ANNEX 3

Part A. Summary of Country Reports on Production and Field/Pot Test of Plant Growth Promoter (PGP) from Chitosan by Radiation Processing

(1) Bangladesh (Md. Emdadul HAQUE, Bangladesh Atomic Energy Commission)

Chitosan from shrimp shell was prepared and the molecular weight of the chitosan was determined corresponding to applied radiation doses of gamma rays using viscosity method. Oligo-chitosan was prepared by applying 25 kGy radiation dose on solid condition and again irradiated by 10 kGy in solution adding 0.3% H_2O_2. The oligo-chitosan was used for pot/field test on rice, tomato, mungbean and maize.

From the results of field test it was found that, for rice yield increases up to 13% by applying chitosan twice at 50 ppm at tillering (25 DAT) and booting stages (50 DAT).

For summer tomato fruit yield increases up to 56% when chitosan was sprayed one time at 75 ppm during vegetative stage (25 DAT).

For mungbean yield increases up to 30% by applying chitosan twice at 50 ppm at vegetative (25 DAS) and flower initiation stages (40 DAS)

For maize yield increases up to 24% by applying 100 ppm chitosan three times (40, 55 and 70 days after sowing).

(2) China (Dr. WU Guozhong, Shanghai Institute of Applied Physics)

Radiation degraded chitosan has been tested in chicken broiler, pig and cow as a feed additive. It was found that low molecular weight has a good effect for all the animals tested. Compared to antibiotics, low molecular weight can enhance the immune activity and biochemical index of blood for pigs. It is also effective to enhance the growing of broiler and to cure mastitis of milk cow. In conclusion, radiation degraded chitosan is safe and effective for the use as an animal feed additive.

(3) Indonesia (Dr. Darmawan DARWIS, National Nuclear Energy Agency of Indonesia)

Oligochitosan produced by BATAN using gamma irradiation was applied on chili at Kerinci District, South Sumatra Province to overcome diseases due to virus. Chili (TM 99 chili variety) was planted in plot area of 1,400 m^2 consist of 1,950 plants. The results show that oligochitosan with average concentration of 50 ppm sprayed once a week effectively prevented the plant from severe Gemini virus attack whereas the chili plant was destroyed without oligochitosan. The harvesting times for one planting cycle

increased two times compared to that without oligochitosan. The overall production of chili for 25 times harvesting was 2,670 kg. Assuming that 1 kg of chili is US$ 2, the farmer can earn about US$ 5,340 with production cost only around US$ 850. Another experiment using YM chili variety planted in 4500 m^2 area (about 6000 chili plants) was done by different farmer. Considering that only 20 % of plants were recovered after virus attack, treatment with pesticides did not give significant impact. However, treatment with oligochitosan 50 ppm at once a week was effectively significant. It was found that chili with length of about 30 cm were produced, and the total production during the 3 times harvesting was 280 kg. On December 6-8, 2011 and May 24-26, 2012, two seminars on the Application of Radiation Technology for improvement of Agriculture sectors in Kerinci were done, attended by 11 participants, including farmers, staff of Agriculture Department District, and farmer supervisor. Oligochitosan was disseminated to the farmers association and local government. To continue and expand the application area of oligo-chitosan, the Memorandum of understanding (MoU) between BATAN and Local Government of Kerinci and also BATAN and Indonesian Plantation Research Agency has been signed.

(4) Japan (Dr. Naotsugu NAGASAWA, Japan Atomic Energy Agency)

Oligo-chitin derived from gamma-irradiated fine grinded crab shell is effective as a plant elicitor. The radiation-induced degradation behavior is different from the enzymatic hydrolysis from TLC result. The oligo-chitin significantly increased the plant elicitor in the rice, Kinmaze. Compared to enzymatically degraded oligo-chitosan, which is already well known to have plant elicitor activity, oligo-chitin obtained by ganma-irradiated at 1000 kGy has higher elicitor activity such as phytoalexin induction and defense protein production.

In collaboration research with FNCA biofertilizer project group (PL: Prof. Yokoyama; Tokyo University of Agriculture and Technology), the synergy effect of biofertilizer and oligochitosan in Japan has been studied. Oligo-chitosan has positive effects on developing nodule number in soybean pot experiments and plant pathogenic resistance to tomato seedlings treated with bio-pesticide strongly.

(5) Kazakhstan (Prof. Grigoriy A. Mun, Kazakh National University)

The grafting of 2-hydroxyethyl acrylate (HEA), 2-hydroxyethyl methacrylate (HEMA) and N-vinyl pirrolidone (VP) onto chitosan using γ-radiation and real initiation has been investigated. The general peculiarities and optimum conditions of the grafting have been studied. It was possible to control the extent of grafting by varying the reaction

conditions such as the initiator and monomer concentration, reaction temperature and time. Morphology and structure of the grafted materials has been studied using FTIR and SEM. The grafted copolymer samples are soluble in water and diluted acid solutions, describing an enhanced hydrophilic character as compared with control chitosan. It was shown the HEA-grafted chitosan product is soluble in a wide pH range, while the original unmodified chitosan is water soluble only in a narrow pH range.

The complexation of water-soluble and crosslinked chit-g-HEA with poly(acrylic acid) (PAA) was investigated by complex of physicochemical methods in solutions and on the division border hydrogel - solution. The formation of non-stoichiometric polycomplexes stabilized by electrostatic interactions has been found. It was established that the efficiency of complexation depends on polyacid molecular weight, pH and ionic strength of surrounding medium. It was established that the efficiency of interaction of new copolymers with PAA and their polycomplexes compositions significantly depend on content of nonionic HEA groups in polymer structure, polyacid molecular weight and pH. The stability of polycomplexes to low molecular salt additives is higher in comparison with polyelectrolyte complexes on the basis of chitosan and PAA.

Polymer hydrogels by using γ-radiation graft-polymerizaton of HEA, HEMA and VP onto chitosan have been obtained. It was found that polymer networks can swell or contract in solutions of anionic surfactants (DDS) and these effects depend on the concentration of surfactant. Presence of the complex bonded DDS is caused in great modification of copolymer thermo-sensitive properties.

(6) Malaysia (Dr. Kamaruddin BIN HASHIM, Malaysian Nuclear Agency)

Agarwood plant is being plant commercially due to demand of resin or oil extract from the plant that can be use as perfume which is very expensive. Field test study of oligochitosan on agarwood shown that improvement on the growth, based on data of diameter and height of the plant. The results reveal that 80ppm oligochitosan is the optimum concentration to apply on the plant for PGP. Apply 80ppm oligochitosan will double the diameter and increase 79% in height of the plant compare to control without oligochitosan after 21 month. 2 times spray per month oligochitosan 80ppm will increase 34% diameter and 25% height of the plant for the duration of 21 months. Promotion performed by Nuclear Malaysia and company Avid Focus Resources together with collaborator for the field test of oligochitosan, indicate that there is a potential of oligochitosan as plant growth promoter and elicitor.

(7) Philippines (Ms. Charito ARANILLA, Philippine Nuclear Research Institute)

Production of oligochitosan was done according to the FNCA guideline with slight modification. Preliminary field test on oligochitosan application (40 ppm) in combination with Bio N was conducted in collaboration with BIOTECH-UPLB (Ms. Anarna, PL for Biofertilizer Project)using sweet corn as crop. Six treatments were applied as follows: T1-Control (no fertilizer, no innoculant); T2-Full Recommended Rate Fertilizer (FRR); T3-Bio N Only; T4-FRR/Bio N; T5-FRR/Oligochitosan; and T6-Bio N/ Oligochitosan. Results show that yield of plots treated with T2, T4, T5 and T6 are statistically different to control and are higher by more than 30%. However, yield of plots treated with T5 (FRR + oligochitosan) and T6 (Bio N + oligochitosan) are not significantly different. Even so, application of Bio N/oligochitosan is a better alternative to chemical farming in view of economics, health, and environmental impact.

For oligocarrageenan, production was done using the synergistic effect of gamma radiation and H_2O_2. Initial small scale field experiments were conducted in the premises of PNRI. The first field test aimed to determine the effect of oligo-k-carrageenan (OkC) application on the yield and yield components of sweet corn. Treatments applied were T1 - Control (no fertilizer, no innoculant); T2-Full Recommended Rate Fertilizer (FRR); T3-FRR/100 ppm OkC SW; and T4-FRR/100 ppm OkC RF. Yield components did not vary statistically for all treatments. Only T4 influenced the yield significantly compared to control (T1). Yield of plots treated with T2, T3, T4 are not statistically different but T4 gave the highest yield among the treatments. Yield of plots treated with T4 are higher by 43% compared to control and by 11% compared with fertilizer treatment only (T2). The second field test aimed to determine the effect of application frequency on the yield and yield components of white corn. Treatments used were T1 - Control (no fertilizer, no innoculant); T2-Full Recommended Rate Fertilizer (FRR); T3-FRR/100 ppm OkC (3x application); and T4-FRR/100 ppm OkC (5x application). Yield components of plots treated with oligo-kC are significantly different to T1 and T2. Total yield for all treatments are not significantly different, but highest percentage was obtained in plots treated with T4. Marketable yield for treatments T2, T3, and T4 are not significantly different, but highest was obtained in plots treated with T4. Unmarketable yield for all treatments are not significantly different but lowest percentage was obtained in plots treated with T4. Influence of number of spray (T3 and T4) on the yield and yield components are not significantly different but T4 gave higher percentage in marketable yield (83%) and lower percentage in unmarketable yield (17%).

(8) Thailand (Dr. Phiriyatorn SUWANMALA, Thailand Institute of Nuclear Technology)

Chitin was prepared from local shrimp shells. The prepared chitin was changed into chitosan, by chemical reactions. Radiation-induced degradation was used to reduce the molecular weight of the prepared chitosan, yielding oligochitosan. The obtained oligochitosan was tested for its potential use as plant growth promoter. Effects of oligochitosan on growth and productivity of Thai chili plants were investigated. The experiment was carried out with randomized complete block design (RCBD) with ten replications. The foliar spraying of oligochitosan (molecular weight ~ 15,000 Da) with the concentration of 20, 30, 40 and 80 ppm mixed with fertilizer was applied. The growth and productivity of these oligochitosan-treated chili plants were compared with those of untreated chili plants. The effects of oligochitosan on Thai chili's growth and productivity were investigated in term of plant height, total number of chilies, total weight of chili, total number of green chilies, total number of red chilies, harvest time and weight per chili. The results showed that the application of oligochitosan, at the concentration of 80 ppm, mixed with the fertilizer displayed significant effects, statistically, on chili height, total weight of chili, total number of chilies, total number of green chilies, total number of red chilies and weight per chili. The results showed that productivity was increased up to 34%. The oligochitosan exhibited the ability to protect not only aphid infection but also the ability to shorten the harvest time of chili plants. The treatment of chili plants by oligochitosan clearly displayed positive effects on chili's growth and productivity. These results suggest its potential use in agriculture purposes as growth promoter for Thai chili plants. The field tests were done further with mango plum and the results showed noticeable effects on yield and sweetness.

(9) Vietnam (Dr. Nguyen Quoc HIEN, Vietnam Atomic Energy Institute)

Oligochitosan plant growth promoter/elicitor has been applied for production of breeding rice seeds in Mekong delta rice growing region. Result indicated that the rice yield increased to about 20% and quality of rice seeds is good. Leaflet of "direction of use" of oligochitosan for rice has been prepared. Degradation of chitosan in swelling state with 5% H_2O_2 solution by gamma Co-60 irradiation has been systematically studied. Results showed that irradiation of mixture of 1 gam chitosan/5 ml H_2O_2 solution at 5kGy can be applied to prepare chitosan with low molecular weight (Mw ~ 20,000-30,000). The biological effect low molecular weight chitosan and oligochitosan for chicken and fish has been carrying out. In addition, preparation of oligoglucan from the waste of mushroom and beer industry has been started.

(10) Myanmar (Mr. Myo Min Thant, Department of Atomic Energy, Ministry of Science and Technology)

In future, Myanmar has a plant to investigate the gamma radiation effect on seaweed plant growth promoter for Chick-pea. A method for promoting plant growth consists of exposing an aqueous solution of sodium alginate (oceanic natural resource) or a powder of sodium alginate to 10-100 kGy of gamma rays and diluting this product in water. By this process, nature care balances the pH of land by reducing the acidic part due to which (1) increases land fertility and (2) provides carbon and protein to the soil.

(11) Pakistan (Mr. Muhammad SHAFIQ, Pakistan Institute of Engineering & Applied Sciences)

In Pakistan, there is an abundance of crab shell, shrimp shell, starch and rice husk. These raw materials are aimed to be potentially employed in agriculture sector. We have explored the application of radiation processed CS as a growth elicitor and shelf life promoter in flowers and fruits. In Zinnia flowers, varying amounts (0-300 ppm) of gibberalic acid, irradiated CS and seaweed were used to investigate their effect on the nutrition uptake, plant growth and shelf life. The results have revealed significant improvement in the afore-mentioned parameters with radiation degraded CS. The number of leaves, buds, flowers and stalk length was increased remarkably using 100 ppm CS, whereas, vase life and respiration showed significant improvement at 200 ppm CS. Phosphorous level increased in leaves sprayed with 300 ppm CS. Likewise the combination of either low molecular weight CS (LMWC) or medium molecular weight CS with oleic acid (OA) was used in orange fruits (succari) to improve its pre- and post-harvest quality. The fruit treated with 1% LMWC and 4% OA showed best response in improving the post-harvest quality of orange fruits throughout the storage period with minimum weight loss, least decline in firmness, minimum increase in soluble solid contents, least decline in titratable acidity and sugar content even after 12 weeks of storage than the other treatments and proved to be best coating material. The field tests are being carried out and the products have been planned for commercialization.

(12) Sri Lanka (Ms.Samantha Samalatha KULATUNGE, Atomic Energy Authority)

Sri Lanka conducted research work by the National Research team comprising of Atomic Energy Authority, Horticultural Research and Development Institute, Rice Research & Development Center, Regional Rice Research Institute and Industrial Technology Institute was established. to develop a plant growth promoter/elicitor(oligomer), fungicide using chitosan which is an extract of shrimp shell

and to carryout field test trials. The field test trials were conducted on horticultural crops (tomatoes, okra) and rice at the three institutions under Department of Agriculture. The overall objective of the above studies is to control major disease & pest of horticultural crops, rice and also to improve the yield by using environmental friendly plant growth promoters/elicitors, fungicides.

•Chitosan fungicide and oligomer were developed by the above team.

•Developed chitosan fungicide was tested for different pathogens responsible for diseases in different crops and fruit and find out the optimum concentration of chitosan fungicide that can be fully controlled the respective pathogens in-vitro.

•Field test trails on disease control yield and fruit quality of tomato, and rice was conducted with irradiated Chitosan. It was observed that the fruit yield has been increased by 25% with chitosan application on Tomato cultivation. It does not improve the post-harvest quality parameters of tomato fruit. Disease severity of Leaf Blight of Tomato plant has also been reduced by application of chitosan. Farmer field trials were conducted for tomato using the developed chitosan product.

•Farmer field test trials have been conducted to evaluate the impact of chitosan on growth & yield of rice are being under evaluation stage.

•Sri Lanka has signed an MOU with the Dragon Fruit Growers association for large scale application of the developed chitosan fungicide to control the diseases which cannot be controlled by the available pesticides in the market in Sri Lanka.

•Sri Lanka is in the process of applying product registration for the developed products of chitosan fungicide and oligomer.