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FNCA2021 放射線加工・高分子改質プロジェクトワークショップ
バイオ肥料分野および高分子改質分野におけるサマリーレポート

Zoom Web Meeting

2021 年 11 月 29 日 - 30 日

Session 3 and 4: Progress Report on Biofertilizer

Subject: 1. Degraded Chitosan for Animal Feed, 2. Hydrogel for Medical Application, 3. Environmental Remediation, 4. Synergistic Effect among Plant Growth Promoters, Super Water Absorbents and Biofertilizer, 5. Plant Growth Promoters and Super Water Absorbents, inclusive Process development, 6. Mutation Breeding of Biofertilizer Microbe using radiation, and 7. Sterilization of Biofertilizer Carrier using radiation

1) Bangladesh (Dr Md. Kamruzzaman Pramanik, Bangladesh Atomic Energy Commission)

Subject: 4. Synergistic Effect among Plant Growth Promoters, Super Water Absorbents and Biofertilizer

Short summary

i) A semi-field level experiment was carried out to study combined effect of chitosan as PGP and *Azospirillum* species as biofertilizer on rice plant (Rice variety, BRRI-129) with six different treatments. Results showed that grain yield of rice (t/ha) was increased up to 1.77% and 4.45% in T_4-treatment (T_4: 50% chemical fertilizer +100ppm chitosan+ biofertilizer) in the experiment of the year 2018 and 2019, respectively.

ii) To screen potential nitrogen fixing symbiotic bacteria for soybean, we isolate bacteria from root nodule of *Glycine max* which can be used as biofertilizer in combination with PGP like oligo-chitosan. Twenty Isolates were classified into 9 different groups on the basis of their cultural, morphological and biochemical characteristics which were further subjected to molecular identification by 16S rDNA. Isolates were identified up to 99.72% similarity upon BLAST analysis.

Results for 2018-2021

i) In **2018**, an experiment was carried out to study combined effect of chitosan as PGP and *Azospirillum* species as biofertilizer on rice plant (Rice variety, BRRI-129) at the semi-field level with six different treatments with triplicates viz, T_1: 100% chemical fertilizer, T_2: 40% chemical fertilizer, T_3: 40% chemical fertilizer +100ppm chitosan, T_4: 40% chemical fertilizer +100ppm chitosan+ biofertilizer, T_5: 40% chemical fertilizer + biofertilizer and T_6: Control (native nutrient). Results showed that grain yield of rice (t/ha) was increased up to 1.77% in T_4-treatment (T_4: 50% chemical fertilizer +100ppm chitosan+ biofertilizer). In **2019**, the same experiment was carried out like 2018. Results showed that grain size was increased up to 3.07% in the T_4-treatment as measured by 1000-grain weight but not at a significant level. Grain yield of rice (t/ha) was increased up to 4.45% in T_4-treatment in comparison to control but not as synergistically. In **2020**, an experiment was set up and initiated at the field level in Rangpur district. Replica plots were used for each type of treatment. But the experiment was initiated and implemented up to seedling stage and then postponed due to the COVID-19 situation.

ii) In 2021, the COVID-19 pandemic situation aggravated a few times than 2020 in Bangladesh and most of the months of this year our organization was shut down and it was not possible to carry out any experiment at that time. We only could conduct some experiments in our lab when Govt. permitted to run R&D works. However, results of the research works done this year are summarized below-

Root nodule bacteria have many potentials such as nitrogen fixation, phytohormone production etc. Hence, this study was carried out to screen potential nitrogen fixing bacteria from root nodule of *Glycine max* which can be used as biofertilizer in combination with other growth factor like oligo-chitosan. Twenty isolates were isolated following standard procedures and were subjected to a number of cultural, morphological and biochemical tests. Stress tolerance tests were also carried out allowing isolates to grow at different temperatures (35°C, 40°C and 45°C) and salt concentrations (1%, 2%, 3% and 4% NaCl). Isolates were classified into 9 different groups on the basis of their colony characteristics. From each group16S rDNA of one isolate was sequenced for the purpose of molecular identification. Analysis of 16S rDNA sequence by using Basic Local Alignment Search Tool (BLAST) revealed that group 1 showed 97.49% similarity with *Bacillus megaterium* strain ML065-1, group 3 showed 98.66% similarity with *Bacillus aryabhattai* strain QH16-25, group 4 showed 99.27% similarity with *Acinetobacter variabilis* strain FDAARGOS, group 5 showed 97.95% similarity with *Bacillus ginsengisoli* strain J8M8LARS, group 6 showed 98.99% similarity with *Acinetobacter* sp. strain ESA 658, group 7 showed similarity with *Microbacterium chocolatum* strain Atecer1I1, group 8 showed 99.72% similarity with *Microbacterium* sp. ST2 16S, group 9 showed 86.14%similarity with *Bacillus aerophilus* strain 0204. In the future, isolates will be tested for their efficacy in nitrogen fixation and the most potential strain will be used for synergy experiment.

Future plan for 2022-2024

The combined effect of chitosan and *symbiotic* biofertilizer on soybean with respect to nodulation, dry matter production and yield of soybean (variety BARI Soybean-6) will be studied in laboratory scale in 2022. If a promising result is found, the same experiment will be carried out in semi-field level experiment in 2023. Finally, we will go for field level experiment to assess the synergy-phenomena in 2024.

2) China (Prof Zhang Ruifu, Chinese Academy of Agricultural Sciences)

Subject: 6. Mutation Breeding of BF Microbe using radiation

Short summary

Screened three high efficient phosphate solubilizing microorganisms (PSB) strains, valued in pot experiments (2018), applied in winter wheat in field (2019). Irradiation breeding of a biofertilizer strain of *Trichoderma guizhouenase NJAU4742* by X-ray to get improved mutants for stress tolerance, plant growth promotion and fungal pathogens suppression (2020), obtained the mutation sites by resequencing the mutants and cloned the acid-resistant gene (2021). For the next phase, I plan to do the synergy of Super Water Absorbents (SWA) and biofertilizer in rhizosphere.

Results for 2018-2021

Three high efficient PBS strains were screened and characterized, pot experiments showed they can promote the maize growth effectively. Then one of the Bacillus PBS Bacillus megaterium X-14 was selected for large scale industrial production of PBS biofertilizer, and tested in the field experiment of winter wheat, which demonstrate increased yield. To get enhanced *Trichoderma guizhouenase* 4742 strains for different characters: Stress tolerance and antagonism, breeding of this strain with X-ray radiation was carried out, Irradiation dose was: 84Gy, 84Gy and 82Gy for three times, and the total was 250Gy, After irradiation, the spores of *Trichoderma guizhouenase* NJAU4742 was coated on different screening media. About 3500 different mutants were isolated and screened, after irradiation, the salt tolerance of different mutants was significantly improved, especially for the mutants of M4 and M5. For acid tolerance, some mutants which can tolerance the low pH of 2.5 were obtained. For fungal pathogen antagonist, mutants with improved biocontrol ability for *Fusarium moniliforme*, *Fusarium oxysporum*, *Phytophthora capsici* and *Rhizoctonia sclerotiorum* were obtained. Sequenced the mutants, obtained the mutation sites cloned the acid-resistant gene.

Future plan for 2022-2024

(1) Solid fermentation of these breeding strains of *Trichoderma guizhouenase* NJAU4742;

(2) Commercialization of these breeding strains of *Trichoderma guizhouenase* NJAU4742 in agricultural production.

(3) Synergistic Effect of Super Water Absorbents and Biofertilizer

3) Indonesia (Dr Tita Puspitasari, Nuclear Energy Research Organization)

Subject: 3. Environmental Remediation, 4. Synergistic Effect among Plant Growth Promoters, Super Water Absorbents and Biofertilizer, 6. Mutation Breeding of BF Microbe using radiation, 7. Sterilization of BF Carrier using radiation

Short summary

The application of biofertilizer (BF) for environmental bioremediation has been carried out in several places in Indonesia. Due to differences in conditions or initial land status, spatial planning, environmental resources and target vegetation types, several types of functional microorganism inoculants have been developed in BATAN such as IMR, Compostar, Trichomix, Deligno, HCDec, IPLB. The results of this study are expected to be an alternative solution in supporting the development of food safety and security through community empowerment-based land bioremediation. The research of mutation breeding of BF using radiation aimed to study the stability of the characteristics of mutants to dissolve P and K which were less available after storage for more than one year. The result shows that the mutants of BPK5 which irradiated at 7.5 kGy and FPF4 which irradiated at 1.0 kGy have the highest ability to solubilize phosphate and potassium in liquid medium. Regarding the synergistic effect between BF and oligochitosan, the result shows that the highest nutritional status by B. circulans and T. harzianum increased 90 times and 2.8 times higher for NH4+ accumulation, 1.9 times and 2.4 times higher for phosphate solubilization compared to the application without oligochitosan substrate. The data shows that the synergistic effect of using oligochitosan biocontrol and biological fertilizers clearly has a positive synergistic effect.

Results for 2018-2021

The main challenges in Indonesia in terms of agricultural productivity is the low availability of P and K in the tropics (Indonesia); the soil has a low pH which is around 4-4.5; The P fertilizer and K fertilizer are mostly imported and the P uptake efficiency is low. Biofertilizers, organic fertilizers and bio-organic fertilizers are increasingly popular for stabilizing soils that are severely damaged by chemical fertilizers. Biofertilizers consisting of microbes, produce organic acids, vitamins, growth factors, and increase solubility, stimulate plant growth, and increase yields. Biofertilizers usually consist of multistrains that can act as N fixers, P solubilizers , K solubilizers, antagonists, PGPR, and hormone producers. While the antagonists only consisted of a single strain with multifunctionality such as P and K solubilizers. National Nuclear Energy Agency (BATAN) Indonesia, currently named Research Organization for Nuclear Energy has successfully prepared biofertilizer (BF) for environmental bioremediation; carried out of mutation breeding of BF microbe using radiation, sterilization of BF carrier using radiation, and also conducted the research of synergistic effect between plant growth promoters and biofertilizer.

In term of environmental bioremediation, the application of 2 isolates of the rhizosphere bacteria *Sonneratia alba* (SB33, and SB36) showed the potential as N fixers and P-solubilizing in high salinity conditions of the mangroves of the Hurun Bay in Lampung. Furthermore, the Fungi *Trichoderma reesei* exposed to gamma rays at a dose of 750 Gray had a specific cellulase activity 1.5 times higher than that of the control (wildtype). Fungi *P.chrysosporium* exposed to gamma rays at a dose of 600 Gray have high lignin peroxidase (LiP) activity and can adapt well in a liquid medium containing Pb and Cd. The LiP activity in 1% alkaline lignin medium was about 2.34 times from 1291 to 3025 U/ml and in medium 5% Gmelina wood (*Gmelina arberoa* Roxb.) substrate about 2.07 times from 1089 to 2258 U/ml. The initial treatment of 1% NaOH solution and solid fermentation with the fungi *P.chrysosporium* 600 Gray can optimize the reduction in lignin content and produce a substrate with high levels of cellulose and microbial biomass so that it can optimize the ability to increase Pb uptake around 31.94% from 10.41 to 13.74 mg/g and Cd uptake increased by about 32.62% from 0.86 to 1.15 mg/g. The results of this study are expected to be an alternative solution in supporting the development of food security and security through community empowerment-based land bioremediation. The research of mutation breeding of BF using radiation also aimed to study the stability of the characteristics of mutants to dissolve P and K which were less available after storage for more than one year. The result shows that the mutants of BPK5 which irradiated at 7.5 kGy have the highest ability to solubilize phosphate and potassium in liquid medium, with the values of 165.67 ppm and 18.89 ppm consecutively, at pH of 4.8 and 4.5 respectively. Whereas the mutant of FPF4 which irradiated at 1.0 kGy showed the highest ability to solubilize phosphate and potassium in liquid medium, with the values of 379.57 ppm and 11.54 ppm consecutively, at pH of 4.2 and 2.81. The stability of bacterial BPK5 mutants to solubilize the insoluble phosphate and insoluble potassium after 12 months storage at room temperature was achieved by mutant 7.5. Due to differences in conditions or initial land status, spatial planning, environmental resources and target vegetation types, several types of functional microorganism inoculants have been developed such as IMR, Compostar, Trichomix, Deligno, HCDec, IPLB.

The use of biological agents as fertilizers and biocontrols has a great influence on environmental sustainability. The use of these two applications is carried out separately so that there is a need for application development to obtain a formulation for plants with dual effects, namely as Plant Growth Promoter (PGP) and environmentally friendly biocontrol. This research was conducted to evaluate the synergistic effect of using rhizosphere microorganisms (biological fertilizers) and chitin-based substrates as plant growth enhancers and soil phytopathogen controllers. The research was conducted by formulating a biofertilizer consisting of B. circulans and T. harzianum which were each grown in a chitin-based substrate consisting of colloidal chitin, colloidal chitosan and colloidal oligochitosan. The phytopathogen used was Fussarium spp. with the test plant of cayenne pepper, coal variety. Evaluation of the synergistic effect between biofertilizers and chitin-based substrates showed that oligochitosan substrate had a positive effect on plant growth and resistance to pathogens. The nutritional status of the plant was evaluated as accumulation of NH_4^+ and dissolved phosphate, while the pathogen control effect was evaluated as specific chitinase activity. The highest nutritional status by B. circulans and T. harzianum increased 90 times and 2.8 times higher for NH_4^+ accumulation, 1.9 times and 2.4 times higher for phosphate solubilization compared to the application without oligochitosan substrate. Chitinase activity by B. circulans in oligochitosan substrate increased 7.4 times while T. harzianum was 5.9 times higher than without oligochitosan substrate. The data shows that the synergistic effect of using oligochitosan biocontrol and biological fertilizers clearly has a positive synergistic effect.

Future plan for 2022-2024

Due to the reorganization of several research centers in Indonesia including BATAN to the National Research and Innovation Agency. The continuity of the research of Biofertilizer will depend on the support of the agency.

4) Malaysia (Dr Phua Choo Kwai Hoe, Malaysian Nuclear Agency)

Subject: 3. Environmental Remediation, 4. Synergistic Effect among Plant Growth Promoters, Super Water Absorbents and Biofertilizer, 6. Mutation Breeding of BF Microbe using radiation, 7. Sterilization of BF Carrier using radiation

Short summary

Malaysia's biofertilizer market is currently focusing on liquid multifunctional biofertilizer products. Malaysia focuses on carrier sterilization, commercialization of biofertilizer, synergistic effects of biofertilizer with oligochitosan, mutagenesis of biofertilizer microorganisms by using gamma irradiation, seed treatment biofertilizers, biofertilizer toxicity test, and bioremediation.

Results for 2018-2021

Malaysia successfully commercialized four biofertilizer products (Bioliquifert, GoGrow BioNPK Biofertilizer, M99 Biofertilizer, and Bioliquifert M100). A total of 770,000 L Bioliquifert were distributed to paddy growers in West Malaysia from year 2020 to 2021. Total sales were RM 13,090,000.00. Synergy effects of biofertilizer with oligochitosan had been tested on chili fertigation and maize (Positive synergy effects), rice and strawberries farm (Negative synergy effects). Mutation Breeding of BF Microbe using radiation had isolated of full pqq gene dan development biomarker of

M100 in progress. Optimization doses of gamma irradiation on new isolates (Gram positive and negative isolates) will carry out. Seed treatment test on optimisation and shelf life in progress. Biofertilizer toxicity test with zebra fish and seed model showed biofertilizers are safe to use. Carrier sterilizations were developing new carrier. Digestate was produced by fermenting organic waste (eg. food waste) anaerobically in a biodigester. Sterilization by using gamma irradiation will carry out. Soil sampling of bioremediations in progress. Bioremediations chemical profiling and isolation bioremediations will carry out. One trademark and three copyrights had been registered. Seventeen publications and eight awards were obtained. In conclusion, biofertilizer project were successfully delivered R&D products into market. New R&D such as mutagenesis, bioremediation and carrier developments are in progress.

Future plan for 2022-2024

Current biofertilizer projects (commercialization, mutagenesis and seed treatment carrier sterilization and bioremediation) will be continued.

5) Mongolia (Ms Sunjidmaa Otgonbayar, Institute of Plant and Agricultural Sciences)

Subject: 4. Synergistic Effect among Plant Growth Promoters, Super Water Absorbents and Biofertilizer

Short summary

Since 2001, in our laboratory produce Rhizobium and Rhizobacterial fertilizer. Showed the application Rhizobium bio-fertilizer increases crop yield by 21.7-65% and the number of Rhizobium nodules on crop roots by 2 times.

The liquid Rhizobacteria fertilizer increases yield of wheat grain by 25 -33%, seed potato yield by 42.3-45.6% and yield of vegetable crops /carrot, onion, tomato, sweet pepper et.c/ by 22-50 %.

Results for 2018-2021

In 2018, showed the application Rhizobium biofertilizer increases soybean yield by 5.6 centner/ha more than control.

An experiment to evaluate the effects of multifunctional biofertilizer, irradiated oligochitosan, the synergistic effect of both plant promoters on the growth of sweet pepper and tomato plants was conducted in the greenhouse.

In 2019-2020, showed synergy effect of liquid Rhizobacterial fertilizer and Oligochitosan increase crops yield by 50-80%. Biofertilizer and Oligochitosan are significantly effective for plant growth promotion.

In 2021, evaluated the effects of multifunctional biofertilizer, irradiated oligochitosan, the synergistic effect of both plant promoters on the early maturity potato.

Synergy effect of liquid Rhizobacterial fertilizer and Oligochitosan increase potato yield by 46-50%

Future plan for 2022-2024

Greenhouse gas (CO_2) has emitted significantly by fallow affection in Agricultural field. In crop rotation, planting green manure or legumes may affect nitrogen fixation and absorb C in soil by the effect of photosynthesis instead of fallow. Planting legumes requires rhizobium bacterial fertilizer because of Mongolian low nutrient soil. Since 2006, we have been cultivating and storing legumes host plant's rhizobium bacteria in our laboratory (Soybean, peas, alfalfa, etc.). We may utilize these rhizobium

bacteria in further studies.

6) The Philippines (Ms Julieta A. Anarna, University of the Philippines Los Banos)

Subject: 4. Synergistic Effect among Plant Growth Promoters, Super Water Absorbents and Biofertilizer

Short summary

The need to improve plant growth and thereby increase the yield, need to address the nutrients needed by the plants with the objective of reducing chemical usage. Among the major nutrient needed by the plants are nitrogen (N), phosphorous (P) and potassium (K). The institute has produced wide arrays of microbial inoculant products from *Azospirillium*, *Mycorrhiza*, *Rhizobium*, PGPR and *Trichoderma* species. The combined application of biofertilizers, PGP, and carrageenan will supply nutrient requirements of plants for sustaining desired crop productivity. This year 2021 interaction effects among BIOTECH different commercially and proven technologies was conducted to determine the effect of mixed inoculation as compared with chemical fertilizer using corn, tomato and eggplant at the test crops. Economic analysis among treatments was also recorded.

Results for 2018-2021

(2018) Research on the combined inoculation of microbial inoculant was conducted to determine the efficacy of combined inoculation of Bio N and Mykovam, Bio N + Oligochitosan, and Bio N + Carrageenan. Results of these studies showed that oligochitosan and biofertilizer have synergy effect on yield of rice and corn under field condition and it can be recommended for agricultural practices to achieve sustainable agriculture.

(2019) In 2019 three experiments were conducted using different corn varieties as the test crops to compare the effect of Bio N (brand name for nitrogen fixing bacteria *Azospirillum*) in combination with other biofertilizers, chemical fertilizers and carrageenan. Plants treated with Bio N whether in combination of 100% or 50% chemical fertilizers increased the mean weight of corn. It was also observed from the plants treated with ½ chemical fertilizer combined with Bio N and Mykovam were comparatively similar to the fully fertilized plot.

(2020) Two experiments were conducted first, the effect of combined inoculation of Bio N and Vamri on the dry weight of grain of rainfed rice and second Combined inoculation of Bio N and Mykovam with chemical fertilizer using corn as the test crops. The heaviest weight was observed from plants with full amount of chemical fertilizer followed with 50% chemical Bio N.The results of the second experiment showed that the mean weight of corn from the plants treated with ½ chemical fertilizer combined with Bio N and Mykovam were comparatively similar to the fully fertilized plot.

(2021) Production and Utilization of Bio-organic Fertilizers Enhanced with Bio fertilizers (*Bio N* and Mykovam) for Organically Grown Crops (on going). Formulation of the enhanced bio-organic fertilizer for tomato and eggplant was conducted and pilot testing is on -going in different sites. Initial evaluation for tomato was recorded. Based on the data gathered the enhanced bio-organic fertilizer composed of Bio N and Mykovam contributed to the increase in yield of the test crop. The data also revealed that farmers can harvest enough even without application of chemical fertilizer with the right amount of bio-

organic fertilizers.

Project 2. Impact Assessment of *Bio N* Technology Utilization.

The results obtained from different studies showed a positive effect when Bio N was combined either with VAMRI or Mykovam. The biofertilizer used is matured technologies that had been evaluated and had proven the effect/benefits on agricultural crops.

Future plan for 2022-2024

1. Capacity building
2. Improvement of Bio N technology (liquid form, multistrain product)
3. Conduct research on gamma irradiation of Bio N carrier for lower dosage rate
4. Enhancement technology transfer, marketing and commercialization
5. Conduct more research on biofertilizers and carrageenan application in the field

7) Thailand (Dr Kunlayakorn Prongjunthuek, Department of Agriculture)

Subject: 7. Sterilization of BF Carrier using radiation

Short summary

Sterilization of carrier as autoclave and γ-irradiation used in the production of PGPR biofertilizer should be studied and developed methods of sterilization suitable for other genus. Meanwhile, should be studied carrier and sterilization method for liquid biofertilizer. Then transfer technology to private company to help distributes products thoroughly and have sufficient quantities to meet the needs of farmers.

Results for 2018-2021

The experiment in development of biofertilizer, sterilization of the carrier (autoclaved and γ-irradiation) used in the production of PGPR-I influence of growth and survival of all three genera. It should be studied and developed methods of sterilization suitable for all three genus and other. For research on the development of liquid PGPR bio-fertilizer, all strains had low number of viable cells after storage at room temperature but storage at 25^{o}C contain only *Azospirillum brasilense* TS29 and *Gluconacetobacter diazotrophicus* BR11281 that had the number of viable cells in accordance of quality set by the Fertilizer Act.

The experiment in climate change project, we get new isolates of PGPRs isolated from areas affected by climate change in Pai river basin for develop new biofertilizer that can be used in all areas. Now, we continue to conduct in the same area and expanded to neighboring areas.

The experiment in sweet corn project, it was concluded in the development of PGPR biofertilizer for maize that the TS13 isolates (original) with the LB1-3 isolated from Hi-brix 3 had no difference in the growth and yield of sweet corn.

After expand PGPR bio-fertilizer production to the region. In 2020, DOA start to transfer PGPR biofertilizer technology to private company and in 2021, DOA can transfer all 3 types of PGPR biofertilizer production technology to 5 private companies and currently have 3 companies registered for commercial biofertilizers for rice.

Future plan for 2022-2024

Conduct product testing in farmer plots to improve, issue recommendations on the use of biofertilizers in the production of crops. Meanwhile, testing of product manufacturing and performance testing in laboratories and greenhouse. Furthermore, develop of new PGPR biofertilizer for rice with Rice Department and study of biology and ecology of soil microorganisms in rice production systems and implementation. In addition, continuing the transfer of all 3 types of PGPR biofertilizer production technology to private companies.

8) Vietnam (Dr Tran Minh Quynh, Vietnam Atomic Energy Institute)

Subject: 6. Mutation Breeding of BF Microbe using radiation, 7. Sterilization of BF Carrier using radiation

Short summary

Wide-area field trials to evaluate the effectiveness of bio-fertilizer (RAPOL-V) on the growth and development of vegetable (complete);

Screening the high cellulase producing Trichoderma mutants for preparation the rice straw degradation products (in progress)

Results for 2018-2021

During 2018-2021, a bead based biofertilizer (Rapol-V) was designed and applied for plant growth promotion, and their efficiencies on the growth of vegetable crops including tomato, cabbage and radish were investigated. The results showed that the combination of these BF with NPK as local recommendation much increased the crop production. Moreover, the use of Rapol V biofertilizer can also reduce at least 20% NPK without any negative impacts to the yield and quality of vegetable.

Gamma irradiation was applied to *Aspergillus* sp. and *Trichoderma* sp. for inducing radiation resistant mutants. The highest mutation rates were recorded with the cultures irradiation at dose range of 700-1500 Gy. After screening, two high cellulase producing mutants (VTCC(k) I-1 and VTCC(r) I-1) were used to prepare rice straw decomposition product IRTr, and its rice straw degradation capacity was investigated in pilot scale. Preliminary results revealed a higher decomposition rate of this product compared to some existing products in local market.

Future plan for 2022-2024

1. Wide area trials to evaluate the long-term effects of Rapol V biofertilizer on other crops (green tea, herbs, and fruit plants)
2. Investigate the effects of BF to some crops fertilized with lower NPK to mitigate the pollution caused by chemical fertilizers.
3. Set up a procedure to preparation the rice straw decomposition products from radiation induced *Trichoderma* mutants
4. Field experiments for evaluating the efficiency and applicability of the obtaining rice straw decomposition products

Session 5 and 7: Progress Report on Polymer Modification

1) Bangladesh (Dr Salma Sultana, Bangladesh Atomic Energy Commission)

Subject: 5. Plant Growth Promoter

Short summary

The plan which we were supposed to accomplish in 2021 is now also postponed to the COVID-19 pandemic situation. For COVID-19, the last week of March to July, 2021 there was a lockdown in our country. Lockdown period, we couldn't attend our office and that's why our all research activities were postponed.

Results for 2018-2021

Foliar application of oligo-chitosan increased the growth and yield of crops (rice 7-10%, mung-bean 30%, maize 52%), fruits (capsicum 68%, strawberry 54%) and vegetables (egg-plant 48%, tomato 41%) over control. The oligo-chitosan also shows the ability to shorten the harvest time in contrast to control and it can also display antibiotic activity against microorganisms including bacteria and fungi on tomato and egg-plants.

Achievements:

1. Rayhanur Jannat, Milon Shaha, M. Tanbir Rubayet & **Salma Sultana**, Role of Chitosan in Induction of Defense Response against Phomopsis vexans and Augmentation of Growth and Yield of Eggplant, Global Journal of Science Frontier Research: C Biological Science, Volume 18 Issue 3 Version 1.0, p. 6-16, 2018.
2. Nusrat Jahan Nitu, Md. Mahidul Islam Masum, Rayhanur Jannat, **Salma Sultana,** Md. Khurshed Alam Bhuiyan, Application of chitosan and *Trichoderma* against soil-borne pathogens and their effect on yield of tomato, International Journal of Biosciences (IJB), Vol.9 No. 1, p. 10-24, 2016.
3. **Salma Sultana,** Mahfuza Islam, Mst Afifa Khatun, Md Afzal Hassain and Roksana Huque, Effect of Foliar Application of Oligo-chitosan on Growth, Yield and Quality of Tomato and Eggplant, *Asian Journal of Agricultural Research* ISSN 1819-1894, Vol. 11(2), p.36-42, 2017.

Future plan for 2022

- We have already discussed with our higher authority and collaborator Bangladesh Agricultural Research Institute (BARI) regarding Field-level trial.
- We have been able to extend our collaboration with BINA as part of us to commercialize our PGP. From Bangladesh Institute of Nuclear Agriculture (BINA) it would be very much easy for us to reach the farmers.
- We have been trying on building up awareness about using the environment friendly agricultural practice

Future plan for 2022-2024

(1) Degraded chitosan for Animal Feed

Conventional poultry feed has detrimental elements such as chromium which causes cancer in human bodies. In this regard we have to think about alternative so we have chosen bio-feed product like degraded chitosan. As animal we have selected chickens to explore this research.

(2) Environmental Remediation

Arsenic is a heavy metal which is responsible to cause arsenicosis in the human body. Arsenic has been found beyond the permissible limit in drinking waters of southern part of Bangladesh. Moreover, Saline water is one of the major problems for the people living in the southern part of Bangladesh. To counter these existing problems we will try to explore the efficacy of hydrogel to get a better remedy.

2) China (Prof Ma Hongjuan, Shanghai University)

Subject: 3. Environmental Remediation

Short summary

Functional adsorbents were prepared and applied to extract metal ions such as uranium et al... Cotton fabric for highly efficient and stable solar-driven interfacial evaporation was prepared. Waste water treatment using EB irradiation entered the stage of large-scale commercial application. Super Water Adsorbent were prepared and used for desertification control in west China. Novel Coronavirus irradiation inactivation test moves to pilot stage.

Results for 2018-2021

1. Several types of novel fibrous adsorbents totaling 30 kg were synthesized for uranium extraction from seawater. More than 100 g uranium was extracted from seawater. (Shanghai University, University of Science and Technology of China, Shanghai Institute of Applied Physics)
2. Cotton fabric for highly efficient and stable solar-driven interfacial evaporation was prepared with radiation technology. (Shanghai University, Shanghai Institute of Applied Physics)
3. Waste water treatment using EB irradiation entered the stage of large-scale commercial application. The project realized the online operation of 7 electronic accelerators, with a daily wastewater treatment capacity of 30,000 tons. (CGNPC, China Guangdong Nuclear Power Company & Tsinghua University)
4. Cooperate with many companies on pilot scale up of adsorption materials prepared with irradiation induced graft polymerization.
5. Super Water Adsorbent were prepared with radiation technology and used for desertification control in west China. (Shanghai Institute of Applied Physics)
6. Novel Coronavirus irradiation inactivation test move to pilot stage, radiation technology is expected to be used in cold chain Novel Coronavirus eradication. (CNNC, China National Nuclear Corporation, CGNPC, China Guangdong Nuclear Power Company)

Future plan for 2022-2024

1. Novel fibrous and membrane adsorbents will be synthesized for the uranium extraction from seawater. 1 kg uranium will be expected to be extracted from seawater. (Shanghai University, University of Science and Technology of China, Shanghai Institute of Applied Physics)
2. Pilot scale cotton fabric for solar-driven interfacial evaporation will be prepared with radiation technology.(Shanghai University)
3. Cooperate with companies on pilot scale up of adsorption materials prepared with irradiation induced graft polymerization. (Shanghai University)

3) Indonesia (Dr Tita Puspitasari, Nuclear Energy Research Organization)

Subject: 1. Degraded Chitosan for Animal Feed, 4. Synergistic Effect among Plant Growth Promoters, Super Water Absorbents and Biofertilizer, 5. Plant Growth Promoters and Super Water Absorbents, inclusive Process development

Short summary

The application of oligochitosan as for PGP was conducted in pepper and chrysanthemum plant. The result shows that oligochitosan can increase the pepper plant by increasing the circumference of primary branch (CPB), number of primary branch (NPB), length of primary branch (LPB) and chlorophyl index. Furthermore, the chrysanthemum (PN variety) treated with oligochitosan have shorter harvesting time compared to the chrysanthemum treated with commercial PGP. The application of oligochitosan as animal feed additive has been implemented for Indonesian poultry which are local duck namely Cihateup duck, and local hen variety namely Sentul hen. The result showed that the administration of oligochitosan was able to increase nutrient digestibility and metabolic energy. Improved digestion by giving oligochitosan irradiation is a manifestation of repair of ileal tissue, as part of the small intestine that acts as a place for nutrient absorption. The same tendency was also observed for increasing body gain in Indonesian local variety cow namely Pasundan cow.

Results for 2018-2021

National Nuclear Energy Agency (BATAN) Indonesia, currently named Research Organization for Nuclear Energy has successfully prepared irradiated chitosan (oligochitosan) from shrimp shells using demineralization, deproteination, and deacetylation process to produce chitosan and it was followed by irradiation using gamma rays to produce oligochitosan. It is well known that oligochitosan is used as PGP (plant growth promoter), plant elicitor, animal food additive, pharmaceutical and cosmetic product, as well as biomedical materials for application in medicine. In this report, oligochitosan was used for PGP, and plant elicitor on the pepper and chrysanthemum plant and as an animal feed additive for ruminant (cow and sheep) and poultry (duck and hen). As for PGP application in pepper plant, the result showed that treatment of oligochitosan can increase the circumference of primary branch (CPB) as 4%; number of primary branch (NPB) as 24%; length of primary branch (LPB) as 25.54%; chlorophyl index (9-16)%. Furthermore, the chrysanthemum (PN variety) treated with oligochitosan have shorter harvesting time (112.33 days) compared to the chrysanthemum treated with Hyponex (121.33 days) and Evergreen (123 days).

Application of oligochitosan as an animal feed additive for Indonesian local duck namely Cihateup duck showed that oligochitosan increased blood glucose, albumin, globulin, number of goblet cell, number of villi, length of villi, Hb, erythrocytes, lymphocytes and egg weight. Whereas triglycerides, urea, cholesterol, apoptosis, leukocytes and N/L ratio was reduced by oligochitosan. The administration of irradiated oligochitosan was able to increase nutrient digestibility and metabolic energy. Giving the level (350 ppm in solution/kg ration) has been able to improve the digestibility of Indonesia local variety of hen namely Sentul chickens. Improved digestion by giving oligochitosan irradiation is a manifestation of repair of ileal tissue, as part of the small intestine that acts as a place for nutrient absorption. Improvement of dry matter digestibility, as well as to organic matter digestibility, is the impact of

increasing the volume of ileal microvilli. Overall, the administration of irradiated oligochitosan was able to increase glucose, non-esterified fatty acid (NEFA) and HDL levels, which was followed by a decrease in cholesterol, triglyceride and LDL levels of Indonesian local hen.

Furthermore, application of oligochitosan as an animal feed additive showed increasing body gain in Pasundan's cow with the increasing of concentration of oligochitosan. It was shown that the increase of body gain after 40 days application of oligochitosan with the concentrations of 0, 300, 400 and 500 ppm are 5.2, 9.8. 10 and 13.8 kg, respectively. The same tendency was also observed for increasing body gain in sheep. The highest individual body weight gain of Pasundan cow was achieved in the application of oligochitosan of 500 ppm. This is probably because there is a decrease in amino acid levels in the rumen which implies a decrease in the NH_3 content. This means that amino acids can be properly absorbed by the system. Furthermore, the increase in Dry Matter Digestibility (DMD) and Organic Matter Digestibility (OMD) showed that oligochitosan accelerates the digestion process.

Future plan for 2022-2024

Due to the reorganization of several research centers in Indonesia including BATAN to the National Research and Innovation Agency. The continuity of the research of application of oligochitosan on Indonesia local hen variety will depend on the support of the agency. The support funding proposal will be submitted for fiscal year of 2022, by the end of November 2021.

Another research activity that will be conducted is reuse plastics in which recycle plastics will develop to be use as functional product.

4) Japan (Dr Taguchi Mitsumasa, National Institutes for Quantum Science and Technology)

Subject: 2. Hydrogel for Medical Application

Short summary

Functional hydrogels of gelatin for bio- and medical-applications were prepared by using the radiation crosslinking technique. The obtained hydrogels were applied to three-dimensional cell culture substrates that can control the arrangement of muscle fibers, and to nanoparticle-type MRI contrast agents that are rapidly discharged from the body without accumulating in the brain.

Results for 2018-2021

In vivo, the extracellular matrix (ECM) composed of proteins, sugars, and water stimulates cells and regulates gene expressions due to its composition, stiffness, and microstructure. On the other hand, there is a problem that cells cultured on the plastic dish, which is six orders of magnitude harder and flatter than the ECM, respond differently than in vivo. Proteins were gelled by the radiation crosslinking technique and applied to cell culture. Myotubes were obtained on the hydrogel with a line shape on the surface and oriented in one direction, which can be used as a muscle model. Nanoparticles were also produced by γ-ray irradiation under controlled conditions of gelatin concentration and dissolved oxygen in the water. The obtained nanoparticles keep their biodegradability and low cytotoxicity. By loading Gd onto the 20 nm diameter particles, a safer MRI contrast agent was developed that does not enter the brain and eliminates quickly from the body.

Future plan for 2022-2024

We investigate the radiation crosslinking technique to develop functional devices in medical applications. In particular, we aim to develop 3D cell culture hydrogels that can be applied to drug discovery and regenerative medicine, nanosensors that can be used in MRI and PET, and microfluidic chips that can perform multiple types of simultaneous tests on a palm-sized chip, and implement into society.

5) Kazakhstan (Mr Kassymzhanov Murat, JSC "Park of Nuclear Technologies")

Subject: 4. Synergistic Effect among Plant Growth Promoters, Super Water Absorbents and Biofertilizer

Short summary

Within the period from 2016 to 2021, the project advanced from the idea to the industrial production and was taken out into a separate subsidiary enterprise with the involvement of a private investor with a share of 91%. The product has a full list of permitting documents. The five-year SWA project is officially completed.

Implementation of project «Modification of polymer cable insulation based on the electron accelerator ELV-4».

Results for 2018-2021

Over three years of observations of the experimental plot in the nursery "Semei Ormany" farm, the seedlings of two-year-old pine with the use of SWA not only outdid the control area in root taking by two times, but the growth of some seedlings reaches up to 40 cm, which is 10 cm above the seedlings in the control area.

National Standard of the Republic of Kazakhstan ST RK 2696-2015 "Radiation cross-linked water-absorbing superabsorbents" was developed and implemented.

The "BetaSorb" trademark is registered and entered in the State Register of Trademarks.

Patent for the invention "Method of synthesis of polymeric hydrogel for agricultural crops" was received.

Industrial production of SWA is well established.

A subsidiary enterprise "BetaSorb" LLP was established with the involvement of a private investor with a share of 91%.

A certificate of conformaty has been obtained dated July 01, 2021.

The five-year SWA project is officially completed.

Future plan for 2022-2024

Implementation of project «Modification of polymer cable insulation based on the electron accelerator ELV-4».

Within the framework of the project, it is planned to develop new wires and cables that are not currently produced in the Republic of Kazakhstan. It is planned to develop two types of cables, cables for photovoltaic systems and cables for oil submersible pumps.

The first stages of the production of photovoltaic and oil-submersible cable will be carried out on the existing equipment in Kazelectromash LLP, namely drawing, twisting, laying insulation. The next stage of production will take place in JSC "Park of Nuclear Technologies", namely, the launch of an isolated

workpiece, by means of a rewinding line, under the influence of radiation.

6) Malaysia (Ms Maznah Mahmud, Malaysian Nuclear Agency)

Subject: 1. Degraded Chitosan for Animal Feed, 2. Hydrogel for Medical Application, 4. Synergistic Effect among Plant Growth Promoters, Super Water Absorbents and Biofertilizer, 5. Plant Growth Promoters and Super Water Absorbents, inclusive Process development

Short summary

Chitosan, carrageenan, and sago waste are natural resources-derived polymers utilized in our RPPM project for development of hydrogel for medical application, PGP, animal feed supplement and absorbent for agriculture. The polymers are treated with gamma radiation to induce whether degradation, crosslink, sterilization, or combination of these reactions depending on the process requirement and product designs. Radiation implies various modification of polymer chains and networks to fulfill and expand the product characteristics.

Results for 2018-2021

Project 1: Oligocarrageenan as PGP

In 2018, Nuclear Malaysia has initiated the development of a new PGP from carrageenan. The irradiation of carrageenan in dry and solution states indicated the carrageenan molecular weight reduces to below 20kDa at the dose of 75kGy and 50kGy, respectively. The type of carrageenan used in this project are characterized as kappa-carrageenan based on the low intensity peak at 805cm-1 which attributes to sulphate ester at position C2 in anhydro-D-galactose. Application of high radiation dose indicates no changes in the carrageenan bone structures. Study on the performance of oligocarrageenan as PGP is still in progress. Due to pandemic issue since end of 2019, the pot test was unable to run according to the plan scheduled. We completed the Mw study and proceeded with formulation development of oligocarrageenan solution. Suggested concentration is 10000ppm compared to oligochitosan solution is 20000ppm. Viscous solution formed after dissolved oligochitosan powder at 15000ppm. Addition of sodium salt induces the better solubilization of oligocarrageenan powder in water.

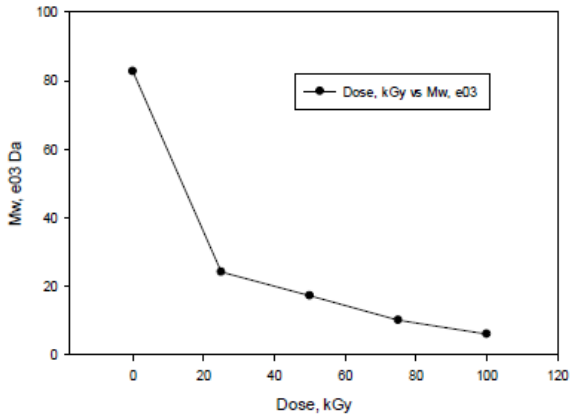

Figure 1; Effect of absorbed dose on Mw of carrageenan.

Measurement was done using SEC-MALLS at 40°C of column temperature.

Figure 2. FTIR spectras of carrageenan after treated with gamma ray at various doses

Figure 3: Seed treatment (80ppm) shows mustard seed 100% germinated and 100% survived after 2 weeks at nursery and seedlings developed 3 - 4 leaves before being transferred to the pot. C 80% seed germination, 90% survived with development of 2 leaves before transferred.

Project 2: Synergistic effect of PGP-SWA

Experiments were carried out around July 2020. 5 parameters were carried out in this study, 1) Control, 2) SWA only, 3) SWA with Oligochitosan, 4) SWA with Oligocarrageenan and 5) SWA with VitaGrow (commercial PGP). Chinese Kale was used in this study. For plants heights, the synergistic of SWA with Oligochitosan gave the highest value at 20.85 cm. While, for the length of the roots, treatment of SWA only gave the highest result.

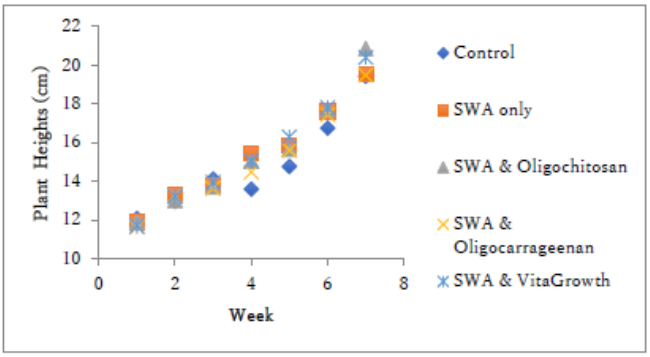

Figure 4. Synergistic effect of SWA and PGP of growth on Chinese Kale.

We found out that the results for roots length were not as expected even though the synergistic effects of SWA and PGP on growth of the plant were significant. This may be due to the location of the pot test

study. The green house was located behind a big building and close to a big tree.

We assumed that the plants were not getting enough sun light throughout the study. In future we would like to repeat the experiments to confirm the results obtained this year.

Project 3: Oligochitosan as fish feed supplement

Radiation degraded chitosan or oligochitosan has proven to be one of the successful outputs throughout RPPM project. Besides it plays as great PGP, oligochitosan also indicates outstanding results as fish feed supplement. The 0.1% chitosan in AcOH irradiated at 25 kGy (Figure 3) was selected to use as fish feed supplement as its molecular weight is optimized (5.3 kDa). Oligochitosan increases tilapia growth rate and mortality after adding 0.025% and 0.05% oligochitosan in feed.

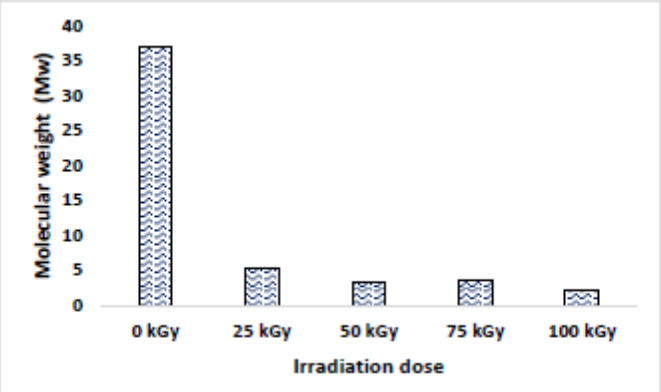

Figure 5. The effect of gamma irradiation dose on Mw of chitosan

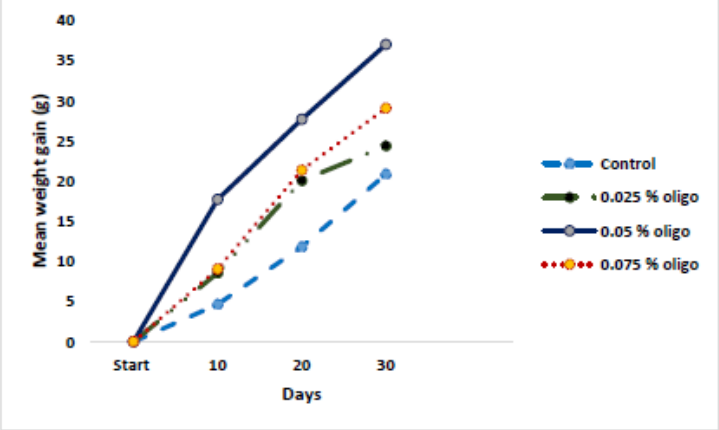

Figure 6. Weight gain of the tilapia fed with oligochitosan

Project 4: Carradish 3D cell culture

The Carradish project as the hydrogel-based cell culture has been initiated in 2019. The carrageenan indicated its potential cell culture media after hybridization with PVA and PVP. The network developed retains its form until day 10 and it started to dissolve after that. The challenges are to induce strong network formation through polymers hybridization and radiation treatment, to provide cell-friendly environment and to support the development of cell in 3D.

Figure 7. Hydrogel-based media cell culture from carrageenan-PVA-PEG

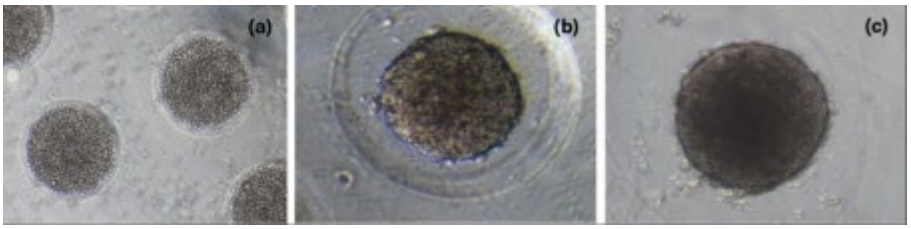

Figure 8. HTB43 spheroid formation at (a) day-1 (b) day-5 and (c) day-10

Community Project & Corporate Social Responsibility Project (CSR)

Malaysian Nuclear Agency (Nuclear Malaysia) always commits to the government programs especially on helping community to increase their income. In 2018, Nuclear Malaysia granted with USD 71000 from MOSTI to help community in Kedah, northern part of Peninsular Malaysia to increase production of corn-silage. The oligochitosan (Mw below 10000Da) has been introduced to mixed with silage as feed supplement and increase the fermentation rate of corn silage. In 2021, Nuclear Malaysia team-up with Mutation Breeding group supplied oligochitosan and mutant rice to the farmers who were affected by floods, in Pahang, eastern part of Peninsular Malaysia.

Figure 9. Oligochitosan solution was mixed in the silage formulation to fasten the fermentation rate and acts as feed supplement.

Figure 10. Team members together with community with silage machine

Figure 11. Mutant rice and oligochitosan were transported to the farmers.

Figure 12. NMR 152 able to survive and produce high yield even in soil with excess water and oligochitosan increases plant quality and yield.

Current Problem

March 2020 – October 2021 was the challenging time for us to carry out all the works planned. Due to pandemic, almost one and the half year time, our workstations have been shifted from office to home. We were prohibited to access the premises and most of our R&D work were unable to be carried out at home. Some of the material procurement and facility repair/maintenance can't be done as planned due to restriction to access the premise during MCO. At that time, we were more concentrate on attending webinars, online programs (conference and courses) and focusing on writing articles and guidebook.

Future plan for 2022-2024

For 2022 – 2024, we will resume all the unfinished work which was planned before the pandemic hit. The work will be focusing on completing shelf-life and performance studies of oligocarrageenan. The synergistic study also will be carried out next year which will concentrate on utilization of different PGP. As oligochitosan proved its promising activity to increase and improve yield and quality of the fish, 2022 will be suitable time to approach fish farmers for collaboration. The work on development of Carradish should be resume as planned.

7) Mongolia (Dr Chinzorig Radnaabazar, National University of Mongolia)

Subject: 2. Hydrogel for Medical Application, 3. Environmental Remediation, 7. Sterilization of BF Carrier using radiation

Short summary

During last 4 years we are successfully experimented with subject 2, 3, 7 and most promising results obtained from Synergistic Effect of Plant Growth Promoters, Super Water Absorbents and Biofertilizer. The amount of yield from chili, tomato and potato increased at least 50%.

Results for 2018-2021

- "Hydrogel for Medical Application" subject is now in ongoing study. We prepared PVA based hydrogel by cryo-polymerization method and synthesized ZnO, Ag2O nanoparticles. Up to 60nm nanoparticles were effective against pathogenic bacteria (MRSA) in Mueller-Hinton disk diffusion method but during storage in 4^0C degree, aggregation of nanoparticles occurred. Also, pore size analysis of Hydrogel and swelling test is not done.
- "Environmental Remediation" subject is performed successfully last 3 years. Plantation of endemic plants in mining area with supplementation of biofertilizers were tested. Among dozens of plants *Allium mongolicum* was most salt tolerant and dominant. Bioremediation of degraded soil using biofertilizer and phytoremediation achieved 89% of square with good and 10% lower effect in gold mining site.
- Under the theme of "Sterilization of BF Carrier using radiation" we tested several different type of carrier materials including bentonite, zeolite charcoal and cow dung. All carrier materials were prepared according to FNCA guide and with and without biofertilizer tested on cucumber seedlings. Depending our results dung and bentonite shows 5% higher effect on growth of cucumber.

Future plan for 2022-2024

In 2022-2024 we are aiming to perform the following subjects:

1. Develop and transfer ZnO nanoparticle containing Hydrogel to clinical trial.
2. Heavy metal adsorption of Cryogel-Zeolite composite material should be tested in laboratory scale. Currently, we are only tested adsorption of Pb and efficiency rate was 87.47 mg/g after 20 hrs, room temperature.
3. We are not tested SWA yet. In 2022 we are going to develop starch based SWA according to FNCA guideline and test in field of desertificated area.

8) The Philippines (Dr Charito T. Aranilla, Philippine Nuclear Research Institute)

Subject: 2. Hydrogel for Medical Application

Short summary

Radiation-crosslinked hydrogels based on carboxymethyl cellulose and k-carrageenan/polyethylene oxide were prototyped into hemostatic granules and dressing for application to control bleeding in traumatic wounds. Both hemostats have high clotting capability, no cytotoxicity and acute systemic toxicity, non-irritant and are weak dermal sensitizer. Pre-clinical studies demonstrated faster and more effective clotting in four, moderate to severe, animal bleeding models resulting to higher survivability rate compared to commercial hemostats. The prototypes are ready for clinical trials.

Results for 2018-2021

Whole blood clotting and platelet adhesion tests of several polymers have managed to identify potential formulations based on carboxymethylcellulose (CMC) and polyethylene oxide-kappa carrageenan blends (PEO-KC). Consolidation of all analyses identified 20% w/w CMC irradiated at a dose of 40 kGy to be the best formulation for hemostat granules, with comparable effectiveness to the commercial

product Celox granules. For the dressing-type hemostat, PEO-KC blends (5% PEO, 2.5% KC with 2.5% PEG irradiated at 25 kGy) pressed onto a gauze showed promising efficiency in platelet adhesion and coagulation time tests compared to Celox and Quikclot gauze brands. Bioburden and sterility studies showed that 25 kGy irradiation dose is sufficient to achieve sterility in ten out of ten products that were tested. The stability studies of the prototypes showed product shelf-life up to 18 months with no significant changes in the efficacy and physico-chemical properties. However, it is recommended to store the samples at an ambient or cool temperature. The biocompatibility and efficacy tests revealed very promising results that supported their suitability as medical devices for bleeding control. The cellular response (MTT assay) in both prototypes showed cell viability between 85-87% and rated as non-cytotoxic based on ISO standard 10993-5. Acute systemic toxicity of the extracts, investigated by a single intravenous injection in female and male rats, did not produce any systemic toxic signs like reduction in feed and consumption and body weight. Blood chemistry assay showed neither hepatotoxic (normal ALT level) nor nephrotoxic (normal creatine and BUN levels). During the 14-day testing period, no rats in any of the treatment groups manifested behavioral, respiratory, and neurologic changes indicative of systemic toxicity. In addition, no mortality occurred in all male and female test rats regardless of the treatment given. The skin sensitization or allergic potential of the granules and dressing hemostats were assessed using the Guinea Pig Maximization Test of Magnusson and Kligman method. Based on test findings, both hemostats were classified as weak sensitizers - Grade 1 since minimal sensitization was observed in evaluation of challenge application. Findings of efficacy tests to control bleeding of punctured femoral artery, aorta, deep wound, and caudal pole nephrectomy, in healthy 8-week-old male Sprague-Dawley rats, revealed that the prototype dressing was more efficient than Celox gauze in controlling bleeding time with a 100% survival rate while the prototype granules was as efficient as Celox granules in controlling bleeding time but with better survival rate, lesser adhesion and easier removal.

Future plan for 2022-2024

The following are proposed activities for 2022-2024: (1) Up-scaling of production of CMC granules and KC/PEO/PEG hydrogels in a GMP facility; (2) Validation and substantiation of 25 kGy as radiation sterilization dose; (3) Preparation for clinical trials in cooperation with the Industry partner.

Subject: 2. 5. Plant Growth Promoters and Super Water Absorbents, inclusive Process development

Short Summary

Super water absorbents (SWA) based on cassava starch and acrylic acid (AAc) were prepared by gamma irradiation for agricultural applications. The SWA effectively retained water in clay-rich soil while its water absorption in sandy loam increased with time. Microbial degradation, phytotoxicity and genotoxicity tests showed biodegradability and safety of SWA for agricultural use. Performance evaluation of SWA done in greenhouse demonstrated the effectiveness of the optimized SWA. The use of SWA can save irrigation water and resources (labor and time) as the frequency of watering is reduced from every 6 days to every 10-11 days. Cost benefit analysis showed that this environment friendly SWA

can improve farmer's productivity, especially during El Niño or in drought-prone areas and help conserve irrigation water.

Results for 2018-2021

The influence of synthesis parameters on the gel properties of SWA and its effect on the soil water retention were investigated. Gel fraction of the different SWAs ranged from 40% to 98% and degree of swelling reached up to about 483 g water/g dry gel and SWAs exhibited gel strength up to 1000 kPa. The SWA with superior gel and soil water retention properties was found to be the formulation of 20% AAc, 30% DN, 7.5% starch and radiation dose of 20 kGy. This SWA effectively retained water in clay-rich soil while its water absorption in sandy loam increased with time. It has a biodegradation rate of 46% in 218 days determined by microbial oxidative degradation analyzer. The safety of the developed SWA were evaluated by phytotoxicity (seed germination) and *Allium cepa* tests. These assays indicated that the optimized SWA is safe for agricultural use. There were neither genotoxic effects observed from its mitotic activity nor chromosomal aberrations. Real-time aging test showed that stability of SWA was dependent on the storage temperature. The shelf life of the developed SWA is 6 months at room temperature storage. Performance evaluation of SWA done in greenhouse demonstrated the effectiveness of the optimized SWA. Pot testing with okra showed that 1.0 g SWA per plant had significant effect on the fresh shoot biomass (21% increase) and very significant effect on the fresh root biomass (81% increase). For corn pot experiment, 0.75 g of SWA is sufficient to promote higher fresh biomass of shoot and root. The best treatment found for lettuce is the use of 0.50 g SWA per plant. The developed SWA can save a lot of irrigation water and resources (labor and time) as the frequency of watering is reduced from every 6 days to every 10-11 days. Cost benefit analysis showed that this environment friendly SWA could help farmers increase their productivity. Meanwhile, cultivation of okra in the field with SWA significantly induced earlier flowering. Despite substantial decrease of irrigation frequency in treatments with SWA (i.e., from 9 reduced to 3 for okra: and from 9 reduced to 6 for cucumber), there was no discernable difference in harvested cucumber fruit and okra fruit production when compared to control, thus making SWA more ideal to use during events of water-crisis or extreme drought phenomena such as El Niño. The function of the PNRI optimized SWA in maintaining and controlling the soil moisture in the field trials projected 33% savings from reduced volume of agricultural water and labor time used during irrigation.

Future Plan for 2022-2024

The following are proposed activities for 2022-2024: (1) Comparison of microbial oxidative degradation between EB and gamma-irradiation SWA and (2) Performance evaluation of SWA in vertical farming.

9) Thailand (Dr Phiriyatorn Suwanmala, Thailand Institute of Nuclear Technology)

Subject: 5. Plant Growth Promoters and Super Water Absorbents, inclusive Process development

Short summary

This project is aimed at scaling up the production of starch-based super water absorbent (SWA) and to validate the practical benefits of SWA for agricultural applications. Results from the field tests, performed for two seasons, revealed that the prepared SWA increased the survival rate of young rubber

trees planted in arid areas by up to 40%, while simultaneously enhancing the growth characteristics of the young rubber trees.

Results for 2018-2021

Cassava starch-based SWA was successfully synthesized in an up-scaling production by radiation-induced graft polymerization of acrylic acid (AA) onto cassava starch (CS). The change from the thermal gelatinization in the lab-scale synthesis to the alkaline gelatinization in the up-scaling production simplified the process and rendered it practical as well as energy-saving. For the first time, two seasons of a field testing of starch-based SWA with young rubber trees was carried out in a comparatively sizable scale, with a large sample of trees. The field tests with young rubber trees planted in an arid area without any watering except from natural rain demonstrated that SWA increased the survival rate of the young rubber trees, and concurrently enhanced their growth characteristics. The optimized amount of SWA required to maximize the survival rate of the young rubber trees planted in arid areas in the dry season was 30 g of SWA per one rubber tree. The ability of SWA to retain a large amount of water and subsequently and gradually release water to the root zone of the plants can result in a higher survival rate of plants grown in arid areas, while the increased soil porosity and better oxygenation can bring about enhanced growth characteristics. The benefits therefore include reduced water consumption, decreased frequency of watering, increased soil porosity and better oxygenation. Consequently, SWA offers a promising and sustainable way of increasing the survival rate of trees planted in arid areas.

Future plan for 2022-2024

Under the project, SWA beads were successfully prepared at a laboratory scale. The future plan for the SWA beads project includes, [1] the loading and releasing efficiency of fertilizers encapsulated inside SWA beads [2] the up-scaling production of SWA beads and [3] field test of SWA beads in order to compare with previous results from the original SWA hydrogel.

A project proposal entitled, “Preparation of sugarcane bagasse-based SWA incorporated with chitosan for controlled release of plant growth promoter,” has been submitted by TINT for 2021 – 2024. The project will focus on the preparation of SWA from sugarcane bagasse which is an abundant biomass in Thailand. Chitosan will also be incorporated in the prepared SWA and its plant growth promoter attributes will be studied to see the synergetic effects of SWA and chitosan.

10) Vietnam (Dr Nguyen Ngoc Duy, Vietnam Atomic Energy Institute)

Subject: 1. Degraded Chitosan for Animal Feed, 2. Hydrogel for Medical Application, 3. Environmental Remediation

Short summary

- SeNPs/OCS were synthesized by the irradiation method. White leg shrimp and mice were fed with SeNPs/OCS to investigate immune stimulation and recover the total white blood cells of γ-ray irradiated mice. The results indicated that SeNPs/OCS exhibited a good immune stimulation effect and efficient recuperation of white blood cells.
- The cell scaffolds from the mixture of gelatin/CM-chitosan and gelatin/CM-chitin were prepared by irradiation method. The results indicated that both CM-chitosan and CM-chitin much improved the

swelling capacity, porosity, and pore size of the gelatin-derived scaffolds.

- Isolate and select microbial strains from dyeing textile dyes wastewater to combine with an electron beam for eliminating real textile wastewater.

Results for 2018-2021

- SeNPs with a size of 41.8 nm were synthesized by γ-irradiation using oligochitosan (OCS) as the stabilizer. Results revealed that the as-obtained SeNP/OCS powder exhibits high purity. The SeNP/OCS solution's stability test results indicated that the SeNP/OCS solution stored at 4 °C exhibits good stability for 60 days. The level of total WBC in all three groups of irradiated mice, which were even orally supplemented with OCS, SeNPs/OCS, and Phosphate Buffer Saline (PBS), significantly decreased during the 10 days after irradiation. However, the recovery of total WBC in irradiated mice orally supplemented with OCS and SeNPs/OCS for 20 days was already observed. Compared to OCS, SeNPs/OCS exhibited a higher recovery for the total WBCs. White leg shrimp were fed with SeNPs in various concentrations of 0, 0.5, 1.0, and 2.0 mg/kg feed for 30 days and then challenged with Vibrio parahaemolyticus bacteria to investigate immune stimulation and weight gain effects. The results indicated that SeNPs/OCS exhibited a good immune stimulation effect with a suitable 1.0–2.0 mg/kg feed concentration. The survival rate and weight gain of white leg shrimp fed with SeNPs/OCS increased 29% and 4.5% compared to control, respectively.
- The CM-chitosan and CM-chitin exhibited significant amelioration of the gelatin-derived scaffold material features in the same manner. Both gelatin/CM-chitosan and gelatin/CM-chitin accounted for a swelling degree of 7–9 g/g in PBS, a compressive modulus of 46–66 kPa, a porosity of 70–73%, and a pore size of 100–300 mm. They also exhibited acceptable biodegradability in the collagenase enzyme and non-cytotoxicity for adipose-derived stem cells (hADSCs) with an RGR of ~97%, which all met the fundamental requirements for hADSC culture-scaffold materials.
- Real dyeing wastewater was degraded mostly by the electron beam method at a dose of 1 kGy and 1 mM H_2O_2, combined with biological treatment (selected strains from textile wastewater).

Future plan for 2022-2024

- Study the increase of oligochitosan on productivity and content of bioactive substance of medicinal plants.
- Study on the synergistic effect of oligosaccharides and selenium nanoparticles on the immune-stimulation for use in breeding and functional food.
- The production of oligochitosan as an additive to increase the immune system in aquaculture at a pilot scale.
- Commercialization of oligochitosan in livestock and aquaculture