where they live. The highest concentration was recorded in *L. vannamei* shrimp edible tissues is may be due to commercial port of Pipavav on south coast and Alang ship breaking yard on east coast, this bioavailability of trace elements in soil get accumulated and get transfer to all side of sea, where culture pond are located

The mineral composition in *L. vannamei* shrimp flesh cultured during monsoon crop was in the following order: Ca > K> Na >Mg> Fe> Cu>Zn>Mn>Cr.

Table 2: Mineral composition in the flesh of harvested *L. vannamei* shrimp.

n=5	Stocking Density Nos/m²					
	MT1	**MT2**	**MT3**	**MT4**	**MT5**	**MT6**
Major Elements (g Kg⁻¹)						
Ca	18.97±1.62	15.99±3.40	14.57±2.31	14.24±1.62	23.68±1.57	25.22±1.97
Mg	3.36±0.07	2.83±0.07	3.13±0.08	2.56±0.06	3.27±0.09	3.57±0.09
K	9.22±0.43	9.48±0.41	8.51±0.34	8.93±0.64	8.55±0.46	8.67±0.52
Na	6.73±0.21	9.13±0.93	7.57±0.88	8.31±0.94	7.41 ± 0.81	9.08 ± 0.92
Minor Elements (mgKg⁻¹)						
Zn	66.38±0.16	59.60±0.20	64.14±0.12	60.28±0.10	83.72±0.60	87.79±0.12
Cu	128.6±0.40	104.8±0.11	117.8±0.14	98.13±0.25	89.05±0.13	82.89±0.08
Fe	776.7±0.16	538.2±0.35	941.9±0.21	824.2±0.14	846.1±0.1	690.1±0.07
Mn	15.52±0.23	16.55±0.16	20.62±0.07	18.21±0.13	26.13±0.06	24.33±0.14
Cr	9.34±0.16	8.34±0.07	8.82±0.09	8.20±0.08	7.21±0.11	8.77±0.10

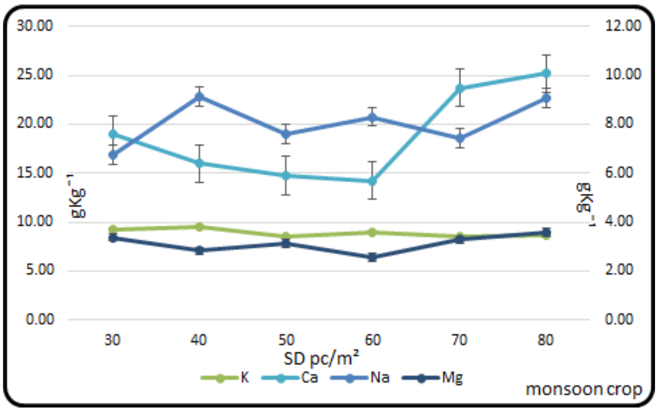

Fig 2: Major elements composition in the flesh of *L. vannamei* shrimp cultured during monsoon crop.

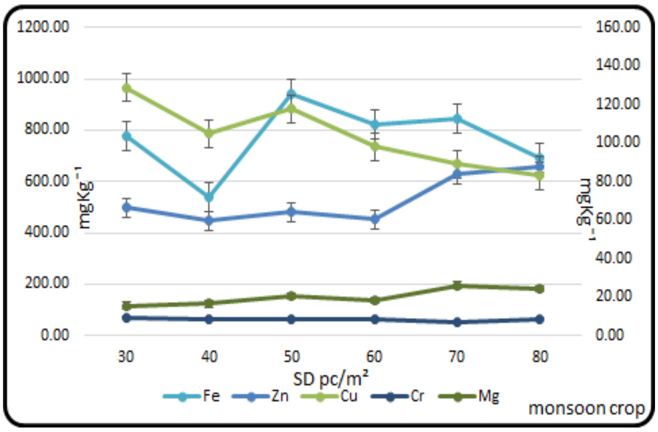

Fig 3: Minor elements composition in the flesh of *L. vannamei* shrimp cultured during monsoon crop.

Fatty acid profile

Fatty acid profiling was detected using Fatty Acid Methyl Ester by Gas Chromatography (GC-MS) in culture harvested shrimp. Fatty acid composition and chromatogram of fatty acid profile (Table 3. and Fig 4 to 9). The total saturated fatty acid (SFAs) composition of *L. vannamei* shrimp ranged within treatment is 41.33 to 45.92 µg/g of FAME, total (MUFAs) Monounsaturated fatty acid within treatment was 23.79 to 27.26 µg/g of FAME, total Polyunsaturated fatty acid (PUFAs) ranges between 28.85 to 28.96 µg/g of FAME. In all treatment, the palmitic acid (C16:0) was dominated than other SFAs, with range between 25.06 to 28.05 µg/g of FAME, the highest quantity of palmitic acid (C16:0) was in MT2 treatment 28.05 µg/g of FAME followed by MT1 (26.86), MT4 (26.41), MT6 (26.21), MT5 (25.87) and low in MT3 25.06 µg/g of FAME. Other SFAs have been detected namely butyric acid, capric acid, lauric acid, pentadecyclic acid, margaric acid, Stearic acid and archidic acid, which totally ranged between 0.15 to 13.14 ug/g of FAME.

The quantity of Un-Saturated Fatty Acid (USFAs) ranged between 55.37 to 59.34 µg/g of FAME from which monounsaturated fatty acid ranged between 23.79 to 27.26 µg/g of FAME. Presence of Oleic acid was relatively in higher among MUFAs with range between 20.54 to 25.4 µg/g of FAME. The quantity of polyunsaturated fatty acid (PUFAs) range between 28.85 to 34.75 µg/g of FAME with highest in MT3 and lowest in MT2 treatment. Eicosapentaenoic acid (EPA) was highest in MT3 (6.67) and lowest in MT2 (0.0 µg/g of FAME). Other USFAs have been detected namely 9,12-octadecadienoic acid, linoleic acid (LA), arachidonic acid (AA), linolelaidic acid, alpha linolenic acid and eicosapentaenoic acid.

Table 3: Fatty Acids in the flesh of *L. vannamei* shrimp cultured

Fatty acid (µg/g FAME)	MT1	MT2	MT3	MT4	MT5	MT6
Butyric acid C 4:0	0	0	0	0	0.15	0.31
Capric acid C 10:0	0.18	0.16	0.28	0.22	1.09	0.23
Lauric acid C 12:0	0	0.08	0	0	0.21	0.34
Pentadecylic acid C 15:0	0.97	1.32	0.83	0.58	1.17	1.07
Palmitic acid C 16:0	26.86	28.05	25.06	26.41	25.87	26.21
Margaric acid C 17:0	0.18	2.58	1.77	2.22	3.15	3.81
Stearic acid C 18:0	13.14	11.7	12.32	12.33	12.28	12.98
Arachidic acid C 20:0	0	0	0.4	0.67	0.71	0.97
∑Saturated FAs	41.33	43.89	40.66	42.43	44.63	45.92
Palmitoleic acid C 16:1 (n-7)	1.78	2.36	1.84	1.39	2.21	2.49
Oleic acid C 18:1 (n-9cis)	25.4	24.9	22.75	22.5	24.2	20.54
Gondoic acid	0	0	0	1.1	0	0.76
∑MUFAs	27.18	27.26	24.59	24.99	26.41	23.79
Omega-6 fatty acids						

9,12-Octadecadienoic acid	0.14	0	0	1.66	0	0
Linoleic acid (LA) C18:2 (n-6)	21.94	24.27	23.84	20.61	21.46	19.26
Arachidonic acid (AA) C20:4 (n-6)	2.07	2.75	2.85	3.77	1.44	4.23
Linolelaidic acid	0	0	0	0	1.85	1.85
Omega-3 fatty acids						
Alpha-linolenic acid (ALA) 18:3 (n-3)	1.5	1.83	1.39	0	0	2.44
Eicosapentaenoicacid(EPA) 20:5 (n-3)	5.84	0	6.67	6.54	4.21	4.36
∑PUFAs	31.49	28.85	34.75	32.58	28.96	32.14
∑Unsaturated fatty acid	58.67	56.11	59.34	57.57	55.37	55.93

Many authors reported that the superior nutritional value of marine oils of sardine, pollack, short-necked clam and cod liver oils over plant oils or animal fats for *Penaeus japonicus*, *Penaeus monodon* and *Penaeus vannamei* [45, 46, 47, 48]. In the present study, shrimp sample among SFAs group palmitic acid was dominating followed by stearic and margaric acids. The concentration of palmitic acid in *L. vannamei* was 25.06 to 28.05%. This statement was in agreement with [47] Abdullah *et al*. stated that the amount of palmitic acid of *P. longirostris* (20.27%) and *P. martia* (20.14%) black tiger shrimp (22.2%) and white tiger shrimp (21.8%) [30, 17] Sriket *et al*.,(2007); Bragagnolo and Rodriguez-Amaya, *M. carcinus* [74] Simon *et al*., *F. schimitti* [75] Moura *et al*. stated that the C16:0 has pinpointed as the key fatty acid (FA) in white shrimp, similar to that reported for other crustaceans species such as *Penaeus brasiliensis*, *Penaeus schimitti* and *Xiphopenaeus kroyeri* and six shrimp species marketed in China [76].

Among the monounsaturated fatty acids (MUFA), the oleic acid C18: 1n-9cis were in majority and ranged between (22.5 to 25.4 µg/gof FAME), as observed in other shrimp species including *P. braziliensis* and *X. kroyeri* [17] (Bragagnolo and Rodriguez-Amaya, *P. monodon* and *P. vannamei* [30] and *X. kroyeri* [77]. Polyunsaturated fatty acids (PUFA) were predominant and ranged between (28.85 to 34.75 µg/g of FAME) in the samples of the present study, EPA (6.67 to 4.21 µg/g of FAME). There is no consensus on the predominant fatty acid in shrimps. Although SFA appear in higher concentration in some species, there may be differences within the same species. [75] Moura *et al.* found higher SFA levels in *L. vannamei*. Environmental conditions and diet have been reported as factors that most influence the fatty acid composition in crustacean muscles [76, 78]. Osborn and Akoh reported in their report that n-9 fatty acids, found as oleic acids (C18:1 n-9) plays a moderate role in the body. Moreover, n-6 fatty acids cannot be synthesized by humans and are therefore considered as essential fatty acids.

The interaction and balance between n-3, n-6 and n-9 fatty acids are crucial for maintenance of good health [79, 80] Christensen *et al.* (2001); VonSchacky *et al.* (1999). The n-3 fatty acids have anti-inflammatory and anti-coagulant properties as well as many other important health benefits. The EPA can be considered as the most important for everyone else as it is necessary for continuation of the efficient functioning of the brain and body at the cellular level. The n-6 fatty acids have their own role in female reproductive cycle. The consumption of omega 3 will reduce and minimize the risk of cancer [81] Vila and Calder(2011), heart diseases [82] Kwak *et al.,* (2012), mental disorders [83] (Perica and Delas and insanity [84] (Kawakita *et al* remarkably. [85] Ka He *et al*. reported W3 fatty acids rate at 201 mg and [86] Anderson *et al.* reported W3 fatty acids at 290 mg [87]. Beydoun *et al.* specified the total W3 fatty acids in 100-gram raw shrimp at avg. 31.46 µg/g of FAME. Consistency and inconsistency of results of the above mentioned studies is due to the difference and diversity of management and proportion of daily feeding. The recommended minimum value of PUFA/SFA ratio is 0.45 for a balanced diet [88] (Anon, 1994). This ratio is lower than that found for all treatment shrimp (1.41, 1.27, 1.45, 1.35, 1.24 and 1.21), which demonstrates a satisfactory nutritional quality of shrimp *L. vannamei*. Similar results were found for *M. carcinus* [74] Simon *et al.,* and *X. kroyeri* [77] Lira *et al.,* while lower values were reported for *F. schimitti* [75] Moura and higher values for *P. brasiliensis* [30]. A diet with low PUFA/SFA ratio is not recommended as it is a risk factor for the increase in serum cholesterol. Since *L. vannamei* contains considerable amounts of PUFA it can provide a healthy choice of daily diet. Based on the results, *L. vannamei* species can be considered as a good source of fatty acid as well as protein. SFA appear in higher concentrations in some species, there may be differences within the same species [75] found higher SFA levels in *L. vannamei*. Environmental changes and di*et al*together are the factors that most influence the FA composition in crustacean muscles [76].

Amino acid profiling of *L. vannamei* shrimp

The total quantities of amino acids concentration ranges from 1.60mg to 14.86mg amino acids/100g (DW). The total essential amino acids (EAA) concentration was highest in treatment MT4 (66.04%) followed by MT3 (66.03%), MT5 (66.01%), MT6 (65.86%), MT1 (64.61%) and low in MT2 (63.54%) whereas highest total NEAA concentration recorded was in treatment MT2 (36.46%) followed by MT1 (35.39%), MT6 (34.14%), MT5 (33.99%), MT3 (33.97%) and lowest concentration in MT4 (3.96%) amino acid/100g(DW) (Table 4.and Fig.10 - 16).

Highest concentration of individual EAA amino acid was from treatment MT1 Methionine (14.86%) followed by MT4 Isoleucine (14.76%), MT2 Isoleucine (14.39%) remaining all amino acid of this group range between 13.86 to 1.60%/100g (DW). Highest total concentration of individual NEAA was from MT3 Glutamic acid (5.91%) followed by MT1 Glutamic acid (5.86%) other all remaining amino acid of this group range between 5.76 to 1.60%/100g (DW).

Amino acids are the foundation blocks of proteins and serve as protein builders and are key mechanism, which serve as source of energy role in human nutrition and health promotion [89]. The concentration of amino acid varies by intrinsic (species, size, and sexual maturity) and extrinsic factors (food resources, fishing season, water salinity, and temperature) [90, 91]. Crustacean muscles comprise high concentration of free amino acids, such as arginine, glycine, proline, glutamine and alanine [92]. Isoleucine, Histidine, Methionine, Valine, Glycine, Proline, Cysteine, Tyrosine etc. [92, 93, 94, 95]. The free amino acids have shown to function in osmoregulation [96]. Neurotransmitter [97] Mullen and Martin stated metabolic pathways of growth including protein synthesis [93, 95, 98] Padma; Bhavani; Wilson stated allergic and inflammatory reactions. In the present investigation total 19 amino acids were categories in Essential Amino Acid (EAAs), Non-essential Amino Acid (NEAAs), both essential-amino acids and non-essential amino acids in the muscle tissue were quantified (Table 4). In monsoon crop, under Essential-Amino Acids (EAAs) category 10 amino acids in which the maximum mean concentration of methionine was dominating followed by Valine in MT1 and MT2 whereas Isoleucine has replace valine in MT3, MT4, MT5 and MT6 whereas 09 amino acid under Non-Essential Amino Acid (NEAAs) in which mean concentration of glycine was higher followed by glutamic acid in all treatment. So from the study, it is concluded that the amino acid% like Methionine, Valine and Isoleucine varies with stocking density and are active amino acid during monsoon season and hence predicted in maximum (mg/100g (DW).

Table 4: Essential amino acid (EAA) and Non-Essential Amino Acids (NEAA) composition (mg/100g (DW) recorded from *L. vannamei* shrimp flesh during monsoon crop in different treatments.

List of Amino Acid (%)	Abbreviation	Stocking density (Nos/m^2)					
		MT1	MT2	MT3	MT4	MT5	MT6
Alanine	Ala	4.91	5.03	3.14	2.46	2.57	2.54
Asparagine	Asn	1.61	1.73	1.71	1.62	1.60	1.62
Aspartic Acid	Asp	1.94	2.01	1.83	1.74	1.79	1.71
Cysteine	Cys	4.44	4.86	4.20	4.59	4.61	4.57
Glutamic Acid	Glu	5.86	5.59	5.91	4.92	4.94	5.03
Glycine	Gly	5.52	5.76	5.06	5.18	5.25	5.23
Proline	Pro	3.27	3.41	4.08	5.02	4.98	4.97
Serine	Ser	4.88	5.06	4.52	4.48	4.50	4.48
Tyrosine	Tyr	2.98	3.01	3.53	3.97	3.74	4.00
Total NEAA (%) in sample		35.39	36.46	33.97	33.96	33.99	34.14
Threonine	Thr	3.73	3.63	4.21	3.99	4.31	4.19
Arginine	Arg	3.59	3.43	4.07	4.16	4.14	4.02
Histidine	His	5.75	6.60	4.99	5.62	6.29	5.86
Valine	Val	10.92	9.72	7.86	6.03	6.69	7.37
Methionine	Met	14.86	14.39	13.01	10.24	10.73	9.77
Iso leucine	Ile	4.85	4.47	10.82	14.76	13.75	13.86
Phenylalanine	Phe	5.75	5.79	5.47	4.67	4.67	4.86
Leucine	Leu	4.92	4.73	5.86	6.78	6.43	7.11
Lysine	Lys	6.96	7.52	5.93	5.71	5.16	4.95
Tryptophan	Trp	3.28	3.26	3.81	4.08	3.84	3.87
Total EAA (%) in sample		64.61	63.54	66.03	66.04	66.01	65.86

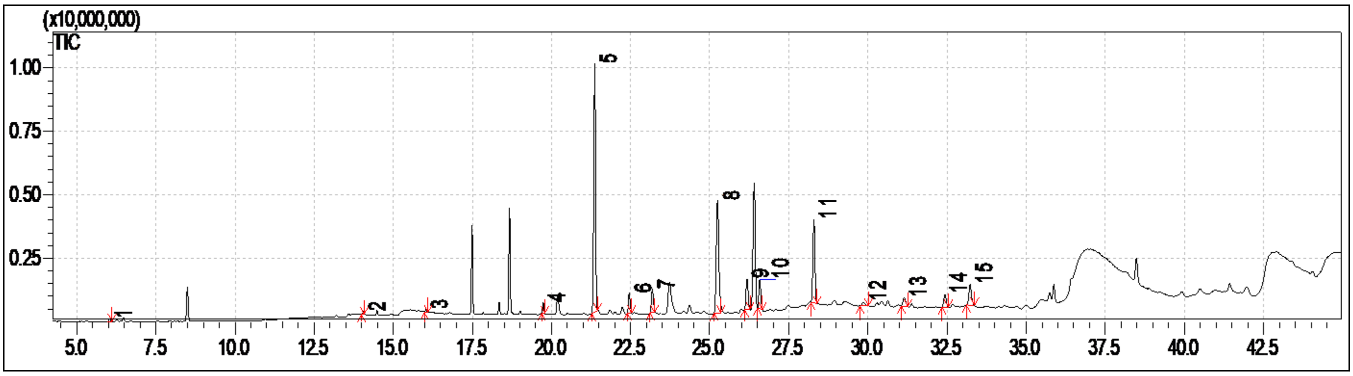

Fig 4: Chromatogram of fatty acid profiling of *L. vannamei* during monsoon crop at 120 DOC of MT1

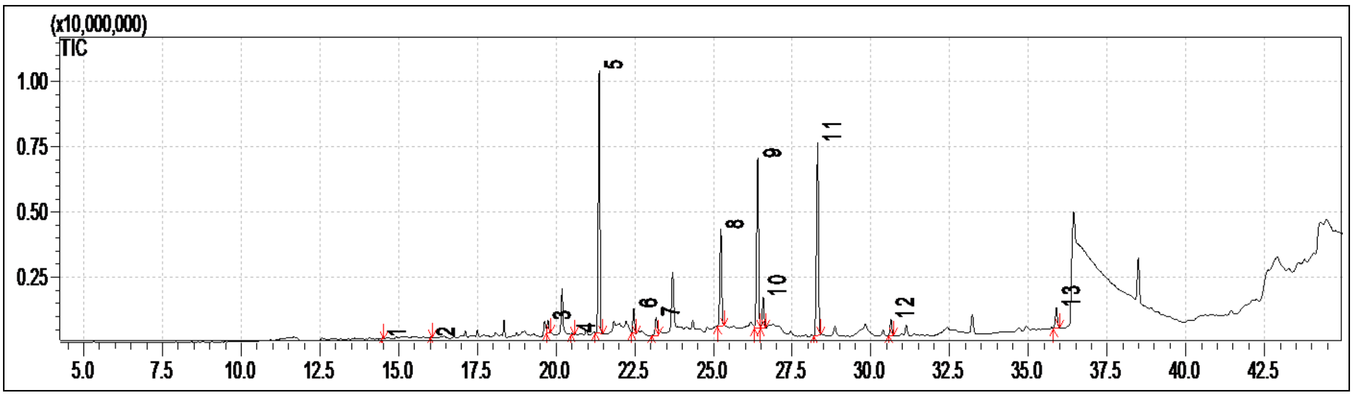

Fig 5: Chromatogram of fatty acid profiling of *L. vannamei* during monsoon crop at 120 DOC of MT2

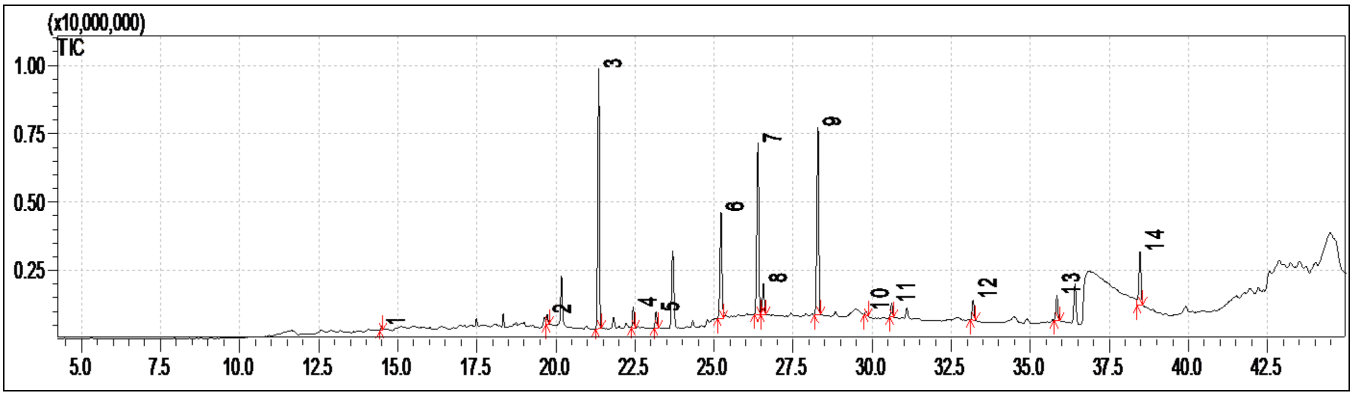

Fig 6: Chromatogram of fatty acid profiling of *L. vannamei* during monsoon crop at 120 DOC of MT3

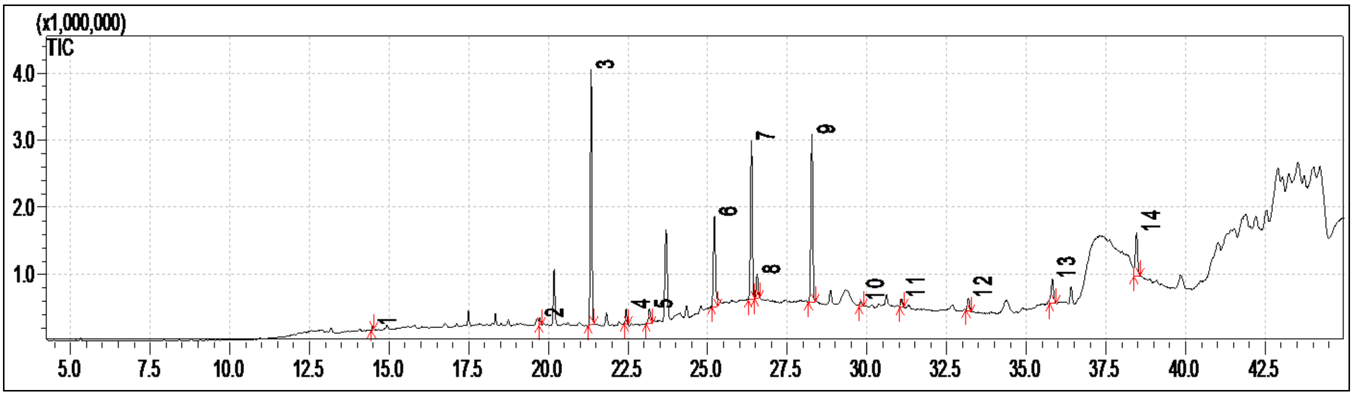

Fig 7: Chromatogram of fatty acid profiling of *L. vannamei* during monsoon crop at 120 DOC of MT4

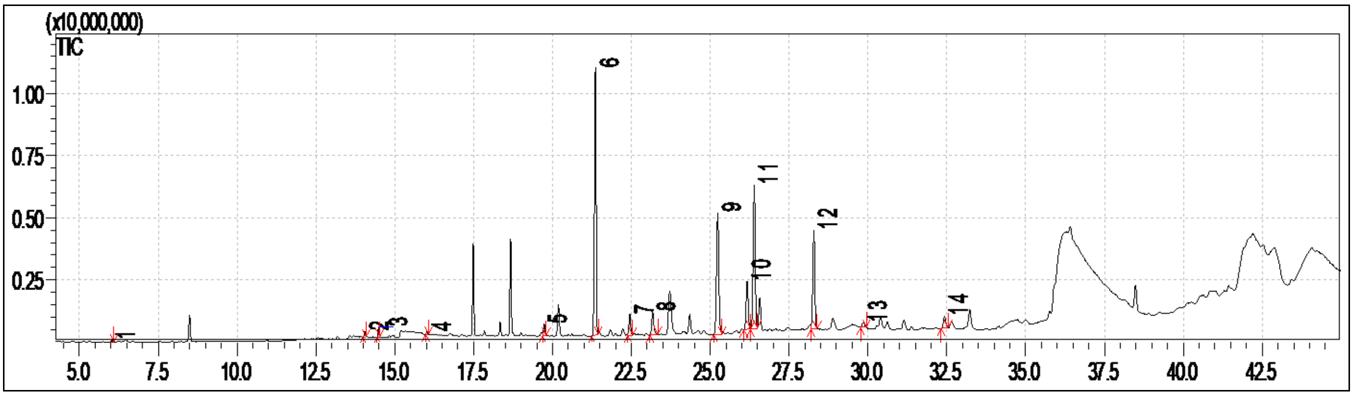

Fig 8: Chromatogram of fatty acid profiling of *L. vannamei* during monsoon crop at 120 DOC of MT5

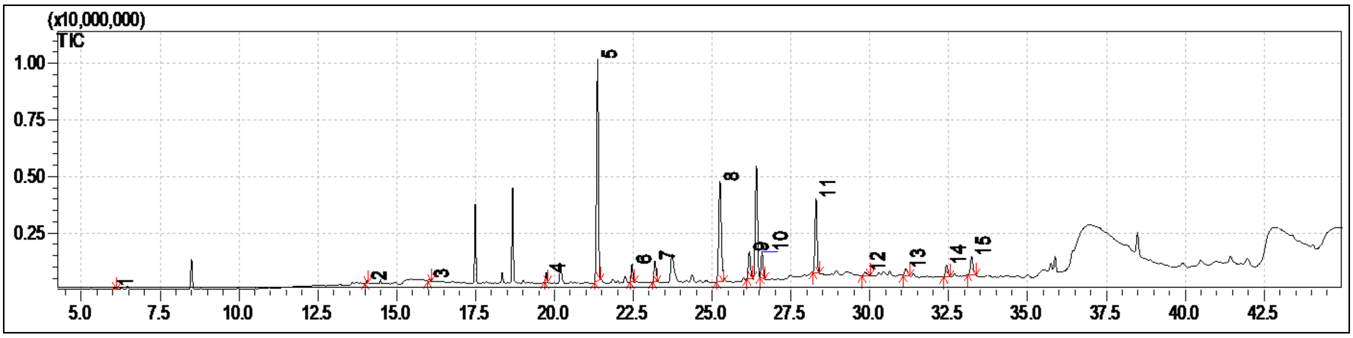

Fig 9: Chromatogram of fatty acid profiling of *L. vannamei* during monsoon crop at 120 DOC of MT6

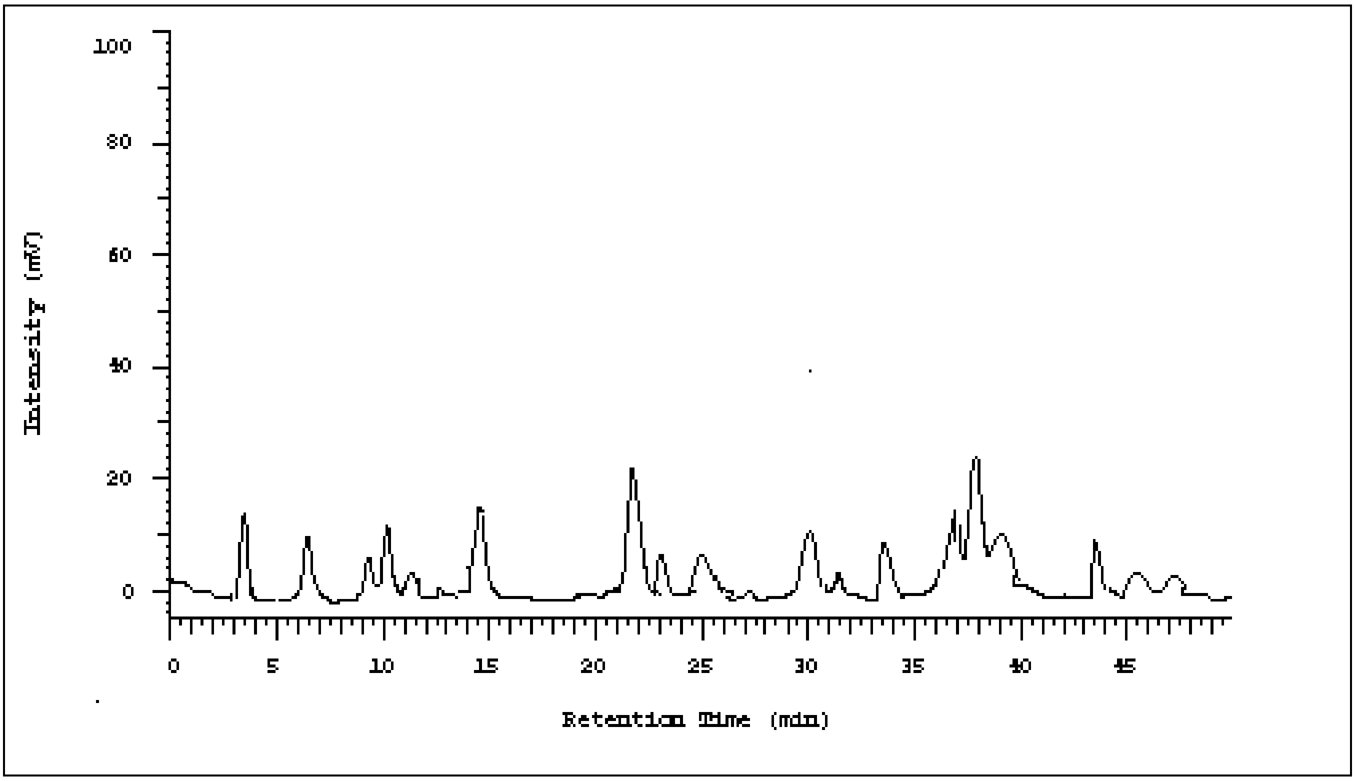

Fig 10: Chromatogram of amino acid profiling of *L. vannamei* during monsoon crop at 120 DOC of MT1

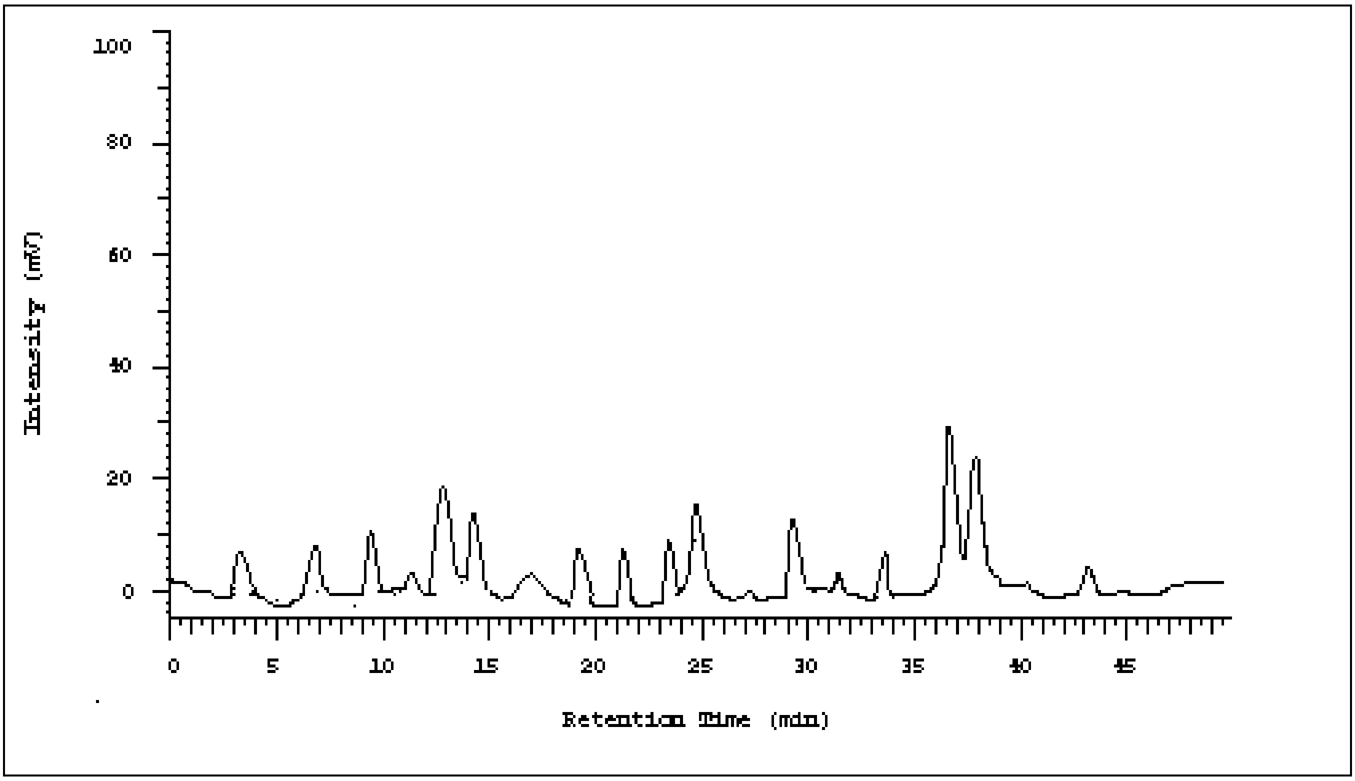

Fig 11: Chromatogram of amino acid profiling of *L. vannamei* during monsoon crop at 120 DOC of MT2

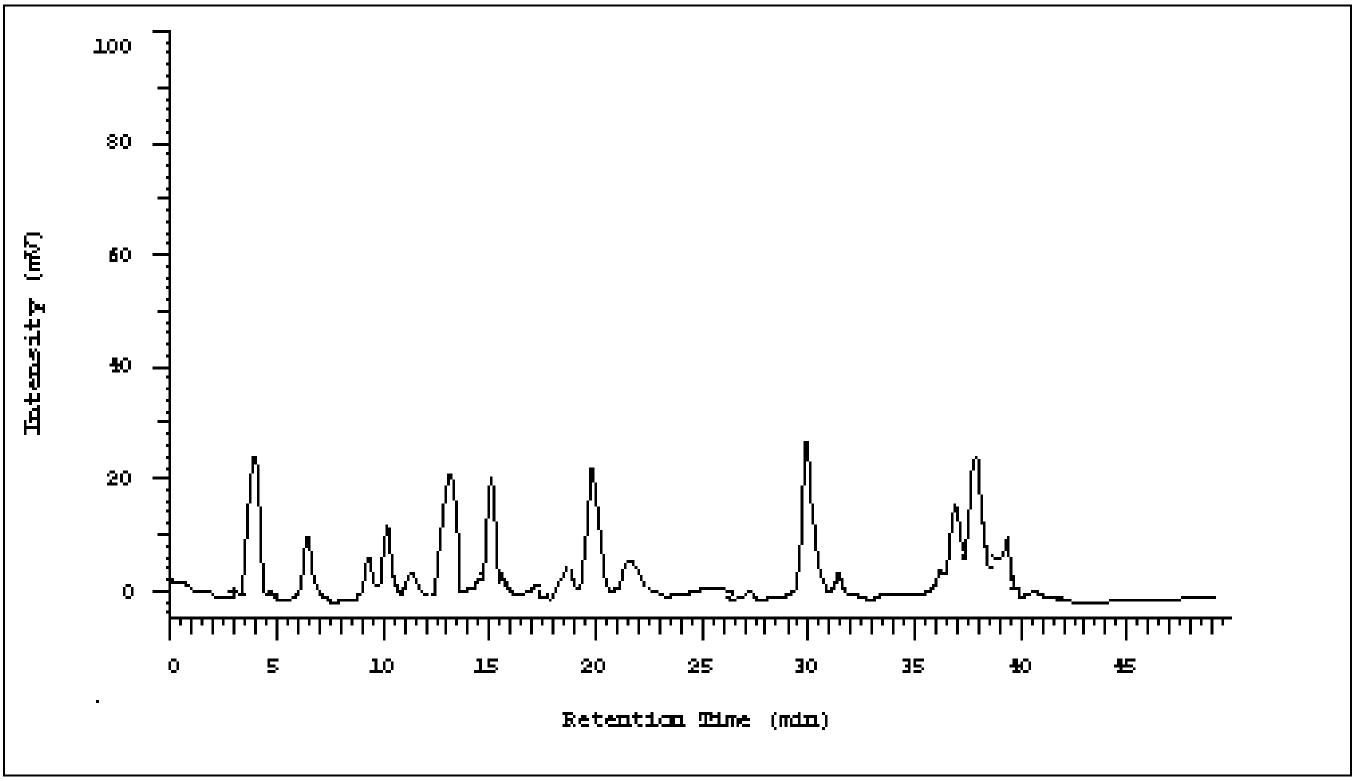

Fig 12: Chromatogram of amino acid profiling of *L. vannamei* during monsoon crop at 120 DOC of MT3

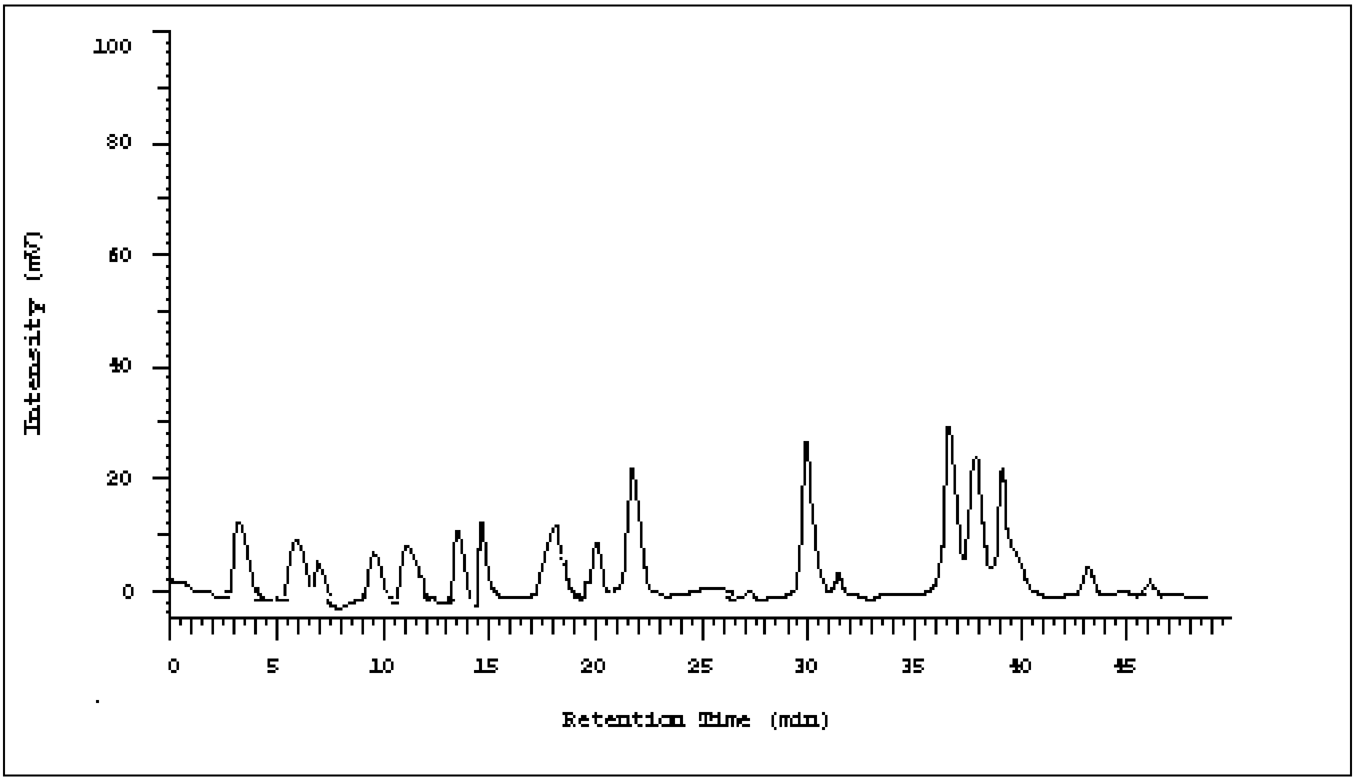

Fig 13: Chromatogram of amino acid profiling of *L. vannamei* during monsoon crop at 120 DOC of MT4

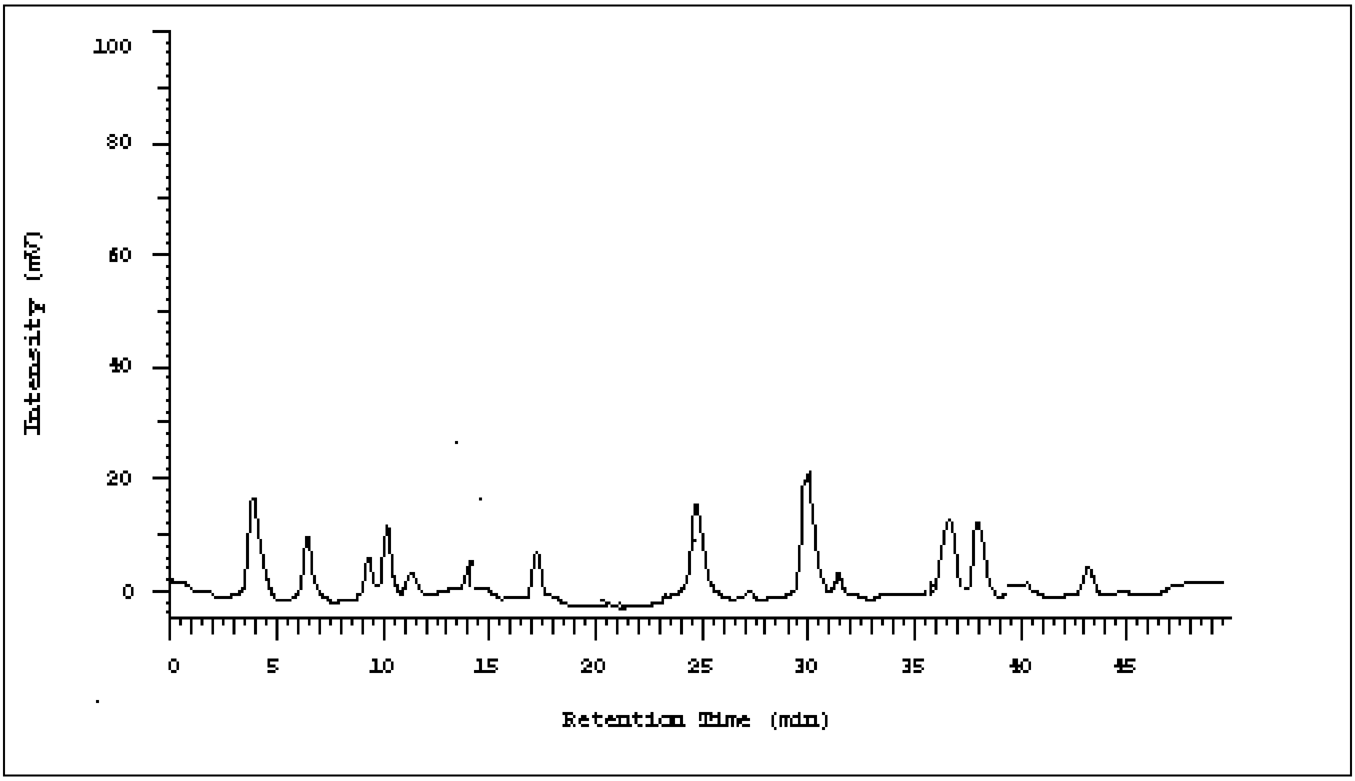

Fig 14: Chromatogram of amino acid profiling of *L. vannamei* during monsoon crop at 120 DOC of MT5

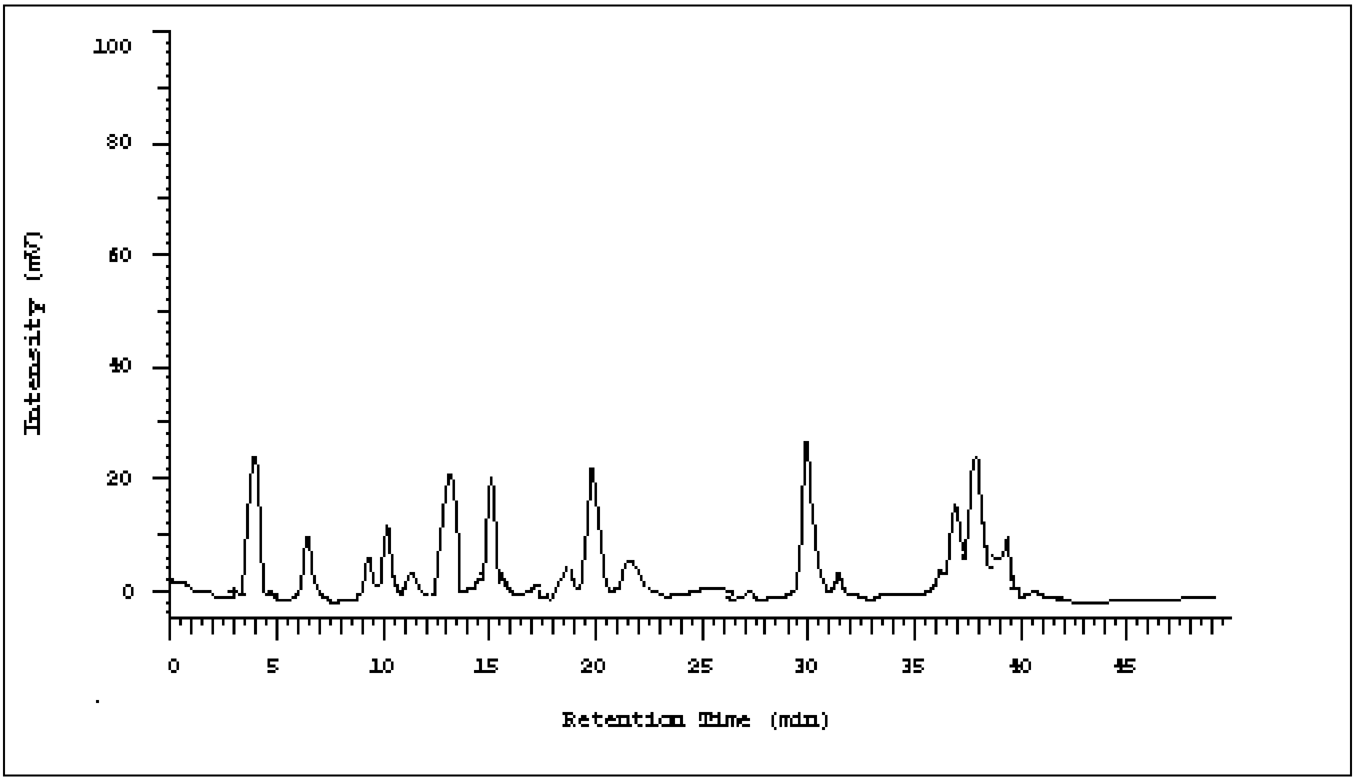

Fig 15: Chromatogram of amino acid profiling of *L. vannamei* during monsoon crop at 120 DOC of MT6

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