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Bio-refinery products from shell fish processing waste: Application of Chitin, Chitosan, Chitooligo saccharides and Derivatives in Organic Agriculture

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Abstract
Shellfish shell waste has high protein (39.6%), Chitin (22.6%) and ash (28.1%). Bioconversion of Chitin into glucosamine through cold fermentation using inherent enzymes and microbes using molasses as a substance was investigated. Cold fermentation has been envisaged as one of the most eco-friendly, safe, technologically flexible and economically viable alternative method for bio-refinement.

Keywords: chitin, chitosan, chitooligo saccharide

Introduction
Chitin is a natural polysaccharide of major importance and most abundant polymer after cellulose. The most important derivative of Chitin is Chitosan produced by deacetylation Chitin and Chitosan have important functional activities but poor solubility makes them difficult to use in food and biomedical applications. Chitooligo saccharides (COS) are degraded products of Chitin and Chitosan prepared by enzymatic and chemical hydrolysis. The greater solubility and less viscosity of COS have attracted the interest of many researchers to utilize COS and their derivatives for various biomedical applications.

Chitin
Chitin is a mucopolysaccharide and supporting material of crustaceans. Chitin is a highly insoluble material and a linear polysaccharide. Naturally occurs in there polymorphic form and orientation of microfibrils known as α, β and ¥ Chitin. A-chitin is prevalent in crustaceans. B-chitin occurs in pens of molluses, d, cuttle fish and loligo. ¥-chitin are present in cocoons or insects. Dietary supplementation of chitin has shown to exert positive immune-modulatory effect and antibacterial activity of Chitin, prepared from shrimp shell waste (Benhabiles *et al*., 2012).

Bio-refinement of shrimp shell waste
Greener industrial method for the hydrolysis of chitin and chitosan is highly desirable as the products are potentially of high commercial value. Even though the specific enzymes as chitosanases and chitinases have shown to have excellent performance in COS production they are too expensive to be utilized in large scale industrial application (Pantaleons *et al*., 1992) [2]. Fermentation with natural enzymes, yeast and starter culture has produced chito-digo-saccharides. Endogenous enzymes present in the shrimp head may be used for the practical purposes of producing chito-oligo-saccharides (CHOS). Chitosanases and other non-specific enzymes including glyconases, proteases and lipases derived from bacterial, fungal, mammalian and plant sources.

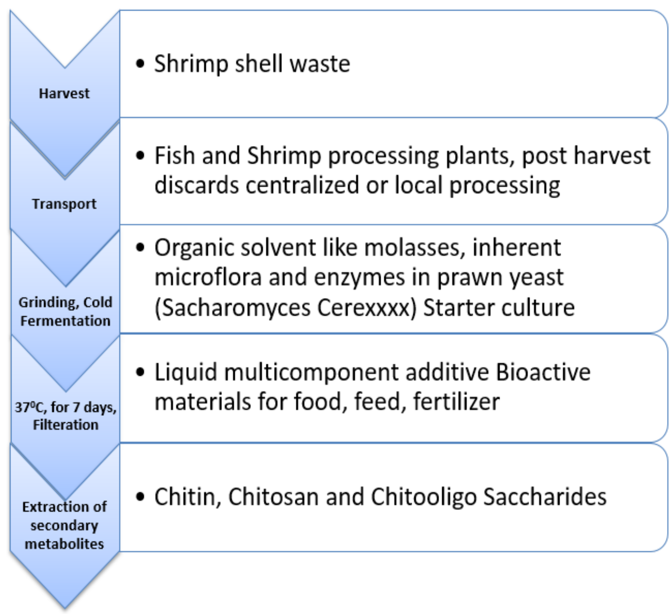

Fig 1: Decision making flowchart for green processing of fishery waste into organic products.

Chitin is the exoskeleton of shrimp shells. Use of native microflora and protaolytic enzymes and sugars for deproteinization is a current trend in conversion of waste to useful biomasses. Deproteinination releases amino acids and act as a nitrogen source phosphates, carbonates and calcium are solubilized for demineralization of the waste (Janarthan *et al*., 2015).
Prawn waste hydrolysate have high quality flavor enhancer aminoacids. Major amino acids are glycine (14.09%), Valine (11.07%), Leucine+ Isoleucine (16.21%), Lysine (18.13%), Glutamic acid, Aspartic acid, Glycine, Algnine constitute 39.27% of the total amino acids (Wassila *et al*., 2013) [39].

Chitosan
Chitosan and Oligochitosan have attracted considerable interest due to their unique biological activity such as anti-

oxidant, anti-microbial these features together with their bio-compatibility bio-degradibility and non-toxivity make them as interesting bio-polymers for application in medicine, cosmetic, biotechnology, food, agriculture and allied activities (Higa and Chinnen, 1998) [50].

Chitosan is a natural, non-toxic biopolymer produced by deacetylation of chitin. Chitosan is normally soluble in acidic pH (<6.0). the free amino groups present in chitosan are protonated and molecules become soluble chitosan solubility, bio degradability, reactivity and adsorption of substrates are dependent upon protonated amino groups, acetylated and non acetylated glucosamine units (Rinaudo, 2006) [1]. Hydrolysis of chitin and chitosan yield mono, di and oligosaccharides. Hydrolusis of colloidal chitin yield chitobiose, a dimer of N-acetyl glucosamine.

Oligochitosan with average molecular weight (mw) of 500g/mol was prepared by gamma CO-60 radiation degradation of 4% chitosan solution containing 0.5% H_2O_2 at 21 kGx for foliar application in agriculture use (Phu *et al*., 2017) [43].

Chitooligosaccharides

Chitooligosaccharides (CHOS) are homo or hetero oligomers of N-acetylglucosamine, and D-glucosamine. Prawn and Shellfish processing waste constitute a good amount of chitin and chitosan. Production of well defined CHOS mixture is of great interest since these oligosaccharides have several interesting bioactivities.

Chitosan with degree of polymerization (DP) < 20 and an average molecular weight less than 3900 Da are called Chitosan oligomers, chitooligomers or chitooligosaccharide. These are generated by depolymerization using acid hydrolysis or hydrolysis by physical methods or enzymatic degradation. But acid hydrolysis results in formation of secondary compounds that are difficult to remove (Maurya *et al*., 2011 and Aam *et al*., 2010) [5, 3].

Chitin and Chitosan Oligomers

Oligomers can be produced from chitin or chitosan as starting material by enzymatic and microbial conversion. Depolymerization under high energy impact and recombinant approaches are being tried (Pantaleone, *et al*., 1992) [2].

Chitosan with high degree of polymerization and molecular weight exibit poor aquous solubility which is as impediment in their applicability. The low molecular weight chitosans (LMWC) and Chitooligosaccharides (COS) can be used to avoid this hurdle. The development of an efficient process of reducing the molecular weight of chitosan, without its chemical structure is of great interest to produce tailore made chitosans of varying degree of acetylation (DA) and degree of polymerization (DP).

Oligomers can be produced from chitin and chitosan as starting material by enzymatic and microbial conversion. Depolymerization under high energy impact and recombinant approach.

Biomedical application of chitooligomers

Owing to the presence of hydroxyl amine and acetylated amine groups, chitoses, low molecular weight chitosan and chitooligosaccharides interact readily with various cell receptions that trigger a cascade of inter connected reactions in living organisms resulting in antiinflamatory (Fernandez *et al*., 2010) [7], anticarcinogenic (Huang *et al*., 2006) [8], antidiabetic (Ju, *et al*., 2010) [9], antimicrobial (Jung *et al*., 2010) [10], anti HIV-I (Artan *et al*., 2009) [11], antioxidant (Ngo *et al*., 2009) [12], antiangiogenic (Quan *et al*., 2009; W U *et al*., 2008) [13, 24], antibacterial (Moon *et al*., 2007) [6, 14].

Hydrolysis of chitin and chitosan yield mono-, di- and oligosaccharides. Hudrolysis of colloidal chitin yield chitobiose, a dimer of N-acetylglucosamine and glucosamine are well known for treatment of osteoarthritis.

Chitin and chitosan oligomers are bioactive and posses antitumurigenie, antifungal and antibacterial properties. Pigs fed with 1.0g chito-oligo-saccharide per kg diet had higher final weight, higher feed intake and lower feed conversion ratio (FCR) than the control group (Hong Kuntod *et al*., 2015) [15, 16]. Pigs receiving Chito-oligo-saccharides (COS) supplemented diet high phagocytic activity dietary supplement of 1g/kg COS improved neutrophil and macrophage capacity for phagocytosis in weaned pig.

Several derivatives of chitin and chitosan were shown to inhibit *E. Coli*, *Staphyllococcus aureus*, some bacillus species and several bacteria in feeding fish. Chitosan inhibits growth of wide range of bacteria. The minimal growth inhibitory concentration vary from 10-1000 ppm for the chitosan, Chito-oligo-saccharide, Quarternary ammonium salt of chitosans (Zia *et al*., 2001; Tsai and su, 1999) [17].

Enzyme hydrolysis of fish waste has been tried as animal feed and fertilizer been tried as animal feed and fertilizer in pigs and shown apprecials result in growth and production (Shahidi *et al*., 1997) [42]

Utilization of fish and shellfish hydrolysate in feed development advantages are replacement of fish meal, decreased storage space, decreased cost, good storage durability, decreased pH of feed, hygienic. Fish and shellfish hydrolysate affects the feed quality and reduce the need for added water. it increases the dietary dry matter and energy density per kg which in turn reduce feed consumption (Sahidi *et al*., 1997) [42].

Sulfuric acid or Taurine deficiency explain the reduction in growth performance when including high level of taurine poor ingredient in fish and animal feed. By supplementing plant based diets with taurine, growth performance has been restored (Aksnes, *et al*., 2006; Gaylord *et al*., 2014) [47, 48].

Natural fermentation of fish and shellfish waste is non-hazardous, easy to use, non corrosive to farm machinery, don’t pollute the environment and were regarded as natural products. Fish waste is a opportunity for feed and nutrient studies. Cats and ducks really like this product and this could really develop into pet food market.

Chitooligosaccharides in plant protection

Chitin and chitosan are naturally occurring products. The anti microbial and eliciting properties of chitin and chitosan and their derivatives are getting more attention in recent years. These products can be used in a number of ways to reduce disease levels and prevent the development and spread of pathogens thus preserving yield and quality (Hadrami *et al*., 2010) [20].

In response to microbialinfection including the accumulation of phytolexins Pathogen Related (PR) proteins and proteinase inhibitor, lignin synthesis and callose formation. Derivatives have been used in agricultural systems to reduce the negative impact of disease on yield and quality of crops (Rabea *et al*.,

2003) [22], Oligomeric chitosans (Pentamers and Heptamers) exibit a antifungal for bacterial disease (Barber *et al*., 1989) [30].

Chitooligosaccharides sehiff base could effectively induce the resistance of tobacco plants to tobacco mosaic virus (TMV) and the contents of chlorophyll and carotene in tobacco leaves were greatly improved after chitooligosaccharide treatment (Longlie *et al*., 2012) [29].

Lipochitooligosaccharide (LCO) or Nodfactor produced by rhizobia are not only important for the establishment of legume rhizobia symbiosis but have a potential to improve somatic embryogenesis, seed germination and plant growth and development of some important crops (Supanjani *et al*., xxxx) [33].

Chitosan is used both as a biostimulant to stimulate plant growth and abiotic stress tolerance and as to induce pathogen resistance. Chitosaccharides stimulate the modulation, growing and yield of legumes as soybean. This could also be used for foliar application or seed coating treatment to boost plant growth and yield.

Chitoligomers are active in-vitro against *Lepidopterous* and *Homopterous* insects. They form a coating on the surface of the insect terminally blocking their respiration. Chitosan oligomer possesses proteinase inhibitor (PI). Seed coating to improve fungal control and seed quality in wheat crop. Proteinase inhibitor are one of the plant response producer to protect from herbivorous insect attack.

N-acetyl and D-glucosamine Co-polymer is a plant stimulator. Seed priming with chitosan makes seedling more resistant to abiotic stress (Pichyangkura and Chadchawan, 2015) [34]. Chitosan inhibits the systemic propagation of viruses and viroids throughout the plant and enhances the hypersensitive response to infection of host (Chirkov, 2002) [35].

Similar observations were reported with potato virus-X, tobacco mosaic, Neero viruses, alfalfa mosaic virus, peanut stunt virus and cucumber moaic virus (Pospieszny *et al*., 1991; Vasyukova *et al*., 2005) [36, 37].

Hydrolyzed chitin and chitosan products provide antiviral and anti bacterial activity on plants. Its derivative prevent late blight diseases in potato and protect from soil borne pathogenie fungi due to its insectieidal and fungicidal activity.

Chitooligosaccharides in organic agriculture

Foliar spray of chitin and chitosan oligosaccharides improve photosynthesis of crops. Reduction of transpiration through foliar application. Antitrnspirant activity is due to abscinic acid dependant stomatal closure (Hemantranjan, 2014) [40].

Fish and prawn hydrolysate enhances resistant of tomato plant to the crown and root rot pathogens *Fusarium Oxysporum* and *Radici lycopernici*. It also provide resistance to powdery mildew in Barley (Wyalt and Mcgoorty, 1990; Yin *et al*., 2010) [41, 44] supposed oligo-chitosan as a plant vaccine that is similar with animal vaccine. Oligo-chitosan is effective at eliciting plant innate immunity against disease in plant such as tomato and grape vine.

Fish and shellfish waste hydrolyasate are ecofriendly:

Toonisari *et al*., 2015 studied the green house gas emission from liquid organic and solid inorganic fertilizers on Lettuce (Lectuca sativa, L.). liquid fish fertilizers having C/N ratio 0.9-1.5 multiple application of liquid fish fertilizer thrice a week had emission factor for N_2O ranged from upto 0-0.1%. Inorganic fertilizer had higher C/N ratio (3.3-3.5) resulted higher CO_2 emission, its emission factor of N_2O ranged from 0.6 to 11% for pre plant applied solid inorganic fertilizer.

Chitooligosaccharides as a coating material for seed and horticultureal products:

Increasing knowledge and concerns as not the environmental consequences of fungicide applications have prompted industry and research scientists to explore the potential natural products.

Prawn waste hydrolysate (Shellfie fert) developed at ICAR-Central Institute of Freshwater Aquaculture, Bhubaneswar. 50ml of shellfire fert diluted with 10 litres of waste sprayed over 100 kg as the seed treatment (Sahu *et al*., 2016) [49]

Chitooligosaccharide based seed treatment agent is a novel environment friendly organic seed coating for rice, wheat, legumes and other commercial seed production units. Priming of seeds with chitooligosaccharides combined with micro fertilization can make up for the shortage of fertilizing and degree to meet nutrient, element requirement to control deficiency diseases.

Chitosan and oligochitosan based novel ecofriendly organic seed coating significantly stimulate the seeding growth of rice, advance the growth of root system, improve root activity and increase the paddy yield (Zeng and Shi, 2008) [45].

Chitosan priming increased the chilling tolerance of maize seeding demonstrated by improving germination speed, shoot and root growth with higher activities of antioxidative enzyme. Oligochitosan exhibit growth promotion of effect for plants as rice and soybean, mumybean.

Application of oligochitosan both by seed treatment or through hyporex solution increased seed yield of soybean from 15-26%. Oligochitosan has better plant growth promotion and elicitating effect that chitosan (Zeng *et al*., 2012; Costaless *et al*., 2016) [55, 56].

Conclusion

Cold fermentation of fish processing waste using inherent enzymes, microbes using molasses as substrate to produce fish and shellfish hydrolysate was envisaged. Deproteinisation release amino acid and act as a nitrogen source, phosphates, carbonates and calcium are solubilized for demineralization of waste. Chitosan and oligochitosan have attracted considerable interest due to their unique biological activity antioxidant, antibicrobial and growth promote. These features together with their biocompatibility, biodegradability make them interesting biopolymer for application in medicine, cosmetic, food agriculture, biotechnology and allied activities. Utilization of fish and shellfish hydrolysate has advantages in replacing the fishmeal in fish feed, decreased storage spaces, decreased cost, good storage durability, decreased pH and hygienic product. Hydrolysate products application in organic agriculture reduces disease levels, spread of pathogens thus processing yield and quality. Foliar application and seed priming boost plant growth and yield. Foliar spray improves photosynthesis and reduction in transpiration.

References

1. Rinaudo M. Chitin and Chitosan: Properties and application. Progress in Polymer Science. 2006; 31(7):603-632.
2. Pantaleone D, Yalpani M, Seollar M. Unusual

succeptibility of chitosan to enzymic hydrolysis. Carbohydrate Research. 1992; 237:325-332.
3. Aam BB, Heggnet EB, Norberg AL, Sortie M, Varum KM, Eijsink VG. Production of chito-oligo saccharides and their potential application in medicine. Marine Drugs. 2010; 8(5):1482-1517.
4. Struszczyk H, Pospieszny H, Kotlinsky S. Some new application of chitosan in: Chitin and chitosan. Skjosk Brok, G., Anthnsen, T and P. Stand ford, Eds, Elsevier Science, London, U.K. 1989, 733-742.
5. Maurya VK, Inamdar NN, Choudhari YM. Chito-oligo saccharides: synthesis, characterization and applications. Polymer Science. Ser. 2011; 53(7):583-612.
6. Moon JS, Kim HK, Kao HC. The antibacterial and immunostimulation effect of chitosan oligosaccharides against infection by *Staphylococcus aureus* isolates from bovine mastitis, Applied Microbiology and Biotechnology. 2007; 75(5):984-998.
7. Fernandez JC, Spindola H, Desousa V. Anti-inflammatory activity of chitooligosacharides in vivo, Marine Drugs. 2010; 8(6):1763-1768.
8. Huang IR, Mendis E, Rajapakse N, Kim SK. Strong electronic change as an important factor for anticancer activity of chitooligosaccharides (COS). Life Sciences. 2006; 78(20):2399-2408.
9. Ju C, Yue W, Yang Z. Antidiabetic effect and mechanism of chitooligosaccharides. Biological and Pharmacological Bulletin. 2010; 33(9):1511-1516.
10. Jung EJ, Youn DK, Lec SH, No HK, Ha JG, Prinyawiwatkul W. Antibacterial activity of chitosan with different degree of deacylation and viscosities, International Journal of Food Science and Technology. 2010; 45(4):676-682.
11. Artan M, Karadeniz F, Karagozlu MZ, Kim MM, Kim SK. Anti HIV-activity of low molecular weight sulfated chitooligosaccharide. Carbohydrate Rescarch. 2009; 345(5):656-662.
12. Ngo DN, Lec SH, Kim MM, Kim SK. Production of chitin oligosaccharides with different molecular weight and their antioxidant effect in RAW 264.7 cells. Journal of Functional Foods. 2009; 1(2):188-198.
13. Quan H, Zhu F, Han X, Xu Z, Zhau Y, Mino Z. Mechanism of antiangiogenic activity of chitooligosaccharide may be through inhibiting heparanase activity. Medical Hypotheses. 2009; 73(2):205-206.
14. Moon JS, Kim HK, Kao HC. The antibacterial and immunostimulation effect of chitosan oligosaccharides against infections by *Staphyllococous aureus* isolates from bovine mastitis, Applied Microbiology and Biotechnology. 2007; 75(5):989-998.
15. Hongkuntod P, Teeranitaytarn K, Areerob P, Chaunchom S, Ruckkwarnnuk T. Dietary chitooligosaccharides. Supplementation 1: Effect on growth performance and nutrient digestibility in weaned pigs. Journal of Kasetsart Veterinarians. 2015; 25(2):66-74.
16. Hongkuntod P, Teeranitaytarn K, Areerob P, Chaunchom S, Ruckkwarnnuk T. Dietary chitooligosaccharides. Supplementation 1: Effect on growth performance and nutrient digestibility in weaned pigs. Journal of Kasetsart Veterinarians. 2015; 25(2):75-86.
17. Zia Z, Shen D, Xu W. Synthesis, antibacterial activities of quaternary ammonium salt of chitosan Carbohydrate Research. 2001; 333:1-6.
18. Tsai GJ, Su WH. Antibacterial activities of shrimp chitosan against Escherichia Coli. J. Food Protec. 1991; 55:916-919.
19. Kendra DF, Hadwinger LA. Characterization of the smallest chitosan oligomer that is maximally antifungal to *Fusarium solani* and elicits pisatin formation in *Pisum sativum* Experimental Mycology. 1984; 8(3):276-281.
20. Hadrami HE, Adam LR, Hadrami IE, Daayi F. Chitosan in plant protection, Marine Drugs. 2010; 8(4):968-987.
21. Makoi JHJR, Ndakidemi PA. Biological, ecological and agronomic significance of plant phenolic compounds in rhizospheres of the symbiotic legume. African Journal of Biotechnology. 2007; 6(12):1358-1368.
22. Rabea EI, Badavy EJ, Stevens MT, Smaggle CV, Steurbaut W. Chitosan as antimicrobial agent: Application and mode of action. Biomacromolecules. 2003; 4:1457-1465.
23. Bautista Banos S, Hernandez LAN, Velazavez DMG, Hernsndez LM, Ait Barka E, Bosquez ME *et al*. Chitosan is a potential natural compound to control pre and post-harvest diseases of horticultural commodities Crop Protection. 2006; 25:108-118.
24. Wu H, Yau Z, Bai X, Du Y, Lin B. Anti angiogenic activities of chitooligo saccharides. Carbohydrate Polymer. 2008; 73:105-110.
25. Zopf D, Roth S. Oligosaccharide anti-infective agents. The Lancet. 1996; 347:1017-1021.
26. Kerton FM, Liv Y, Omari K, Howbolt W. Green chemistry and ocean based bio-refinery. Green Chemistry. 2013; 15:860.
27. Bhatnagar A, Sillannapaa M. Application of chitin and chitosan derivative for the detoxification of water and waste water- A short Review, Adv. Colloid. Interface. Sci. 2009; 152:26-38.
28. Riberio MP, Espiga A, Silva D, Beqtista P, Henriques J, Ferreira C. Development of a new chitosan hydrogel for wound dressing, wound Repair, Regeneration. 2009; 17:817-824.
29. Longlie Z, Cuilian X, Fugen H, Zu Hang L, Wang M, LiHong O. Effect of chitooligosaccharide Schiff base on Chlorophyll Content in tobacco leaves infected by TmV. Acta Agricultural Jiang Xi. 2012; 24(2):113-116.
30. Barber MS, Bertram RE, Ride JP. Chitin oligo-saccharide elicit lignifications in wounded wheat leaves. Physiology Molecular Plant Pathology. 1989; 34:3-12.
31. Felix G, Baureithel K, Boller J. Desensitization of the perception system for chitin fragment in tomato cells. Plant Physiology. 1998; 117:643-650.
32. Lodhi G, Kim YS, Hwang JW, Kim SK, Jeon YJ, Je JY. Chito-oligo-saccharide and its derivatives: Preparation and Biological Application, Bio-medical Research International. 2011. Article ID 654913: 1-13.
33. Supanjani S, Souleimanov A, Smith DL. Lipochito-oligo-saccharide: Handling and Storage. xxxx.
34. Pichyangkura R, Chadehawan S. Biostimulant activity of Chitosan in horticulture. Scientia Horticultural. 2015; 196:49-65.
35. Chirkov SN. The antiviral activity of chitosan: A review. Applied Biochemistry Microbiology. 2002; 38:1-8.
36. Pospieszny H, Chirkov S, Atobekov J. Introduction of

antiviral resistance in Plants by Chitosan. Plant Science. 1991; 79:63-68.
37. Vasyukova NI, Chalenko GI, Ferasimova NG, Perekhod EA, Ozeretskovskay OL, Irina AV. Chitin and chitosan derivative as elicitors of potato resistance to late blight. Applied Biochemistry and Microbiology. 2005; 36:372-376.
38. Janarthanan G, Nagalaxmi K, Sathis Kumar K, Chakraborti R, Venkateswarulu G. Protein hydrolysate from shrimp (*Metapeneus dobsoni*) head waste: optimization of extractions conditions by response surface methodology. Journal of Aquatic Food Product Technology. 2015; 24(5):15-19.
39. Wassila A, Adour L, Amrana A. Chitin extraction from crustaceans using biological method Food Technology and Biotechnology. 2013; 51:12-25.
40. Hemantaranjan A. A future perspective of crop protection: chitosan and its oligosaccharides Advances in Plants and Agricultural Research. 2014; 1:1-8.
41. Wyatt B, Mc Goorty A. use of marine by-products as Agricultural crops. International By-products Conference, April 1990, Anchorage, Alaska. 1990, 187-195.
42. Shahidi F, Jones Y, Krilts D. Enzyme hydrolysis of fish waste for animal feed and fertilizer in Pigot, G. M., Edt. Seafood Safety, Processing and Biotechnology, Technomic Publication, Pensylvania, 1704, USA. 1997.
43. Phu DY, Du BD, Tuan LNA, Tam HV, Hien NO. Preparation and foliar application of oligo-chitosan nano silica on the enhancement of soybean seed yield. International Journal of Environment Agriculture and Biotechnology (IJEAB). 2017; 2(1):421-428.
44. Yin H, Zhao X, Du Y. Oligochitosans: A plant disease vaccine- A review, Carbohydrate Polymer. 2010; 82(1):1-8.
45. Zeng D, Shi Y. Preparation and application of a novel environment friendly organic seed coating for rice. American Eurasian Journal of Agronomy. 2008; 1(2):19-25.
46. Toomisiri P, Del Grosso SJ, Sukor A, Davis JG. Greenhouse gas emission from liquid and solid organic and inorganic fertilizer applied to Lettuce Journal of Environmental Quality. 2015. doi 10.2134. Jeq 2015: 12.0623.
47. Aksnes A, Hope B, Jonsson E, Bjornsson BT, Albrektsen S. Size fractioned fish hydrolysate as feed ingredient for Rainbow trout (*Onchorhynchus mykiss*) fed high plant protein diets. 1: Growth, growth regulation, feed utilization Aquaculture. 2006; 261(1):305-317.
48. Gaylord TG, Gatlon III DM, Barrows FT, Nistler A, Pohlenz C. Stability of synthetic taurine through extrusion processing and subsequent bio availability to Rainbow trout (*Onchorhynchus mykiss*) Aquaculture America, Seattle, W. A. 2014.
49. Sahu BB, Mohapatra BC, Barik NK, Sahu H, Sahoo P, Biswal NC *et al*. *In-vitro* assessment of plankton production using fish hydrolysate. International Journal of Innovative Studies in Aquatic Biology and Fisheries (IJISABF). 2016; 2(1):14-25.
50. Higa T, Chinen N. E. M. Treatment of Odair, Waste water and environmental problem. College of Agriculture, University of Ryukyus Okinawa. 1998.
51. Benhamou N. Elicitor induced plant pathway. Trends in Plant Science. 1996; 1(7):233-240.
52. Das SN, Madhuprakash J, Sharma PV, Purusothan P. Biotechnological approach for field application of Chitooligosaccharides (COS) to induce innate immunity in plants. Critical Reviews in Biotechnology. 2015; 35(1):29-43.
53. Khan W, Prithviraj B, Smith D. Chitosan and Chitin oligomers increase phenylalanine, ammonia lysage and tyrosine- ammonia-lysage and tyrosine-ammonia-lysage and tyrosine-ammonia-lysage activities in soybean leaves. Journal of plant physiology. 2003; 160(8):859-868.
54. EI-Sawy NM, EI-Rahim HAA, EI-Barbary AM, Hegazy EA. Radiation induced degradation of chitosan for possible use as growth promoter for agricultural purposes. Carbohydrate polymers. 2010; 79(3):555-562.
55. Zeng D, Luo X, Tu R. Application of bioactive coating based on chitosan for soybean seed protection. International Journal of Carbohydrate Chemistry. 2012. Article ID 104565.
56. Costaless D, Falem AB, Napoles MC, dewinter J. Effect of chittosaccharides in modulation and growth *in vitro* inoculated soybean. American Journal of Plant Sciences. 2016; 7(9):1380-1391.