FNCA Guidelines

— On Chitosan PGP Application for Rice, Chilli and Other Crops —

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PREFACE

Plant growth promoter (PGP) is a substance which enhances the growth of plants. If PGP is applied to crops, the increase of crop yield causes economical impact in agricultural production. Such PGP can be produced by radiation processing of polysaccharides such as chitosan and carrageenan which are originated from crustacean shelf and seaweed, respectively. Hence, electron accelerator application project in Forum for Nuclear Cooperation in Asia (FNCA) has investigated the PGP synthesis as an application of radiation degradation of natural polymers and revealed the effect of PGP originated from chitosan and carrageenan on various crops since 2006. These positive data of yield increase in size and weight of edible crop part have been shared among the 10 participating countries of Bangladesh, China, Indonesia, Japan, Kazakhstan, Malaysia, Mongolia, Philippines, Thailand, and Vietnam in FNCA. Especially, it was found that PGP originated from chitosan induced elicitor activity against fungal disease. Chemical pesticide may be replaced by environmentally-friendly PGP. To promote the technology transfer of PGP, the accumulated data of crop yield should be opened to end-users like farmers in each country. These years our group obtained the PGP data for rice and chilli which are beneficial crops in Asia countries.

This guideline is composed of 5 chapters and appendix; Chapter 1 is introduction of PGP to understand what PGP is, effect of PGP on plants, preparation by radiation processing, and advantages of PGP prepared by radiation processing. Chapter 2 dealt with preparation, physical and chemical properties, dilution before usage, preservation. End-users can get brief information of production process and handlings of PGP. Chapter 3 and 4 are protocols of timing in plant growth stage, PGP concentration, foliar spray frequency, etc. for rice and chilli, respectively. Chapter 5 is Q&A for general matters and cases of rice, and chilli. PGP effects on various crops exclusive rice and chilli are listed as appendix.

This guideline is expected to be benefit for technology transfer of PGP prepared by radiation processing to end-users as well as sustainable agriculture with economic growth in the Asia region.

Masao TAMADA
Project Leader of Japan

1. Plant growth promoter (PGP)

1.1 What is PGP?

PGP is classified as natural growth stimulant which is non-toxic and soluble in water that enhances the growth of plants and also has elicitor effect against plant disease. It is improved the overall health growth and development of plants that determine the crop quality and productivity. As a result, increase in yield which benefits farmers and agriculture production including floriculture and horticulture crops.

PGP can be either synthetically produced or obtained from the biological derivatives like seaweed for example carrageenan and alginate, and crustacean shelf like chitin/chitosan. PGP from biological derivative is more effective and safe to be used by end user and no hazardous to consumer. It could be easily applied through foliar spray on field crops, fruits, vegetables and flowers to maximize the productivity. PGP either stimulates production of plant hormones in plant or itself content of plant hormones that accelerate the plant growth.

Plant hormones is an organic substance, naturally produces in plant which affects plant growth. There are several plant hormones such as auxins, cytokinins and gibberellins that act as regulator for plant growth. Chitosan contains plant hormones such as indol acetic acid (auxin), cytokinin (kinetin and zeatin) and gibberelic acid which promote plant growth.

1.2 Effect of PGP on Plants

PGP from crustacean shelf or seaweed has an effect on overall growth of plants. It has capability to enhance or accelerate the rate of growth and maturation of crops or plants without interfering natural physiological actions. For example, the existence of auxin hormone in PGP will stimulate cell elongation in stem through lengthwise growth of plants, and root initiation on stem cuttings and lateral root development in tissue culture. It also helps in fruit development by delays fruit ripening, inhibit leaf fall and lateral branching. In the case of cytokinin plant hormones exist in PGP, it will stabilize protein and chlorophyll content in the leaf which produce green leaves, prevent yellowing of leaves. It promotes the conversion of etioplasts into chloroplasts via stimulation of chlorophyll synthesis. Cytokinin is synthesized in roots and move upward in the xylem to leaves and fruits. It stimulates morphogenesis development of shoot initiation/bud formation in

tissue culture. In conjunction with auxin and cytokinin will promote cell division in plant. Gibberellin is another plant hormone which has been identified in PGP to promote plant growth. It mostly found in seeds, young leaves and in roots. It move upward from the root and promote growth of main stems of plant. The PGP will accelerate the growth of plant through cell propagation on stems and roots, increase chlorophyll in green leaves and enhances maturating with early flowering, which increase fruit yield of plant or crops.

Figure 1.1. Effect of PGP on carrot and chilli.

One of the most important defenses of plant tissues against disease infection is the ability to produce phytoalexin which is antibiotics synthesized in response to pathogens or elicitors. Phytoalexins can inhibit the growth and development of phytopathogens at an effective concentration. Momilactones which are phytoalexins in rice leaves were firstly reported by Cartwright *et al.* [1]. Agrawal et al. also indicated that momilactone A induced in rice leaves increased greater than 10,000 ng/50 mg after 72 h treatment of chitosan (Fig. 1.2) [2].

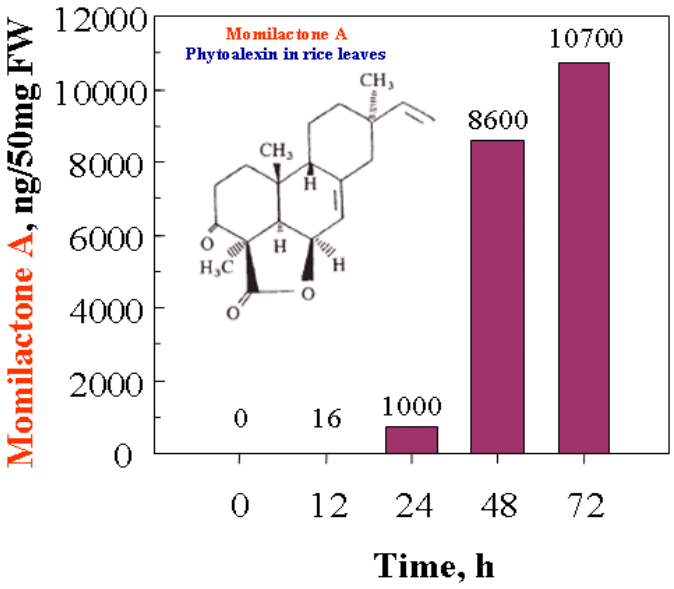

Figure 1.2. Phytoalexin momilactone A content in rice leaves treated with chitosan

Rodiguez *et al.* studied to treat seeds of rice (*Oryza sativa* L.) with chitosan and hydrolyzed chitosan for induction of defense response against blast disease caused by *Pyricularia grisea* [3]. Results revealed that seedlings obtained from seeds treated with chitosan and hydrolyzed chitosan (oligochitosan) showed stronger resistance to blast disease compared with non-treated plants (positive control). More details on elicitation effect of oligochitosan for plants can be referred to the paper reviewed by Ying, Zhao and Du (see Table 1.1) [4].

Table 1.1. The effect of oligochitosan on plant diseases control (survey in China)

Plants	Diseases	Control rates (%)
Tobacco	Tobacco mosaic virus, Potato virus	76.49 – 87.5
Panax notoginseng	Virus diseaseh	>90
Tulip	Peronospora	84.24 – 88.6
Piper nigrum L.	Mosaic virus	73.54 – 81.3

Tomato	Solani, infestans, virus disease, bacterial wilt	84.47 – 88.24
Cucumber	Peronospora	78.96 – 82.65
Pepper	Virus diseases, blight, anthracnose, Pepper phytophthora blight	78.58 – 90
Egg plant	Virus diseases	93.18 – 100
Chinese cabbage	Erwinia carotovora subsp	78.62 – 85
Asparagusplettuce	Peronospora	45.80 – 62.3
Wax gourd	Blight	84.81 – 95
Cauliflower	Black rot	63.60 – 64.2
Geen cucumber	Blight	45.50 – 57.6
Cowpea	Virus diseases	31.13 – 58.8
Papaya	Mosaic virus	70 – 96
Watermelon	Black rot, Didym ella bryoniae, light, virus diseases	81.71 – 85.40
Muskmelon	Powdery mildew	71.34 – 86.26
Banana	Bunchy top	83.70 – 94.6
Apple	Mosaic virus, Venturia inaequalis	76.68 – 93.85
Soybean	Virus diseases	75.10 – 100
Cotton plant	Cotton yellow wilt	85.50 – 87.2
Maize	Sphacelotheca Reiliana, corn northern leaf blight, corn southern leaf blight	23.90 – 45.35
Rice	Rice blast	71.41 – 92.0
Peanut	Virus diseases	23.90 – 26.5

By foliar spraying of oligochitosan with concentration of 20-50 ppm on peanut leaves, the productivity increased to 20-40% compared with the non-treated control (Table 1.2 and Fig. 1.3) [5]. The result indicated that oligochitosan exhibits growth-promotion effect on peanut. Similarly, soybean seeds coated with oligochitosan (0.4%), the productivity also increased remarkably at about 30% compared with the control [6]. In addition, the rate of rust disease on soybean was lower than that of the non-treated control.

Table 1.2. Effect of oligochitosan on productivity of peanut

Oligochitosan, ppm	0 (ctrl)	20	30	40	50
Productivity, kg	3.05	3.64	3.74	4.29	3.81
For control, %	100	119.3	122.6	140.7	124.8

Figure 1.3. Peanut treated with oligochitosan: 0, 20, 30, 40 and 50 ppm

Hien *et al.* studied the elicitation and growth promotion effect of oligochiotsan for sugarcane and rice. Results showed that oligochitosan with moleculer weight (Mw) 6000-10,000 exhibited the most effective elicitation and growth promotion for plant [7]. The optimum oligochitosan concentrations by spraying were 30 and 15 ppm for sugarcane and rice, respectively. The disease index of *Ustilgo scitaminea* and *Collectotrichum falcatum* on sugarcane was reduced respectively to 44.5 and 72.3% compared to control (100%). The productivity of sugarcane increased about 13% (71.4 tons/ha compared to 63.2 tons/ha for control). The disease index of

Pyricularia grisea on rice was reduced to 53.0% for leaf and 34.1% for neck of bloom (panicle) compared to control (100%). The productivity of rice increased for 11-26% (6.0-6.8 tons/ha compared to 5.4 tons/ha for control). The obtained results indicated that oligochitosan is promising to use as a biotic elicitor for plant particularly for sugarcane and rice(Table 1.3).

Table 1.3. The effect of oligochitosan on disease index and productivity for rice

Conc.of oligochitosan, ppm	Leaf DI		Panicle DI		Productivity	
	Index, %	for Ctrl, %	Ratio, %	for Ctrl, %	Tons/ha	Increase, %
15	14.4 b	62.88	10.0 c	34.1	6.8 bc	25.93
30	15.3 b	66.81	12.0 c	40.9	6.0 c	11.11
60	16.0 b	69.87	15.3 c	52.2	6.3 bc	16.67
Ctrl (H_2O)	22.9 a	100	29.3 a	100	5.4 a	-

More recently, Ma *et al.* studied to treat wheat seeds with oligochitosan by soaking seeds in 0.0625% oligochitosan solution for 5 h [8]. The results in Table 1.4 showed that chlorophyll content increased by treating seeds with oligochitosan. It suggested that seeds treatment with oligochitosan had a beneficial effect on photosynthesis. They also confirmed the positive effect of oligochitosan in improving the plant growth and plant's capacity of tolerance to salt stress [8].

Table 1.4. Effect of oligochitosan on growth parameters and chlorophyll content of wheat seedlings

	Control	NaCl (150 mM)	Oligochitosan (0.0625%)	Oligomer(0.0625%)-NaCl (150 mM)
Shoot length, cm	10.62 ± 0.54 a,b	7.81 ± 0.47 c	11.45 ± 0.51 a	9.46 ± 0.61 b
Root length, cm	14.98 ± 0.62 b	10.3 ± 0.53 c	17.94 ± 0.49 a	14.71 ± 0.37 b
Dry weight (g/plant)	0.35 ± 0.02 b	0.28 ± 0.01 c	0.42 ± 0.02 a	0.34 ± 0.02 b
Chl-a, mg/g FW	0.87 ± 0.07 b	0.69 ± 0.02 c	0.97 ± 0.02 a	0.91 ± 0.01 b

Figure 1.4. Field test of elicitation and growth promotion effect of oligochitosan produced by γ-irradiation method on rice in Vietnam

In addition, El-Sawy *et al.* studied on effect of radiation degraded chitosan on growth of faba bean plant [9]. They concluded that the degraded chitosan has positive effects not only on plant growth but also on the productivity of faba bean. Furthermore, a large field test (32 ha) of oligochitosan on rice has been carried out in Malaysia [10]. Oligochitosan used for this field test was produced by continuous flow ^{60}Co γ-ray irradiation system which can be suitably applied on large scale production.

Table 1.5. Results of field test of oligochitosan on rice in Malaysia

Treatment	Average yield, kg	For contl. 1	For contl. 1
Contl. 1 (water)	143	-	-
Contl. 2 (fungicide)	163	13.9%	-
Oligochitosan 40 ppm	163	13.9%	0.0%

Seed soaking in 100 ppm oligochitosan + spraying 40 ppm	181	26.6%	11.0%

The results in Table 1.5 indicated that oligochitosan could be used to replace fungicide, and the combination of seed soaking and spraying oligochitosan on rice plant showed the better effect on increase of the yield. (Fig. 1.5)

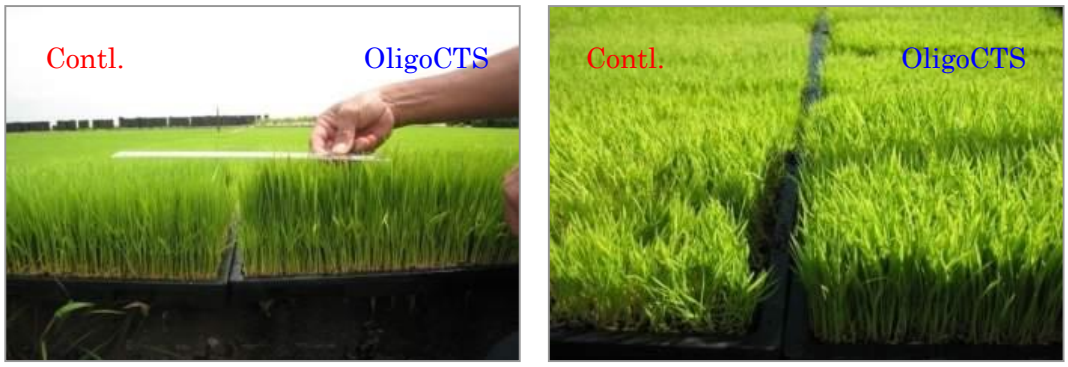

Figure 1.5. Field test of oligochitosan on rice

Furthermore, it was also reported that using 40 ppm oligochitosan in tissue culture media promotes significant growth of pineapple (Fig. 1.6). The effect of oligochitosan on reduction of disease and insect infection, and growth promotion on agarwood has been carrying out.

Figure 1.6. Effect of oligochitosan on tissue culture of pineapple

1.3 PGP preparation by Radiation Processing

1.3.1 Chitosan

Chitosan is linear aminopolysaccharide derived from chemical alkali deacetylation process of chitin. Chitin is natural polymer polysaccharide abundantly found in the outer skeleton of insects, crabs and shrimps.(Fig.1.7) Limitation solubility of chitin in the common solvent hindered it commercial application. Most of chitin/chitosan is produced from the food industrial waste of shrimp and crabs. Chitosan has shown to have wide potential applications in treatment of industrial waste water, health care, food industry and agriculture.

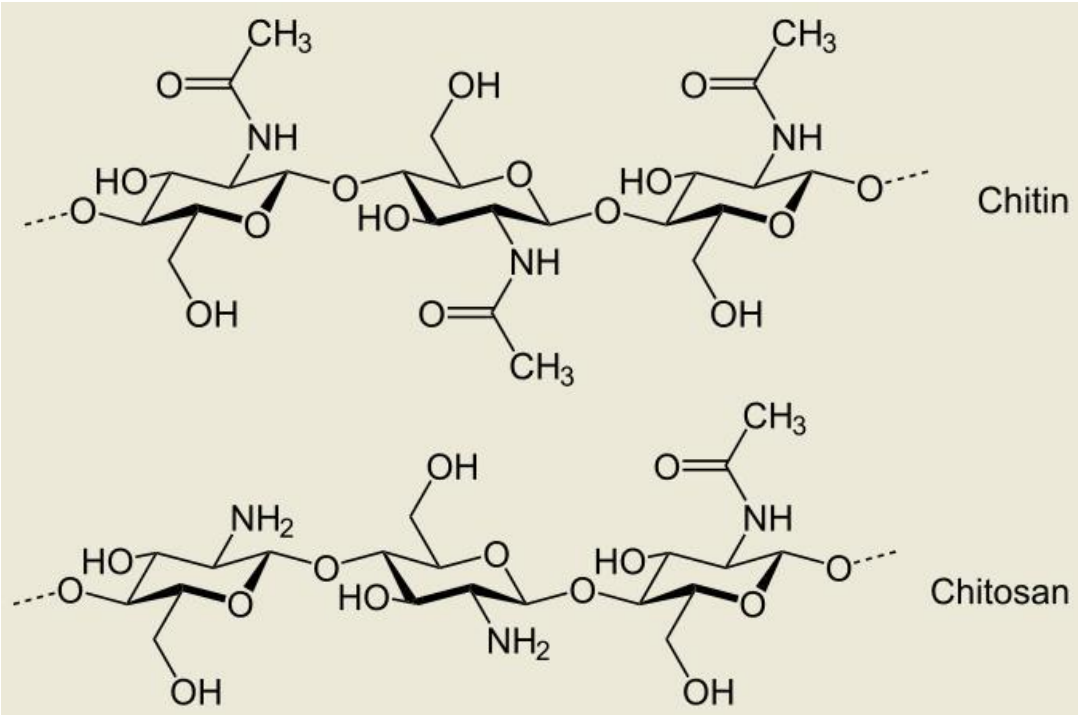

Figure 1.7. Molecular structures of chitin and chitosan.

1.3.2 Degradation by Radiation Processing

Radiation processing technology by γ-rays has been identified as versatile tool in controlling the process of degradation of chitosan and economical way in term mass production, in the preparations of PGP (Fig. 1.8). The degradation of chitosan can be carried out in the form of powder or in solution form by dissolving 3% chitosan in 2% acetic or lactic acid. Irradiation of chitosan in solid state needs very high dose and followed by fractionation process to produce oligochitosan. However, irradiation of chitosan in solution state will only need low dose less than 20 kGy to form oligochitosan compare to powder form. Irradiation of chitosan solution by γ-rays can be carried out either in batch type using drum or continuous flow system using column.

Figure 1.8. Mixing tank for preparation of chitosan (left), and irradiation vessels (center and right).

Addition of hydrogen peroxide about 0.1% to 0.3% into the chitosan solution prior to irradiation reduced further radiation dose needed for formation of oligochitosan with average molecular weight 10 kDa.

Figure 1.9. Oligochitosan solution

Chitosan like other natural polymers such as cellulose, carrageenan and alginates predominantly undergo scission or degradation when exposed to ionizing radiation to form low molecular weight chitosan.(Fig.1.9) Ionization radiation such as γ-rays and electron beam, can be used to perform the degradation process.

High molecular weight (HMw) chitosan has limitation to be used in the agriculture application as PGP due to it less solubility in weak acid. However, oligochitosan with molecular weight about 10 kDa is soluble in water and has been identified as PGP and give elicitor effect to resist disease infection on plant. Further degradation of HMw chitosan into oligochitosan needed much higher irradiation dose which is not economical for production of oligochitosan.

Irradiation of HMw chitosan in solution form of acetic or lactic acid has been identified to improve the degradation process to oligochitosan at low irradiation dose. Study has shown that by adding 0.1 to 0.3% hydrogen peroxide in the HMw chitosan solution will further reduce radiation dose needed to produce oligochitosan.(Fig.1.10)

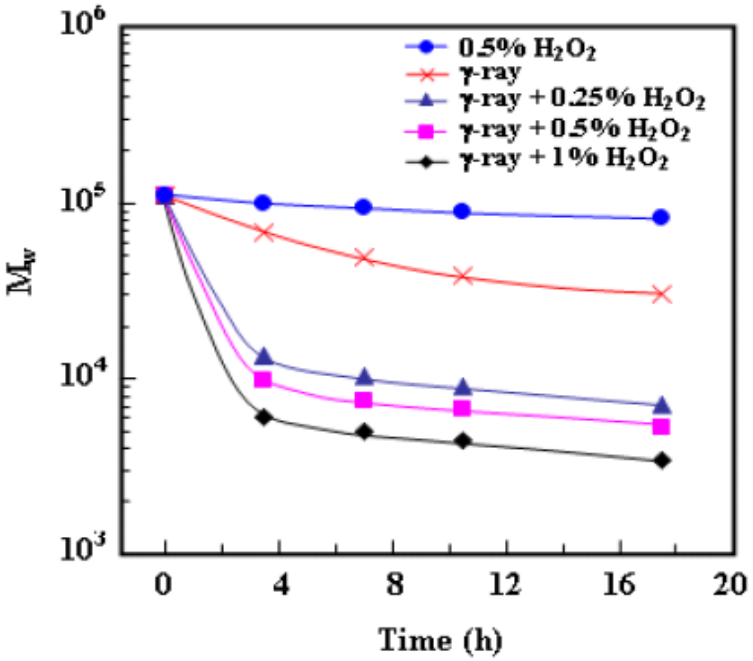

Figure 1.10. Synergistic effect of hydrogen peroxide on degradation of 3% chitosan solution by γ-ray irradiation

1.4 Advantages of PGP prepared by Radiation Process

Degradation process of chitosan to produce PGP can also be carried out by chemical or enzymatic process. However, radiation processing technology has more advantages over chemical and enzymatic processes and preferable process for degradation of chitosan due to several reasons, such as

i) process of degradation can be performed at room temperature,
ii) easy to control the degree of degradation by varying irradiation dose,
iii) practical for large production and cost effective.

1.5 References

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Part 2. PGP (Degraded Chitosan)

2.1 Preparation (Citing Preparation Guideline, Additive, etc.)

Chitosan is a linear aminopolysaccharide (polyglucosamine) derived from chitin, a naturally abundant mucopolysaccharide, poly ß-(1→4)-linked N-acetyl-D-glucosamine by alkali deacetylation.(Fig. 2.1)

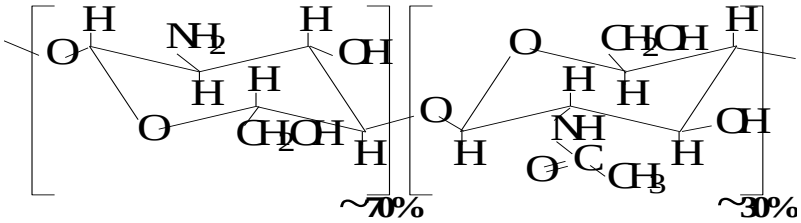

Figure 2.1. The molecular structure of chitosan with degree of deacetylation of ~70%

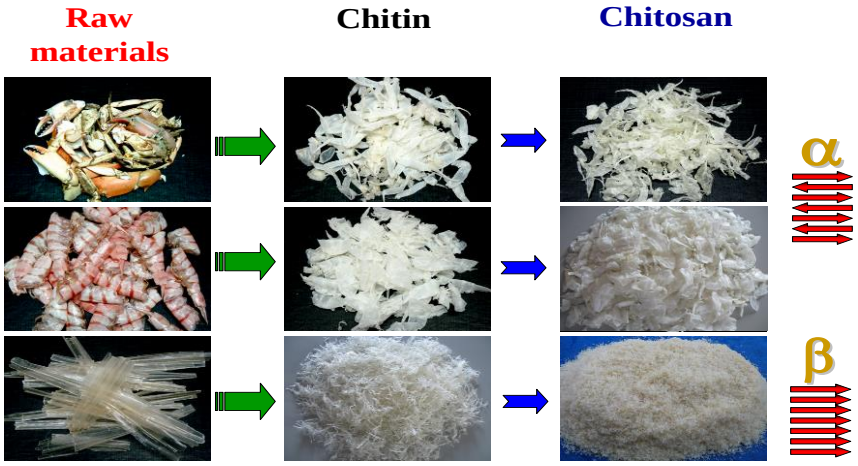

Figure 2.2. The major sources from shrimp, crab shells and squid pens for chitin and chitosan production (IAEA/RCA RTC, 19-23 Oct., 2009, Bangkok, Thailand)

Chitosan has a wide range of applications, such as in waste water treatment, in medicine and cosmetics, in food and functional food and in agriculture [1-4]. The production of chitin/chitosan is currently based on crab and shrimp shells discarded as a food industry waste. The global annual estimate of shellfish processing discards is more than one million metric tons [1]. Thus, disposal of shellfish wastes has been a challenge for most of the shellfish-processing countries. Therefore, production of value-

added products such as chitin/chitosan, oligomers and their derivatives for utilization in different fields is of utmost interest.(Fig.2.2)

Study on preparation of low molecular weight (Mw) chitosan and oligochitosan by irradiation (γ-rays, electron beam) has been carrying out in many radiation processing research centers due to their beneficial properties and potential applications in different fields. The weight-average Mw from 10,000 to about 100,000 is considered for low Mw chitosan, while Mw of oligochitosan is generally less than 10,000. Chitosan prepared from chitin by the deacetylation process has generally high Mw which in many cases limits its applications.

The low Mw chitosan and its oligomer have some special biological properties which are different from that of the ordinary high Mw chitosan such as antioxidant property [5,6], antimicrobial property [7-10], antitumor activity [11], immunity stimulation for animal [12,13] and for plants [14-16]. Comprehensive information of biological activities of chitosan and oligochitosan can be referred to the articles reviewed recently by Kim & Rajapakse [17] and Xia et al. [18]. A variety of techniques including chemical and enzymatic hydrolysis, radiation degradation processes can be used to prepare low Mw chitosan and its oligomer as described by Makuuchi [19]. However, radiation (γ-rays, electron beam) is a useful tool for degradation of polymer from the viewpoint of environmentally friendly processing technology [20].

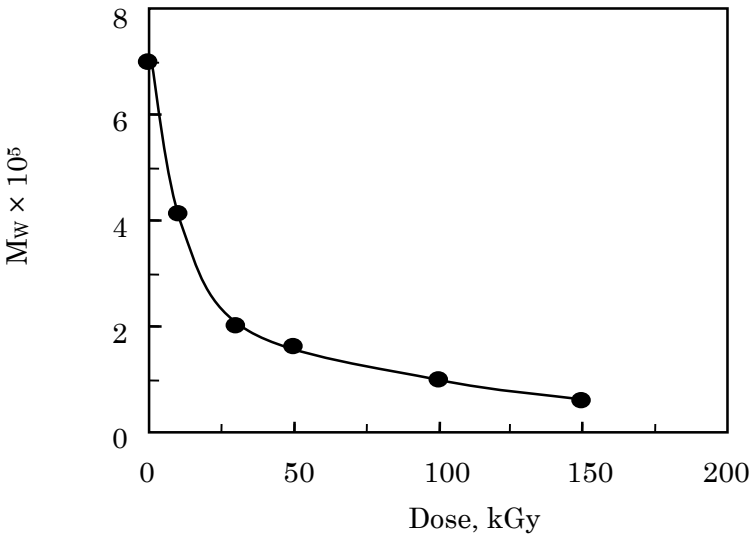

Figure 2.3. Changes in molecular weight of chitosan irradiated by γ-rays in powder and flake form in air with doses

Accordingly, the low Mw chitosan can be conveniently prepared by irradiation of high Mw chitosan in powder or flake form [14, 21-23](Fig.2.3). Ulanski & Rosiak reported Gd-values (scission/dose of 100 eV)at 0.9, 1.1 and 1.3 scissions/100 eV for chitosan powder irradiated in vacuum, in air and in oxygen, respectively [21]. However, Czechowska-Biskup reported the higher Gd value for chitosan irradiated in air (Gd = 0.6 μmol/J eq. to 5.79 scission/100 eV) [24]. The reason for the difference in Gd values may be due to many factors that needs further study to identify.

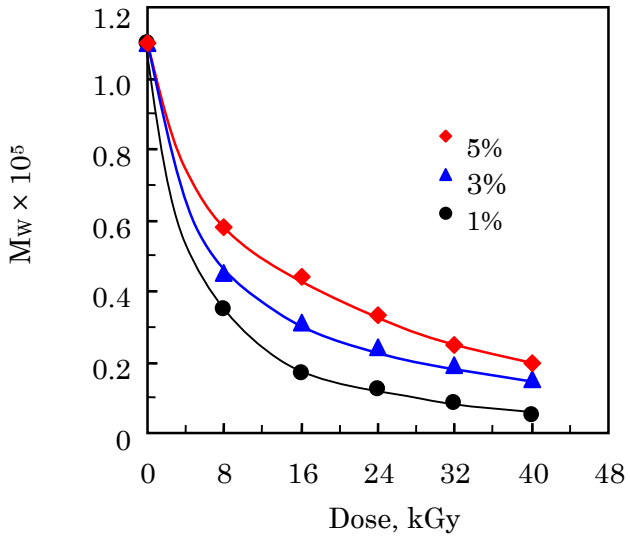

Figure 2.4. Changes in molecular weight of chitosan in acid solution irradiated with γ-rays

Oligochitosan has been produced in most cases by irradiation of chitosan in solution [14, 25, 26]. Gd values were found out to be of 0.058, 0.08 and 0.10 μmol/J for 1%, 3% and 5% chitosan solution, respectively, based on the results of molecular weight presented in Fig. 2.4. Wasikiewicz reported higher Gd value (0.353 μmol/J) for 1% chitosan solution [26]. The reason may be the difference of deacetylation degree of chitosan used for study, because chitin is more stable to degradation than chitosan.

The cost of enzymatic hydrolysis process for production of oligochitosan is generally higher than that of oxidative degradation process [19]. However, oxidative degradation has disadvantages mainly due to chemical structure changes caused by the formation of carboxyl groups, deamination and even breakage of glucoside ring [27]. In addition, chemical

process has some drawbacks due to low production yields and higher pollution risk to environment [17]. In general, radiation degradation to prepare oligochitosan needs high dose so that it is not convenient for large scale production.

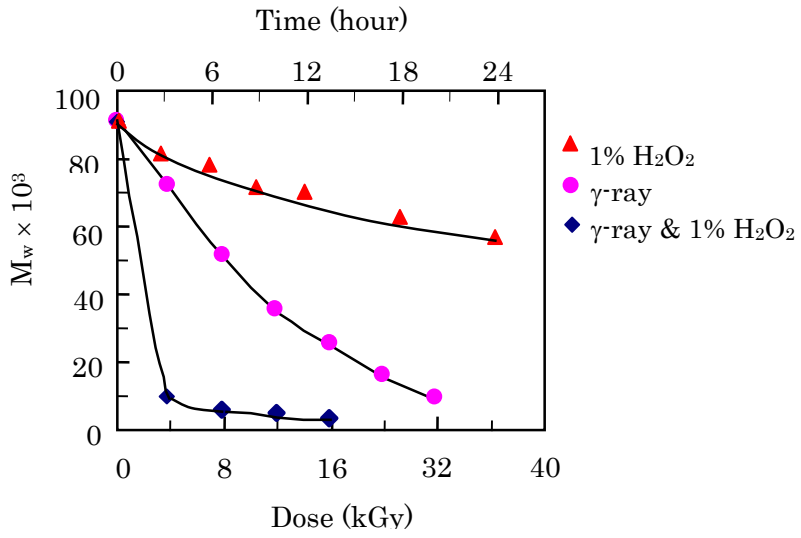

Figure 2.5. Changes in molecular weight of β chitosan treated with H_2O_2, γ-rays and H_2O_2/γ- rays versus treatment time and dose (dose rate: 1.33 kGy/h)

Thus, in order to degrade chitosan effectively, synergistic degradation by combination of hydrogen peroxide (H_2O_2) with γ-rays [28-30], ultraviolet light [31] and microwave [32] has been studied. According to the results reported by Kang et al. [28] and El-Sawy et al. [29], chitosan in the suspension or paste form mixed with high concentration of H_2O_2 (10-30%) was degraded by γ-ray irradiation to prepare oligochitosan in high dose range from 20 to more than 100 kGy. Furthermore, according to the results of the synergic effect of γ-rays and H_2O_2 for degradation of β-chitosan in solution reported by Hien et al. [30], oligochitosan with different Mw less than 10,000 can be produced in low dose range up to 16 kGy with the addition of a small amount of 1% H_2O_2 into 5% β chitosan solution so that it contributes to reduce the cost of production (Fig. 2.5).

However, according to the experiences in setting up large scale of oligochitosan manufacture by γ-ray irradiating chitosan solution (200 litters/batch in Vietnam, 500 litters/batch in Indonesia and 1,000 litters/one run in Malaysia), it was recognized that the highly viscous chitosan solution (>3%) is not suitable for processing. Therefore, study to prepare

oligochitosan with different Mw less than 10,000 by γ-ray irradiation of 3% chitosan solution containing H_2O_2 with various concentration (0.25 - 1%) has been carried out [33]. The influence of varying dose on the changes of molecular weight and radiation degradation yield of chitosan was investigated. The synergistic degradation effect induced by γ-rays and H_2O_2 was also calculated based on the percentage of the reduction (100 × Mw/Mw0) of chitosan molecular weight [30].

Proposed Procedure for Production of Oligochitosan

- Chitosan (powder and/or flake form) with DD 70-90% and Mw 1 – 2 × 10^5 g/mol.
- Dissolving chitosan with concentration of 30 g/L in 1.5% lactic acid.
- Keep standing overnight and then filtering through stainless steel net (100 mesh).
- Adding H_2O_2 into chitosan solution with final concentration of about 0.5%.
- Irradiation of chitosan/H_2O_2 solution at average dose of about 10-15 kGy.
- Neutralization of irradiated chitosan solution by NaOH 2 M to pH 5.0-5.5.
- Adding preservative (0.2% sodium benzoate).
- Packaging final product: biotic elicitor for plants.

Characteristics of the biotic oligochitosan plant growth promoter and elicitor:

Oligochitosan concentration: 30 g/L (30,000 ppm)
Molecular weight (Mw): 5 – 10 × 10^3 g/mol.
pH ~ 5.0-5.5
Appearance: yellowish liquid
Expire date: 2 years

2.2 Preservation

In order to maintain the qualitative stability and for long time storage in ambient condition of oligochitosan solution, appropriate preservatives should be used mainly from the infection of the certain fungus

species. Typical preservative agents are suggested to add to oligochitosan solution: sodium benzoate (0.2% w/v), potassium sorbate (0.2% w/v) or alternatively ethyl alcohol (40% v/v).

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Part 3. Rice

3.1 Cultivations of Rice

Preparation of planting is divided into 4 main steps, based on the farmer practices recommended by FELCRA Berhad, the fully goverment owned company administered by Malaysian Ministry of Rural and Regional Development which own largest area of rice plantation in Peninsular Malaysia. They are :

i. Management of rice straw

After harvesting, rice straw is left sun-dried. Additionally, herbicide is applied to enhance the decomposition of straw. Then the straw was cut using hoe or flail mower. The straw was then burnt in a direction opposite the normal wind flow in order to provide perfect combustion. The burning procedure also helps to kill the pest (e.g snails) in the soil.

ii. Land smoothing

In Malaysia, land smooting procedure is classed into two categories which are minor and major land smoothing. The minor land smoothing is done in every season before planting, while major land smoothing is done once in 3-4 seasons of planting. The equipments are usually used during land smooting are rotovator and tractor backbucket. The rotovator is used to cut the high spots while tractor backbucket is used to filling the low spots and levelling/smoothing the land.

iii. Ploughing

Plouging is for land grading and levelling before planting. Besides, ploughing also promotes elimination of wild weed and reduces the germination of weedy rice (locally known as padi angin), which induces crop losses by reducing the yield and quality of the rice. The rotovator is used to break the soil into smaller soil granules. Meantime, the herbicides and pesticides are applied during ploughing to get rid of the wild weed and pests (e.g snails). The Niplow harrow is used at the end of the ploughing procedure for final land grading and leveling before start planting.

iv. Planting

In Malaysia, there are three types planting methods i.e direct seeding in wet condition, direct seeding in saturated condition and transplanting. All these methods require germination enhancer during seed treatment (seed soaking) to increase the percentage of seed germinate. Most of the

commercial products contain very strong acidic solution such as nitric acid as seed germination enhancer.

Transplanting of rice seedling

Direct seeding in water

Figure 3. 1. Photos of palnting.

3.2 Recommended Application

Nuclear Malaysia has done research on application of oligochitosan on local rice plant, MR 219, for more than 5 years and the research still going on. Oligochitosan is applied at 3 different levels, which are :

i. Seed – seeds are soeked in the oligochitosan solution at concentration of 100 ppm for 24 hours.

ii. Seedling – seedlings are sprayed with oligochitosan 40 ppm at day 3, 7 and 14 after sowing. After first 3 weeks at nursery stage (either on the field or nursery shed), the seedlings are transfered to the real field.

iii. At the field – the oligochitosan is sprayed at the field 4 times (Day 20, 30, 40 & 70) at concentration of 10, 20, 40 and 100 ppm. Spray timing is 2 days prior normal fertilizing. The oligochitosan helps to strengthen the stem of seedlings, promotes growth of new seedlings from the mother plant, enhances the growth of fruit panicles and boosting fruit quality by maintaning the strength and health of panicle and plant overall (protect from blast or bacterial attack).

Figure 3. 2. Photos of Seed soaking (left) and treated rice seed for seedling (right).

3.2.1 Application Protocol for MR 219 and Mutant MR 219-9 and MR 219-4

Rice MR 219 is one of the most planted rice variety by Malaysian farmers. It was developed by MARDI obtained from the cross between of MR 137 x MR 151 and been released on January, 9th 2001. The characteristics of MR 219 are :

a. has short maturation period (105 – 111 days)
b. highly resistance to bacterial, leaf blight, blast and brown plant hopper.
c. long grades grain
d. low amylose content (21.4%) – gives soft texture of cooked rice.
e. able to produce high yield (10 Mt/ha) – requires high fertilizer and good water management

Meanwhile MR219-4 and MR 219-9 are the mutant varieties obtained from the γ-ray irradiation of MR 219 at dose 300 Gy. It is developed by researchers from Nucler Malaysia and UPM (Putra University of Malysia). They copy the characteristics of MR 219 and exhibit other special characteristics such as :

a. Longer panicle
b. High percentage of filled grains
c. Can adapt in flooded and water stress regimes (aerobic soil)

The application method of oligochitosan on MR 219-4 and MR 219-6 are same as for MR 219. The method is described below :

a. Seed treatment
    i. The oligochitosan solution 100 ppm is prepared by dissolving 5ml oligochitosan of 20,000 ppm for preparation of 1 L solution
    ii. Seeds are soked in the oligochitosan solution 200 ppm for 24 hours. Make sure all the seeds are well-immersed in the oligochitosan solution.
    iii. Then the seeds are dripped for 24 hours.
b. Seedling stage (foliar)
    i. The oligochitosan solution 40 ppm is prepared by dissolving 2 ml oligochitosan of 20,000 ppm for preparation of 1 L solution.
    ii. The oligochitosan is sprayed onto the seddling.
    iii. The application is done once a week for 3 weeks (Day 3, 7 and 14).
c. At the field (foliar)
    i. The oligochitosan solution 40, 60 and 100 ppm is prepared by dissolving 2 ml, 3 ml and 5 ml of oligochitosan of 20,000 ppm respectively for preparation of 1L solution.
    ii. The oligochitosan solution is sprayed onto the plants.
    iii. The application is done on each 2 days prior normal fertilizing. Total application is 4 times (Day 20, 30, 40 & 70) starting on day 3 after plants are transferred procedure.

3.3 Effect of Seed Treatment

Table 3.1 shows the percentage of seeds germinate after treated with oligochitosan 100 ppm at different soaking duration. On day 1, soaking duration of 15 min., 30 min. and 60 min. give less than 50% of germinated seeds. For 90 min. and 120 min. the total of germinate seeds are more than 50% but the germination rate is still very low. The seed soaking for 24 h gave the highest percentage of seeds germinate i.e more than 95%. Based on the farmers' experience, the soaking duration of rice seeds is recommended for 24 h and not less than that. It is because the rice husk is very hard and seed treatment requires longer time to make sure the solution treatment can penetrate into the inner layer of the husk. Meanwhile for seed with has

soft outer layer such as Chilli seed, the soaking period should be around 1 h to 2 h and if too long the quality of seeds become deteriorated.

Table 3. 1 Effect of soaking duration on rice seed germination

Oligochitosan 100 ppm		Day 1	Day 2	Day 3	Day 4	Day 7
15 min	a	47	85	91	91	93
	b	53	89	89	91	93
	c	38	84	88	88	92
	Average	**46**	**86**	**89.3**	**90**	**92.7**
30 min	a	53	85	85	90	92
	b	42	81	86	89	91
	c	40	75	84	85	92
	Average	**45**	**80.3**	**85**	**88**	**91.7**
60 min	a	34	77	82	89	96
	b	28	60	78	80	92
	c	32	60	77	81	89
	Average	**31.3**	**65.7**	**79**	**83.3**	**92.3**
90 min	a	56	86	93	95	95
	b	52	82	89	90	92
	c	52	75	84	88	88
	Average	**53.3**	**81**	**88.7**	**91**	**91.7**
120 min	a	60	84	90	92	95
	b	53	83	93	92	96
	c	60	87	92	94	95
	Average	**57.7**	**84.7**	**91.7**	**92.7**	**95.3**
24 hrs	a	95	97	99	99	99
	b	93	98	99	99	99
	c	98	99	99	99	99
	Average	**95.3**	**98**	**99**	**99**	**99**

3.4 Effect of Concentration

Figure 3.3. illustrates the germination rate of the rice seeds after treated with oligochitosan at different concentration. On day 1, the highest

germination rate is treatment oligochitosan 100 ppm which is more than 82%. The lowest germination rate is oligochitosan 200 ppm treatment which is only 61.7% . Based on this result, oligochitosan 100 ppm treatement is considered to be the best treatment for seed germiantion stage.

As for seedling or nursery stage, oligochitosan 40 ppm treatment is selected to be used. It is because the effect of oligochitosan 40 ppm on germination as well as the growth of seedling shows no significant difference with oligochitosan 100 ppm treatment.

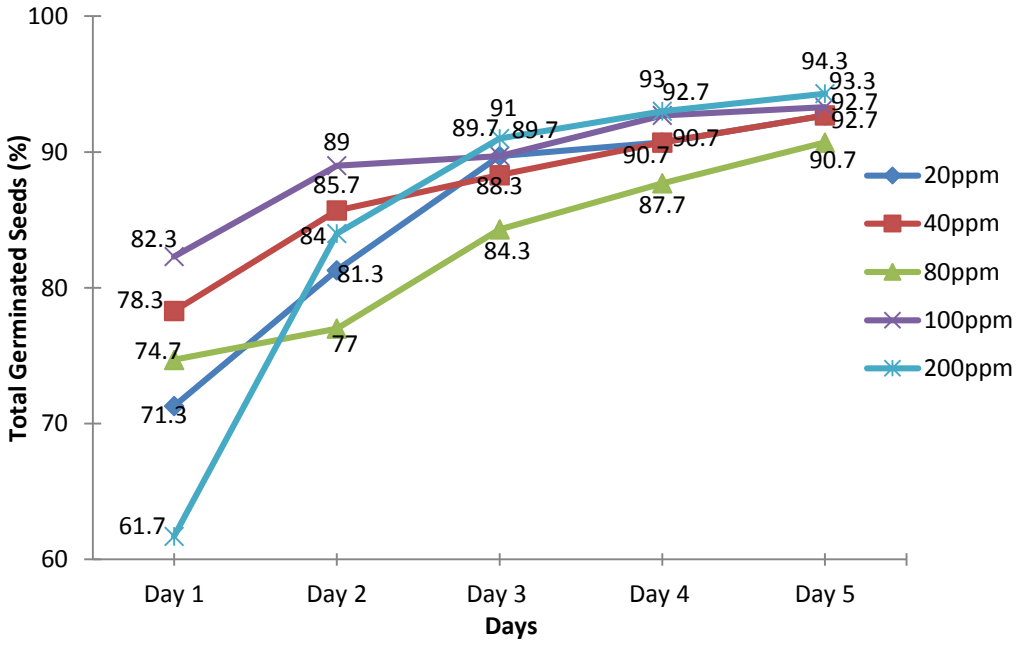

Figure 3. 3. The germination rate of rice seeds after treated with oligochitosan at different concentration

Figure 3. 4 Foliar application of oligochitosan on the field

3.5 Effect of Molecular Weight

In rice project, we never study the effect of oligochitosan molecular weight on the growth of rice. During study, we used oligochitosan with single Mw i.e Mw = 10 000 g/mol. The study by El-Sawy and friends (2010) has shown that degraded chitosan has better effect on the growth of faba bean compare to chitosan.

3.6 Effect of Foliar Spray Frequency

Bittelli *et al.* (2001) reported foliar application of chitosan on pepper plants can reduce 26-43% of plant transpiration or water loss while maintaing biomass production and yield. Chitosan has the self- film forming ability, this property induces formation of thin film on the outer layer of the plant skin. This thin film controls the plant transpiration.

In our case, we recommend to use foliar application of chitosan once in a week for the seedling under the shed (nursery stage). At the early stage chitosan applied for the purpose to enhance the production of early leaf. For the mature plants, e.g Chilli – foliar application of chitosan on mature plants has to be done at least (optimum) twice a week for the growth enhancement and protection purposes. As mentioned before, chitosan thin film which obtained after foliar application can protect the plants against disease or microbes. The foliar application twice a week should be continued until harvest time. This is because besides protecting the plants chitosan thin film also provide protection to the fruits as well. The chitosan thin film which formed on the fruit skin preserves the fruit from deterioration easily.

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Part 4. Chilli

4.1 Cultivations of Chilli

Cultivation of Chilli was adopted from the results of research collaboration between National Nuclear Energy Agency (BATAN) and Development Planning Agency of Kerinci Regency, Jambi province. Chilli cultivation divided into three main steps. They are:

1. Preparation of land

   In the cultivation of Chilli, land preparation should come first, followed by preparation of the seed or seedling. In preparation of land, the following steps should be done:

   a. Management of soil
      Soil management is done by digging the ground to clear the land from debris roots of old crop marks and all kinds of weeds that grow. Land plowed or dug as deep as 40 cm to improve soil aeration and to eliminate the intruder organism plant which hiding in the ground. After land cultivated, prepared the raised beds,120 cm width and 30 cm height, and the distance between beds is around 80 cm. The length of each bed is 12 m or adjusted with the trench width. Create good drainage because red Chilli plants are not resistant to water logging. After preparing the beds, the soil was left around 10-14 days up to dry.

   b. Soil acidity (pH)

      Soil pH should be checked. The ideal pH of soil is around 6.5-7.5 (neutral). If soil pH below 6, add agricultural lime (dolomite) with concentration depending on initial pH. If the soil is very acid, as much as 2 ton/ha of dolomite should be applied. Dolomite is added in the same time with soil digging and beds preparation.

   c. Fertilization

      Compost or manure fertilizers with concentration 20 ton/ha was applied on the beds and mixed homogeneously. Instead of organic fertilizer, the basal dressing, a well-mixed fertilizer containing

urea (300 kg/ha), SP36 (250 - 300 kg/ha), and KCl (potassium chloride) (250 kg/ha) was also applied. The beds was then irrigated accordingly in order to dissolve fertilizer

d. Installation of Silver Black Plastic Mulc

Around 12 rolls of mulch are needed per hectare. The mulch is silver on top, and black on the underside. The silver side reflects the light and repels insects, the dark underside controls weeds). The mulch should be laid on the soil in the middle of the day and in full sunlight. The heat will soften the plastic, so it is easier to stretch. The plastic mulch should be spread tightly over the soil, with the silver side facing upwards. The plastic should be pulled tightly down over the sides of the bed, and fastened in place using thin pieces of bamboo. These are about 40 cm long. They are bent in half, and pushed into the soil to hold the edges of the mulch at intervals of 50 cm. A cross is cut in the plastic mulch for a planting hole where each plant will grow. Installation of silver black plastic mulch is shown in Figure 4.1.

Figure 4.1. Preparation and installation of silver black plastic mulch.

2. **Seedlings**

Choose the quality of seed and growing medium. The medium used to grow the seedlings is a mixture of fertile soil and manure, at a ratio of 1:1. Seedlings are grown in a polyethylene (plastic) bag, or in a well-prepared

seed bed. To prepare the seed bed, the soil is cleaned of weeds and rubbish, and then ploughed. Clods of earth should be broken up by hand. The seedlings should have plenty of light, but should be shaded from direct sun by a simple structure. It has a roof of rice straw or similar materials. To prevent seedling from insect attack, insect screen can be used. The seedlings should be watered every day, or as needed. Before seedling, the seed was immersed in a mixture of warm water (50°C) or Previcur N (1 ml) dan bactomycin (1 ml) solution and oligochitosan with concentration of 25-50 ppm for 3 h. Remove the floating seed. Take out the seeds from the solution and dried under sun light for one day. Seed was then transplanted into prepared polybag, one seed for one polyethylene bag. At 10 days seedling, oligochitosan with concentration 50 ppm was sprayed. When the seedlings are 20 - 24 days old (i.e. 20 - 24 days after sowing) they are ready to be transplanted to the beds as can be seen in Figure 4.2.

Figure 4.2. Preparation of seed and seedling of chilli.

3. **Planting**

Two rows of seedlings are planted in each bed. Seedlings should be planted 60 cm apart, with 60 cm between the rows. Transplanting is best done in the early morning or early evening, when it is cool. Before transplanting, the root is immersed in oligochitosan 50 ppm. After planting, watered the plant as needed with low-pressure spray. Seven days after planting, stake from bamboo with height 100-150 cm was plugged in the soil closed to Chilli plant. (Fig. 4.3) One month after planting, plant stems under the main branch tied to the stake.

Figure 4.3. Installation of bamboo stake.

Supplementary fertilization and maintenance play an important role in Chilli planting. A well-mixed fertilizer containing of NPK, Super bionik D, Super Bionik B, and compost was applied at 3, 6, 9 and 12 weeks after planting. Oligochitosan is sprayed to leaf and all part of plant with concentration of 50 ppm every 10 days starting from 15 days after planting.

4.2 Application Protocol for Chilli TM 999 variety

Based on our result on chilli plants variety TM 999 at Kerinci Regency, Jambi Province, the application of oligochitosan is applied at 3 different levels, which are :

i. Seed: seeds are soaked in the oligochitosan solution at concentration of 50 ppm for 3 h.

ii. Seedling: during seedlings stage, oligochitosan is sprayed only once with 50 ppm at 10th day after sowing. After first 3 weeks at nursery stage, the seedlings are transferred to the real field.

iii. At the field: the oligochitosan is sprayed to the leaf and all part of plants every 10 days starting from 15 days after planting.

Oligochitosan is used along of chilli planting cycle. Other pesticides may also be used to prevent diseases.

Chilli or red pepper or chilli (Javanese) is a fruit and plant member of the genus Capsicum. The fruit can be classified as vegetables and spices, depending on how it is used. As a seasoning, spicy chillies are very popular in Southeast Asia including Indonesia as a food flavor enhancer. Chilli TM 999 is one of hybrid type chilli which is the most planted chilli in Kerinci, Jambi Province. This type of chilli Hybrid comes from Hungnong, Korea.
The application method of oligochitosan on Chilli TM 999 is as follows:

d. Seed treatment
   iv. The oligochitosan solution 50 ppm is prepared by dissolving 1 mL oligochitosan of 50,000 ppm for preparation of 1 L solution
   v. Seeds are soaked in the oligochitosan solution 50 ppm for 3-5 hours. Make sure all the seeds are well-immersed in the oligochitosan solution.
   vi. Floating seeds are taken out and discarded. only the seeds that sink (quality seeds) are used for seedling
   vii. Dry the quality seeds under sunrise for one day.

e. Seedling stage (foliar)
   iv. The oligochitosan solution 50 ppm is prepared by dissolving 1 mL oligochitosan of 50,000 ppm for preparation of 1 L solution.
   v. At 10th day after seedling, oligochitosan with concentration 50 ppm was sprayed onto the seedling.
   vi. When the seedlings are 20 - 24 days old (i.e. 20 - 24 days after sowing) they are ready to be transplanted to beds

f. At the field
   iv. The oligochitosan solution 50 ppm is prepared as described above.
   v. The oligochitosan solution is sprayed onto the plants (leaf, trunk and branch).
   vi. The application is done every 10 days starting from 15th day after chilli transplanted to the field. If an insecticide is needed, it is applied at 5 days before applying of oligochitosan.
   vii. Oligochitosan can be used until chilli is harvested.

4.2.1.Effect of Concentration

Research on the effect of oligochitosan concentration to chilli plants has been done at Kerinci Regency, Jambi Province under collaboration between National Nuclear Energy Agency and Development Planning Agency of Kerinci District, Jambi province. Hybrid TM 999 chilli variety was used. Cultivation system as mention above was adopted in this experiment. Oligochitosan is produced by irradiating of chitosan using γ-rays with dose of 75 kGy. Oligochitosan prepared by BATAN with three different concentrations at 50, and 100 ppm and 0 ppm as control were used. For each concentration of oligochitosan, 80 chilli plants were planted in raised beds, which have 12 m length and 60 cm distance between plants. Each concentration has three replications, so every oligochitosan concentration has 240 plants. Oligochitosan was applied by spraying to leaf and trunk at every 10 days. Insecticides were used when it is needed. Several parameters such as plant height, number of branch, trunk circumference were measured at 30 and 45 days after panting, and yield in every harvesting times as well as total yield were also calculated. For measurement of plant height, number of branch, trunk circumference, the results are the average value from 15 plants for each concentration of oligochitosan while for calculation yield of each harvesting and total yields of each treatment, the results are from total yield of 240 plants for every harvesting. Chilli fruits which show homogeneous red color were harvested. Normally harvesting conducted every 4 to 5 days.

The results show that at 30 days after planting (DAP), number of branch, trunk circumference and plant height were highest value for plants treated with oligochitosan 50 ppm whereas the lowest for control plant. After 45 DAP number of branch, trunk circumference and plant height show the same results as shown in Table 4.1. It was found that at 25 DAP, the plants have disease which the symptom are the curling of leaves, their small size, shortened internodes and general dwarfing of the plant which assumes a bushy appearance, leaves are of pale (light yellow) color and roll downwards. This disease probably attacked by *Cucumber Mosaic Virus* transmitted by aphids and whitefly. To prevent spread out of diseases, insecticides were applied for all plants. However, control plants still showed severe diseases while plants treated by 50 and 100 ppm oligochitosan significantly reduced diseases. These results showed that plants treated by chitosan more resistant to diseases. By continue applying oligochitosan as scheduled, chilli plants treated with 50 and 100 ppm recovered from diseases while control

plant still does not recover.

In Table 4.2, yield of chilli at different harvesting times versus o-chitosan concentration. Chilli without oligochitosan treatment only can be harvested up to 14 times in one planting cycle, while chilli treated with oligochitosan harvested up to 24 times. Chilli treated with 50 ppm oligochitosan showed highest total yield in every harvesting times as can be seen in Tables 4.1 and 4.2. Total yield of chilli without oligochitosan treatment (control), treated with 50 and 100 ppm harvested for each treatment from 240 plants are 82, 292.3, and 251.4 kg, respectively. It is can be concluded that the best concentration of oligochitosan for chilli is 50 ppm.

Table 4.1. Effect of oligochitosan concentration on the average plants height, number of branch and trunk circumference at 30 and 45 days after planting

O-chitosan concentration, ppm	Plant height	number of branch	trunk circumference
At 30 days after planting			
0	45	8.4	4.7
50	70	10	6
100	60	9.5	5.4
At 45 days after planting			
0	72	10.4	6
50	92	13	7
100	85	12	7

Table 4.2. Yield of chilli at different harvesting times versus oligochitosan concentration. Each chitosan concentration has 240 plants

Harvesting times	Yield (kg) at different oligochitosan concentration		
	0 ppm	50 ppm	100 ppm
1	0.17	0.23	0.2
2	0.35	0.7	0.6
3	1.2	2.8	1.8
4	3.5	7	5.2
5	9.3	12.3	10.6
6	11	19	16
7	6.6	11,8	9.1
8	10	26	26.2
9	4.5	8.8	9.2
10	10	24	20.5
11	8	23	20
12	11	15.4	15.1
13	5	9.8	10
14	2	16	15
15	0	15.2	14
16	0	16	13.4
17	0	16.5	12
18	0	17	15
19	0	15	9
20	0	17	15
21	0	18	15
22	0	23	20
23	0	3.7	0
24	0	1.2	0
TOTAL	**82.86**	**292.3**	**251.4**

4.2.2. Effect of Molecular Weight

Chitosan obtained from deacetylation of chitin was irradiated with γ-rays at doses of 25, 50, and 100 kGy with dose rate of 6 kGy/h. Then irradiated chitosan was diluted by using acetic acid to get concentration of 5 % (b/v) named concentrated oligochitosan. The viscosity average molecular weight (Mv) was measured by using viscosimetry Oswald method. The viscosity average molecular weight of oligochitosan decreased with an increase in irradiation dose as shown in Table 4.3.

Table 4.3. Effect of irradiation dose on viscosity average molecular weight of chitosan

Dose (kGy)	Mv (kDa)
0	51.7
25	12.7
50	9.67
100	2.09

The concentrated oligochitosan with various molecular weights then diluted with tap water with ratio of 1:1000 (v/v) to get 50 ppm oligochitosan for foliar spray. The Capsicum annum plant was watered using 50 ppm of oligochitosan once in two days. The height and dried weight of plants were observed at 30 days after planted. The result in Table 4.4 shows that the height of Capsicum annum plants was increasing with decreasing molecular weight (Mv) of irradiated chitosan which used for watering plant. The highest degree of increasing of plant is 79.7% obtained by watering plant with oligochitosan having Mv of 2.09 kDa which irradiated at dose of 100 kGy. The same result has observed at dried weight of plant as presented in Table 4.5.

Table 4.4. Effect of irradiated chitosan on height of capsicum annum plant

No	Mv, kDa	Height of Plant (cm)										Average	Degree of increasing (%)
		No of plant											
		1	2	3	4	5	6	7	8	9	10		
1	51.742	34	36	35	37	33	34	35	39	34	34	35.1±1.34	30.4
2	12.713	37	38	39	37	38	39	38	37	36	38	37.7±0.76	40.0
3	9.665	45	45	44	46	47	44	45	46	47	48	45.7±1.10	69.8
4	2.09	48	48	47	49	50	48	47	48	49	48	48.2±0.68	79.7
5	Control (water)	26	28	28	29	25	26	27	25	28	27	26.9±1.12	0

Table 4.5. Effect of irradiated chitosan on dried weight of capsicum annum plant

No	Mv, kDa	The average of dried weight (g)	Degree of increasing (%)
1	51.742	10.8	31.7
2	12.713	12.2	48.7
3	9.665	15.8	92.6
4	2.09	16.2	97.5
5	Control (water)	8.2	0

The disease index of Capsicum annum and Capsicum frutescens plants shown in Table 4.6. The disease index decrease from 20 to 0.83% for Capsicum annum plant after treated by oligochitosan irradiated with 100 kGy. The decreasing of disease index from 12.5 to 2.08% also observed on

Capsicum frutescens plant.

Table 4.6. Effect of oligochitosan on disease index of capsicum annum and capsicum frutescens plants

1. Capsicum annum

Treatment	No sample	Height of plant (cm)	Amount of shoots (pieces)	Intensity of virus attack (%)	Note
With Oligochitosan: Total plants are 240. Two plants were attack by virus.					
	1	46	59	0.83	2/240 x100% = 0.83 %
	2	44	29		
	3	35	72		
	4	57	146		
	5	54	68		
	6	48	24		
	7	54	119		
	8	50	60		
	9	59	83		
	10	54	55		
Average		**50.1**	**71.5**		
Without Oligochitosan: Total plants are 200. Forty plants were attack by virus					
	1	39	30	20	40/200 x100% = 20%
	2	36	28		
	3	56	29		
	4	51	32		
	5	50	62		
	6	35	28		
	7	42	32		
	8	40	30		
	9	34	30		
	10	30	25		

Average	41.3	32.6		

2. Capsicum frutescens

With oligochitosan: Total plants are 240. Five plants were attacked by virus					
	1	48	10	2.08	5/240 x100% = 2.08%
	2	32	4		
	3	38	4		
	4	29	5		
	5	47	10		
	6	62	12		
	7	26	6		
	8	25	1		
	9	29	12		
	10	63	33		
Average		**39.9**	**9.7**		
Without Oligochitosan: Total plants are 200. Twenty five plants were attack by virus					
	1	20	4	12.5	25/200x100%=12.5%
	2	12	4		
	3	38	9		
	4	40.5	4		
	5	40	6		
	6	25	4		
	7	25	5		
	8	30	5		
	9	25	4		
	10	38	7		
Average		**29.35**	**5.2**		

4.2.3. Foliar Spray

The oligochitosan solution 50 ppm is prepared as described previously. The oligochitosan solution is sprayed onto the plants (leaf, trunk and branch). The application is done every 10 days starting from 15th day after chilli transplanted to the field. If an insecticide is needed, it is applied at 5 days before applying of oligochitosan. Oligochitosan can be used until chilli is harvested.

4.3 Application Protocol for Kulai Hybrid F1 S469

Chilli Kulai (*Capsicum annum*) is originated from Taiwan. The characteristic of Chilli Kulai are :

i. High percentage of seed germination
ii. Plant height : 80 cm – 110 cm
iii. Fruith length : 10-13 cm
iv. Fruit weight : 12-14 g/fruit

Meanwhile Chilli Kulai Hybrid F1 469 exhibit better charactersitics than Chilli Kulai, such as :

i. Very high percentage of seed germination : >80%
ii. Plant height : average 35 cm (fast growing, uniform growth rate)
iii. High yield : 25 g/fruit (able to produce up to 180 fruits per tree)
iv. Fruit length : 17 cm with thick layer of the outer skin
v. Resistant to disease

The method of oligochitosan application on Chilli plant is described below:

a. Seed treatment
    i. The oligochitosan solution 100 ppm is prepared by dissolving 5ml oligochitosan for preparation of 1 L solution.
    ii. Seeds are soaked and immersed in the ooligochitosan solution for 2 hours.
    iii. Then the seeds are dripped and plant on the next day on wet peat moss (tray).
b. Nursery
    i. As normal practice, the seedling are kept under nursery shed for about 30 days.

ii. The oligochitosan solution at concentration of 40 ppm is sprayed on the seedling once in a week

c. At field

i. The seedlings are transfered to the field (in plastic bag).

ii. The oligochitosan solution at concentration of 100 ppm is spray to the plants for twice a week. Recommended on day 1 and day 4 of the week, in the morning.

4.3.1 Effect of Concentration

Data are means of ten replicates. Different superscripts in the same column are significantly different between the treatments at 5% level according to Duncan's new Multiple Range Test (DMRT).

The results in Table 4.7 and Figure 4.5 show that oligochitosan enhanced the height of the Chilli plants. The optimum concentration of oligochitosan for height increase of Chilli plants was 80 ppm. The Chilli plants treated with 80 ppm of oligochitosan showed significant differences, statistically, in Chilli height over the control plants.

Table 4.7: Effect of Oligochitosan on the Height of Chilli Plants.

Concentration of Oligochitosan (ppm)	Chilli Height	
	1 month (cm)	2 months (cm)
0	5.51 ± 0.78^{b}	9.25 ± 1.40^{c}
20	6.00 ± 1.18^{ab}	15.81 ± 1.76^{b}
30	6.04 ± 1.05^{ab}	16.56 ± 2.22^{ab}
40	6.19 ± 1.15^{ab}	17.95 ± 3.39^{ab}
80	6.73 ± 0.77^{a}	18.53 ± 3.05^{a}

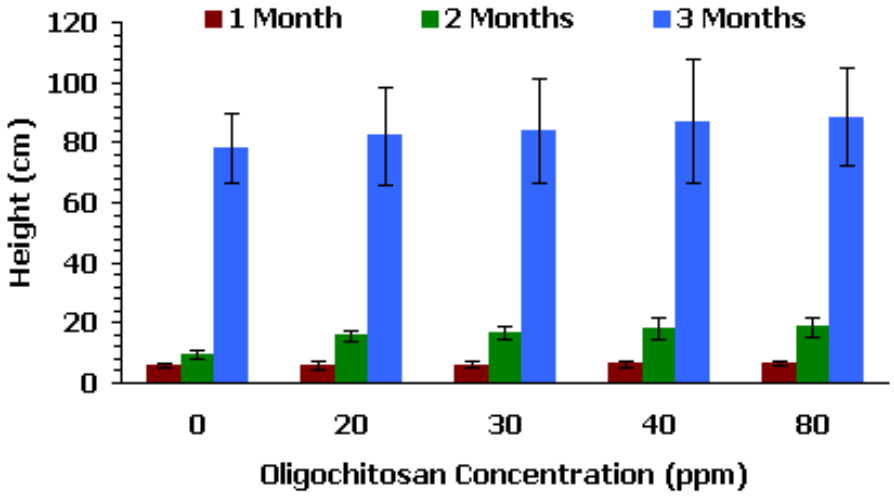

Figure 4.5. Effect of oligochitosan on the height of chilli plants.

Table 4.8: Effect of Oligochitosan on Chilli's Productivity.

Conc.	Total weight of chilli (g)	Total number of chilli	Total number of green chilli	Total number of red chilli	Weight per chilli (g)
0	61.10 ± 54.21^{c}	101.10 ± 78.86^{b}	93.20 ± 77.43^{b}	7.90± 9.52^{b}	0.56±0.13^{b}
20	83.10 ± 63.3	114.10 ± 65.13^{b}	104.20 ± 71.40^{ab}	9.90± 16.03^{b}	0.79±060^{ab}
30	129.80 ± 34.80^{ab}	167.70 ± 51.78^{ab}	147.60 ± 32.81^{ab}	20.10±26.54^{ab}	0.79± 0.12^{ab}
40	131.00 ± 26.13^{ab}	168.40 ± 77.12^{ab}	152.80 ± 63.13^{ab}	9.80±6.77^{b}	0.84±0.23^{ab}
80	180.10 ± 82.18^{a}	192.40 ± 95.36^{a}	158.60 ± 75.62^{a}	39.60±61.41^{a}	0.99± 0.23^{a}

Table 4.8 and Figure 4.6 show the effect of oligochitosan on chilli's production. The results showed that the treatment of chilli plants with oligochitosan enhanced the productivity. Total weight of chilli, total number of chilli, total number of green chilli, total number of red chilli and weight per chilli increased significantly for the chilli plants treated with 80 ppm oligochitosan compared to the control group, as can be seen in Figures 4.7 – 4.10. It was found that there was 34% increase in total weight of chilli compared to the control. Chilli plants treated with oligochitosan displayed

an ability to protect aphid infection. It was found that the untreated chilli plants were badly damaged by aphid infection as shown in Figure 4.7. The untreated chilli plants exhibited various aphid damages such as decreased growth rates, mottled leaves, yellowing, curled leaves and finally death. The removal of sap creates a lack of vigour in the chilli plant and aphid saliva is toxic to chilli plants. Aphids frequently transmit disease causing organisms like plant viruses to chilli plants. Additionally, oligochitosan advanced flowering time. At 70 days after seedling, chilli plants treated with oligochitosan already gave chilli, while the untreated chilli plants just began flowering. The harvest time of chilli plants treated with oligochitosan was three weeks shorter than the untreated chilli plants.

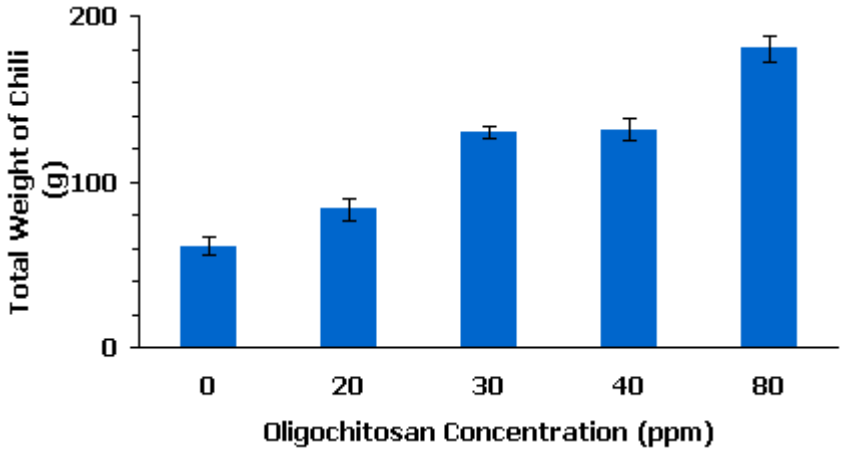

Figure 4.6. Effect of oligochitosan on total weight of chilli.

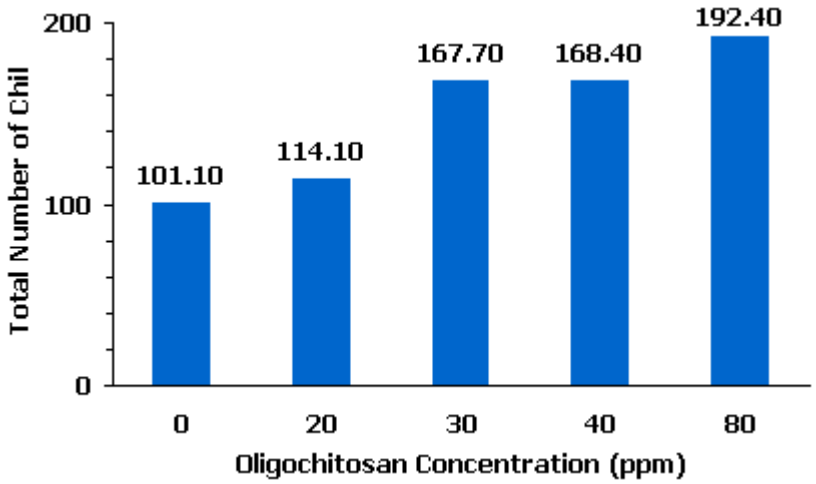

Figure 4.7. Effect of oligochitosan on total number of chilli.

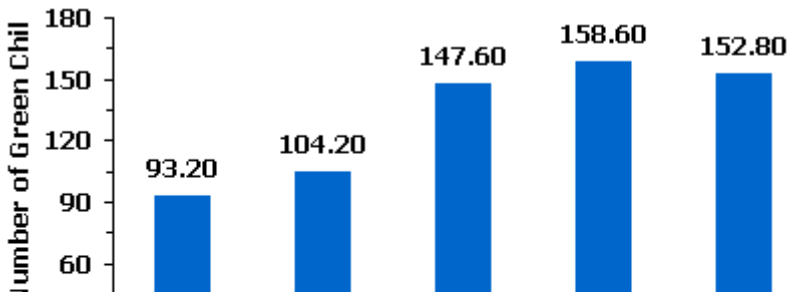

Figure 4.8. Effect of oligochitosan on total number of green chilli.

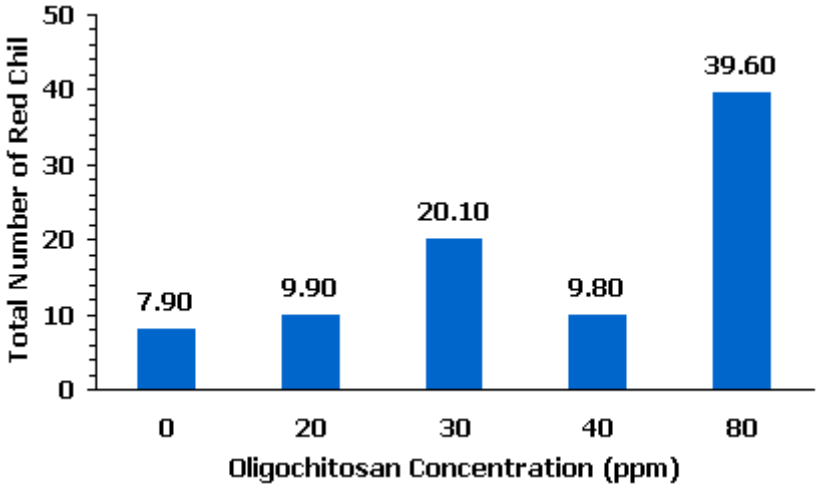

Figure 4.9. Effect of oligochitosan on total number of red chilli.

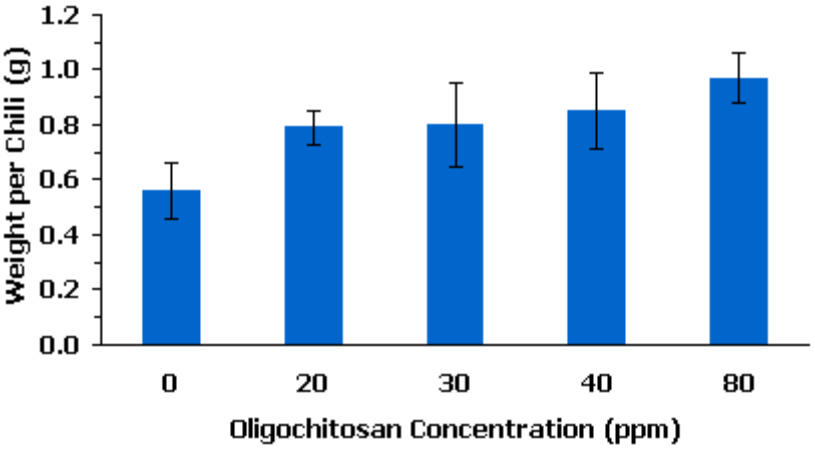

Figure 4.10. Effect of oligochitosan on weight per chilli.

Figure 4.11. Effect of oligochitosan on aphid inflection.

4.3.2 Effect of Molecular Weight

Table 4.9 demonstrates the effect oligochitosan with different Mw on growth of Kulai Chilli Hybrid seedlings. Oligochitosn with Mw at 6.5 kDa shows highest germination rate i.e 85.2% among oligochitosans. Oligochitosan at 6.5 kDa show insignificant difference compare to oligochitosan at 18 kDa on height of plant and number of leaf. The oligochitosan with Mw at 38.8 kDa suppressed the growth of seedlings due to the lowest number in germination (%), height of plants and number of leaf. Based on this result, it be concluded that oligochitosan with Mw value below 20 kDa indicates positive effect on the growth of seedlings and plants.

Table 4.9. Effect of oligochitosans with different Mw on growth of Kulai Chilli Hybrid seedlings.

Mw, kDa	Germination (%)	Height, cm	No. of leaf
Control (Water)	69.1	9.1 ± 1.733	4.145 ±1.12
6.5	85.2	9.4 ± 1.744	4.44 ± 1.11
18	80	9.2 ± 1.629	4.53 ± 0.99
38.8	56.3	7.974 ± 1.51	3.57 ± 0.94

4.4 References

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Part 5. Q&A

5.1 General

Question: Is oligochitosan (PGP) applicable to all kinds of plants?

Answer: In general, PGP can be used for many kinds of plants but the proper concentration, foliar spray, etc. should still be determined.

Question: Is oligochitosan (PGP) safe to use for plants and the environment?

Answer: It is absolutely safe.

Question: Where can I get oligochitosan (PGP) prepared by radiation processing?

Answer: It can be bought from Thailand, Vietnam, Indonesia and Malaysia.

Question: How much does oligochitosan (PGP) costs?

Answer: The cost of PGP is around USD 10-15 per litre for concentration of 20,000 to 50,000 ppm.

Question: How many litres of oligochitosan (PGP) are needed for 1 hectare?

Answer: It depends on the kind of plant. In the case of chilli, it needs around 6 litres of PGP per hectare.

Question: What is the shelf-life of oligochitosan (PGP)?

Answer: The shelf life of PGP is two years.

Final Remarks

This guideline was complied by formulating the outcomes of electron accelerator application project from 2006 under the framework of FNCA. The previous guideline entitled “FNCA Guideline on Development of Hydrogel and Oligosaccharides by Radiation Processing” has been opened since 2006. This guideline dealt with preparation protocols of hydrogel for soil conditioner and oligosaccharides for plant growth promoter (PGP) using radiation technology. FNCA participant countries as well as non-FNCA countries have followed the described protocols to produce the standard performance materials of Hydrogel and Oligosaccharides. The present guideline was described for agricultural users of PGP. The users can get the information of characteristic, proper use against rice, chilli, etc. when they adopt PGP to enhance the yield of farm products. It is a great pleasure for all contributors and editors that PGP prepared by radiation processing will contribute to the socioeconomic benefit in the agriculture in Asian countries.

APPENDIX. Data sheet obtained in each country

BANGLLADESH

PGP Guide Sheet	
Fill-in Date yy/mm/dd	2014 /04/21
Country	BANGLADESH
Name of Plant	Tomato
Species	
Planting time, season	December, 2013
Harvest time, season	April, 2014
Location (Temperature, Humidity etc.)	BINA sub-station Ishurdi District, Bangladesh
Ground conditions, Climate, etc.	Winter (Temperature 19-22°C)
Product information	
Material	Chitosan
Molecular weight (kDa)	Below 10,000
Measurement method	Intrinsic Viscosity
Initial Concentration (ppm)	
other additive	
Methodology	

Cultivations	The experiment was laid out in randomized complete block. Design with 3 replicates. The unit plot size was 4 m ×3 m and spacing was 50 cm ×50 cm.
Recommend applications	
Germination	
Timing	
Foliar spray (Germination stage)	
Concentration (ppm)	0, 50, 75, 100 ppm, sprayed at vegetative and reproductive stage.
Foliar spray (Field stage)	
Concentration (ppm)	

PGP Guide Sheet	
Fill-in Date yy/mm/dd	2014 /04/21
Country	BANGLADESH
Name of Plant	Maize
Species	BARI maize-9
Planting time, season	December, 2013
Harvest time, season	April, 2014
Location (Temperature, Humidity etc.)	Farmer's field of Rangpur district, Bangladesh

Ground conditions, Climate, etc.	Winter (Temperature 19-22°C)
Product information	
Material	Chitosan
Molecular weight (kDa)	Below 10,000
Measurement method	Intrinsic Viscosity
Initial Concentration (ppm)	
other additive	
Methodology	
Cultivations	The experiment was laid out in randomized complete block design with 3 replicates. The unit plot size was 3.9 m × 4.9 m and plant spacing was 70 cm × 30 cm. The grain yield will record on plot basis and will convert in tones/hectare.
Recommend applications	
Germination	
Timing	Foliar sprayed four times starting from 20 days after sowing with 15 days interval.
Foliar spray (Germination stage)	
Concentration (ppm)	0, 50, 75, 100 & 125 ppm
Foliar spray (Field stage)	
Concentration (ppm)	

CHINA

PGP Guide Sheet	
Fill-in Date yy/mm/dd	2014/04/20
Country	China
Name of Plant	cucumber
Species	North Cucumis sativus
Planting time, season	May, summer
Harvest time, season	July, summer
Location (Temperature, Humidity etc.)	Zhejiang Province, Humidity 50-80%, temp. 20-37 degree C
Ground conditions, Climate, etc.	alkali red-yellow soil/ subtropical monsoon climate
Product information	
Material	low molecular weight chitosan
Molecular weight (kDa)	20-30
Measurement method	viscosity and GPC
Initial Concentration (ppm)	50,000 ppm (5 wt%)
other additive	surfactant, small amount of ethanol
Methodology	x
Cultivations	seedling in pot and transplanting.

Recommend applications	promoter & disease resistant
Germination	
Timing	any stage
Foliar spray (Germination stage)	possible but not tested
Concentration (ppm)	20-30
Foliar spray (Field stage)	yes
Concentration (ppm)	40
Effect of foliar spray frequency on Plant Growth & Elicitor （crop yields. length, weight, etc. of Sprayed and Control)	Spraying for the first time after blossom. Sprayed every 2 weeks and total 4 times in field, gain increase 22.8% and the direct fruit rate 90% (control: 40%); sugar degree of cucumber center was increased by about 2.7%. The leaves of plant were much greener compared with the control. Different concentrations of chitosan (20, 40, 100 ppm) were tested and the optimal concentration was 40 ppm.

PGP Guide Sheet	
Fill-in Date yy/mm/dd	2014/04/20
Country	China
Name of Plant	pepper
Species	red capsicum

Planting time, season	March Spring
Harvest time, season	May, Summer
Location (Temperature, Humidity etc.)	Zhejiang Province, Humidity 50-80%, temp. 15-30 degree C
Ground conditions, Climate, etc.	alkali red-yellow solid/ subtropical monsoon climate
Product information	
Material	low molecular weight chitosan
Molecular weight (kDa)	20-30
Measurement method	viscosity and GPC
Initial Concentration (ppm)	50,000 ppm (5 wt%)
other additive	surfactant, small amount of ethanol
Methodology	
Cultivations	seedling in pot and transplanting.
Recommend applications	promoter
Germination	
Timing	any stage
Foliar spray (Germination stage)	possible but not tested

Concentration (ppm)	30-40
Foliar spray (Field stage)	yes
Concentration (ppm)	50
Effect of foliar spray frequency on Plant Growth & Elicitor （crop yields. length, weight, etc. of Sprayed and Control)	Spraying for the first time after blossom. Sprayed every 2 weeks and total 5 times in green house. The average length of chilli was increased by 15% and the gain increase was 9.5%, compared to the control. Virus resistance was significant. Different concentrations (50 and 100 ppm) were tested and the optimal was 50 ppm.

INDONESIA

PGP Guide Sheet	
Fill-in Date yy/mm/dd	2014/03/09
Country	Indonesia
Name of Plant	Rice
Species	Oryza sativa L (MR219)
Planting time, season	2013/08/20
Harvest time, season	2013/11/27
Location (Temperature, Humidity etc.)	Temp (30 -33 C), >80%RH(25C)
Ground conditions, Climate, etc.	type of soil sandy clay loam, tropical climate
Product information	
Material	Chitosan flake/powder from shrimp shell
Molecular weight (kDa)	Oligochitosan 50,000 ppm used with average molecular weight 7 kDa
Measurement method	GPC
Initial Concentration (ppm)	50,000 ppm
other additive	
Methodology	

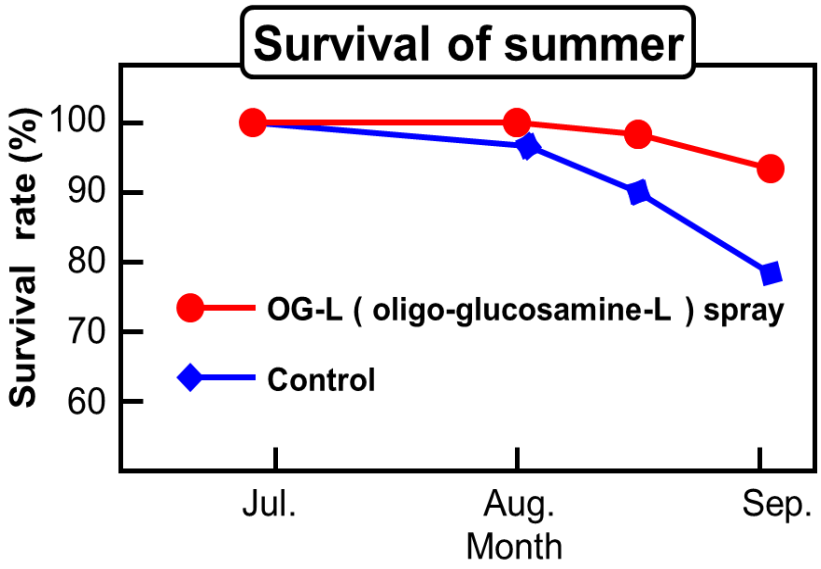
Cultivations	Field test of oligochitosan on rice was performed in collaboration with local farmer located at Pasuruan of East Java. The rice seed soaked with 50 ppm oligochitosan and sprayed one time on seedling tray at 5 days after planting. After transferred to the fields and sprayed 6 times with 50 ppm and 100 ppm oligochitosan on rice plant in field with 7 days for one spraying time interval at vegetative period and 5 times at generative period with 10 days' time interval. The results of field test showed that rice yields increase up 29% for 50 ppm and reached 32% for 100 ppm of oligochitosan compared to that of control without oligochitosan. Results also indicated that oligochitosan can be used as plant elicitor and growth promoter for rice.	
Recommend applications	direct seeding method	transplanting method
Germination		1. Rice seed treatment, dilution 1 mL of 50,000 ppm oligochitosan solution with 1000 times water (1/1000). Rice seeds are dipped in oligochitosan solution about 12 hours and continue with 24 hours dripping. 2. Transfer seed on seedling tray and kept it for another 24 hours before transfer the tray to seedling pond. The seed is watering every day. 3. Spraying rice seed with 50 ppm oligochitosan on day 5. 4. Transfer the rice plant to the rice field on day 12
Timing		

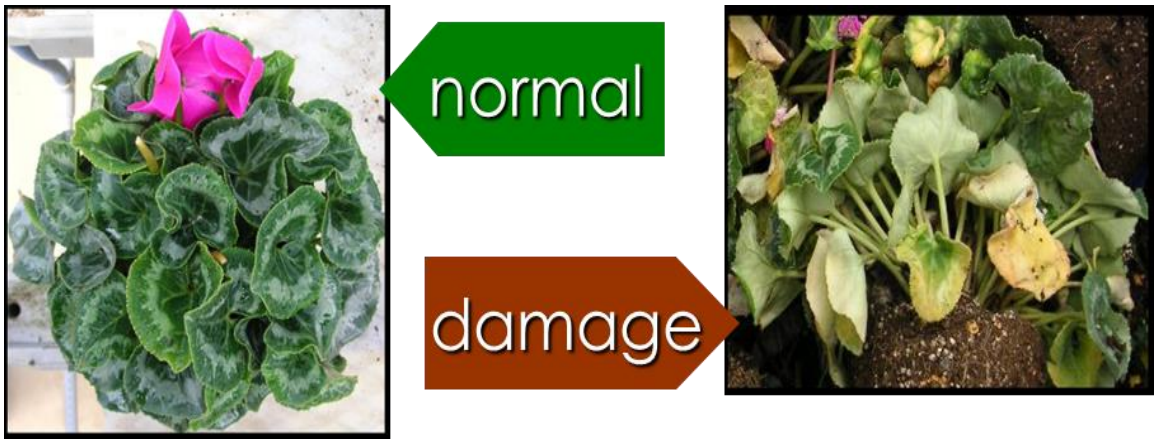
Foliar spray (Germination stage)		Spray 50 ppm oligochitosan on 5th day
Concentration (ppm)		50
Foliar spray (Field stage)		Spray 50 and 100 ppm oligochitosan on 10th, 17th, 24th, 31st, 38th and 45th days after planting at vegetative period and 55th, 65th and 75th days after planting at generative period.
Concentration (ppm)		50 and 100
Effect of foliar spray frequency on Plant Growth & Elicitor （crop yields. length, weight, etc. of Sprayed and Control)		

JAPAN

PGP Guide Sheet	
Fill-in Date yy/mm/dd	2014/1/13
Country	Japan
Name of Plant	Cyclamen persicum
Species	
Planting time, period, season	November~December
Harvest time, period, season	November
Location (Temperature, Humidity etc.)	green house, Temp (25~28 C), ~80%RH(25C)
Ground conditions, Climate, etc.	
Product information	
Material	Chitosan flake from Crab shells
Molecular weight (kDa)	Oligochitosan 40,000 ppm used with average molecular weight 10 kDa
Measurement method	GPC
Initial Concentration (ppm)	40,000ppm
other additive	(N)7: (P)8: (K)8
Methology	

Cultivations	Field test of oligochitosan on cyclamen was performed in collaboration with Azbio Co., Japan. sprayed 100 ppm oligochitosan on cyclamen plant in field with 2 weeks for one spraying time interval. Result of field test, survival ratio of cyclamen in summer improves up 15% compared to control without oligochitosan.
Recommend applications	
Germination	
Timing	
Foliar spray (Germination stage)	
Concentration (ppm)	
Foliar spray (Field stage)	Spray at every 2weeks from July to September
Concentration (ppm)	100
Effect of foliar spray frequency on Plant Growth & Elicitor （crop yields. length, weight, etc. of Sprayed and Control)	Application protocol for cyclamen field 1. Dilution oligochitosan 40,000ppm with 400 times by water to 100ppm. 2. Spraying oligochitosan at every 2 weeks.


Survival of summer
Survival rate (%)
100
90
80
70
60
OG-L (oligo-glucosamine-L) spray
Control
Jul.
Aug.
Sep.
Month

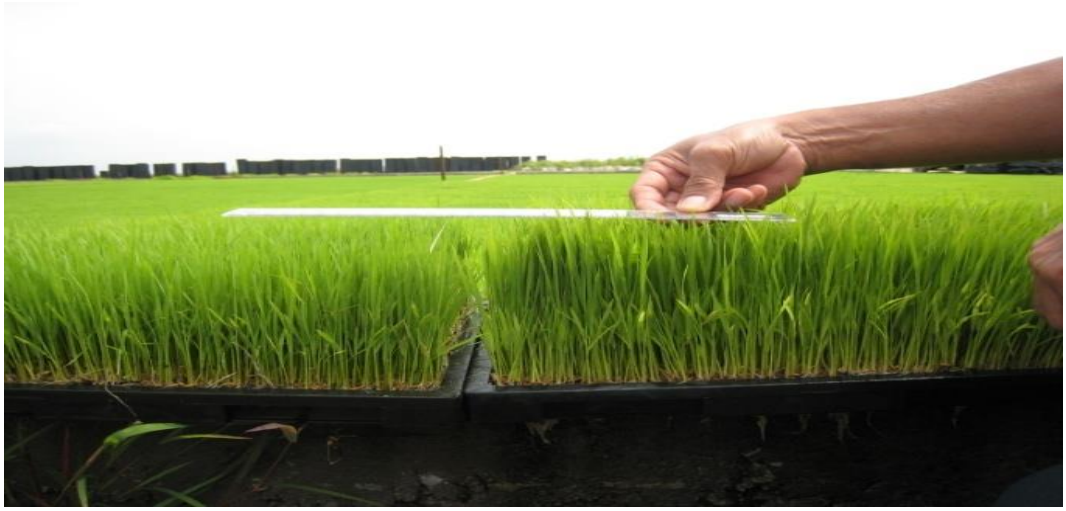

normal
damage

MALAYSIA

PGP Guide Sheet	
Fill-in Date yy/mm/dd	2014/1/13
Country	Malaysia
Name of Plant	Rice
Species	(MR219)
Planting time, period, season	October-November
Harvest time, period, season	February-March
Location (Temperature, Humidity etc.)	Temp (30 -33°C), >80%RH(25°C)
Ground conditions, Climate, etc.	type of soil sandy clay loam, tropical climate
Product information	
Material	Chitosan powder from shrimp shell
Molecular weight (kDa)	Oligochitosan 20,000 ppm used with average molecular weight 10.5 kDa
Measurement method	GPC
Initial Concentration (ppm)	20,000 ppm
other additive	
Methodology	

Cultivations	Field test of oligochitosan on rice was performed in collaboration with FELCRA Bhd (government owned company in agriculture business), Sg. Burung Agriculture Department and Malaysian Agriculture Research Development Institution (MARDI). The germination study on rice seed found that rice seed soaked with 100 ppm oligochitosan follow with sprayed 3 times with 40 ppm oligochitosan shortened seedling period to 10-12 days compare to normal practice 15-17 days using commercial chemical germination product. Result of field test shown that seed soaked for 24 hours in 100 ppm oligochitosan and 4 times spray with 100 ppm oligochitosan during the planting period, increase rice yield up to 26% compare to control without oligochitosan and fungicide treatment. Yield results also revealed that oligochitosan can replace chemical fungicide commonly used by the farmers and at the same time give better yield than using fungicide for disease treatment.	
Recommend applications	direct seeding method	transplanting method
Germination	Rice seed treatment: Require amount of 20,000 ppm oligochitosan solution was diluted with 200 times water (1/200). Rice seeds are dipped in 100 ppm oligochitosan solution for 24 hours and continue with 24 hours dripping. The treated seed was scattered to wet-paddy field	1. Rice seed treatment: Require amount of 20,000 ppm oligochitosan solution was diluted with 200 times water (1/200). Rice seeds are dipped in 100 ppm oligochitosan solution for 24 hours. 2. Transfer seed treatment on seedling tray and kept it for another 24 hours before transfer the tray to seedling pond. The seed is

		watering every day. 3. Spraying rice seed treatment with 40 ppm oligochitosan solution on 3rd, 8th and 13th days. 4. Transfer the rice plant to the rice field on 12th day.
Timing		
Foliar spray (Germination stage)	no	on day 3, 8 and 13
Concentration (ppm)	no	40
Foliar spray (Field stage)	on day 3, 18, 53 and 71, 3 days before apply fertilizer	on day 3, 18, 53 and 71 after transfer to paddy field, 3 days before apply fertilizer.
Concentration (ppm)	100	100
Effect of foliar spray frequency on Plant Growth & Elicitor （crop yields. length, weight, etc. of Sprayed and Control)	Result of oligochitosan performance on plant rice 1. Germination of rice seed treated and spray with oligochitosan could be transfer to paddy field on 12 days compare to control on 15 to 17 days. 2. Root of rice plant treated and spray with oligochitosan at germination stage shown rapid growth compare to control using commercial plant growth promoter. 3. Rice plant treated and spray with oligochitosan shown 26% increase in yield compare to control without oligochitosan. Rice plant spray with fungicide only 14% increase in yield.	



Treated with commercial product

Treated with oligochitosan

Treated with commercial product

Treated with oligochitosan

PGP Guide Sheet	
Fill-in Date yy/mm/dd	2014/5/5
Country	Malaysia
Name of Plant	Chilli
Species	Chilli Kulai Hybrid F1 469 (Capsicum annum)
Planting time, period, season	July to November 2013
Harvest time, period, season	Once a week (Sept. - Nov.)
Location (Temperature, Humidity etc.)	Temp (30 -33°C), >80%RH(25°C)
Ground conditions, Climate, etc.	
Product information	
Material	Chitosan powder from shrimp shell
Molecular weight (kDa)	Oligochitosan 20,000 ppm used with average molecular weight 10.5 kDa
Measurement method	GPC
Initial Concentration (ppm)	20,000 ppm
other additive	White onion extract
Methodology	

Cultivations	Field test of oligochitosan on chilli was carried out in collaboration with Nurezqi Enterprise agriculture company. The germination study of chilli plant was performed by soaking the chilli seed either with 100 ppm oligochitosan or chemical consist of previcure plus tonic and water as control, for 2 hours, follow with sieve and dry prior to seedling process in peatmoss. Nutrient film technique was used for germination of young chilli plant where plant was watering with nutrient 2 times per day for 5 minutes. During germination, young chilli plant was sprayed with 40 ppm oligochitosan, one a week. After 26 days, healthy young plant was transfer to plastic bag content coconut coir dust for field test using fertigation system. Under fertigation system, the chilli plants were dripped with certain amount of water and liquid fertilizer every 5 minute, on coconut coir dust which replacing sand, in plastic container. Chilli plant was foliar spray every week with mix solution of 100 ppm oligochitosan with white onion extract. The harvest of red chilli fruit was carried out twice per week. Average yield of chilli plant spray with mix solution of 100 ppm oligochitosan and white onion extract is 1598g, increase 15% compare to chemical treatment (1387g).
Recommend applications	

Germination	1. Chilli seed was soaked in 100 ppm oligochitosan, for 2 hours, follow with sieve and dry prior to seedling process in peatmoss. 2. The seed was laid down in the peat moss in plastic container. 3. Nutrient film technique was used for germination of young chilli plant where plant was watering with nutrient 2 times per day for 5 minutes. 4. During germination, young chilli plants were sprayed with 40 ppm oligochitosan, once a week. After 26 days, healthy young plant was transfer to plastic bag content coconut coir dust for field test study using fertigation system.
Timing	
Foliar spray (Germination stage)	One a week
Concentration (ppm)	40
Foliar spray (Field stage)	One a week
Concentration (ppm)	10 ppm oligochitosan plus white onion extract.

Effect of foliar spray frequency on Plant Growth & Elicitor （crop yields. length, weight, etc. of Sprayed and Control)	Result of oligochitosan performance on chilli plant at germination stage 1. Germination of chilli seed treated with 100 ppm oligochitosan and spray with 40 ppm oligochitosan one a week, gave average highest plant length and root, 35.8 cm and 7.4 cm, respectively compare to 80 ppm (35.2 and 5.8 cm), 100 ppm (33.8 and 5.6 cm), and control using chemical (33.3 and 5.6 cm) and were water (32 and 4.7 cm). Result of field test application of oligochitosan on chilli plant 1. Foliar spray of chilli plant with 100ppm oligochitosan plus white onion extract produce average highest yield 1598g per plant compare to chemical (1387g), 100 ppm oligochitosan plus half chemical (1345g), 100 ppm oligochitosan (1191g), white onion (1142g) and control water (573g). 2. Field test also reveal elicitor effect of PGP toward insect and disease attack on the chilli plant. Foliar spray with 100 ppm oligochitosan plus white onion extract shown the lowest infected only 22.3% plant from total 9 plants compare to treatment using chemical, 100 ppm oligochitosan and 100 ppm oligochitosan plus half chemical which gave 33.3%, white onion 44.4% and control using water 88.8%.

Chilli plant recovery from Mozac disease

Oligochitosan produce 3 chilli fruits develop at one V stem of chilli plant

Insect attack chilli plant

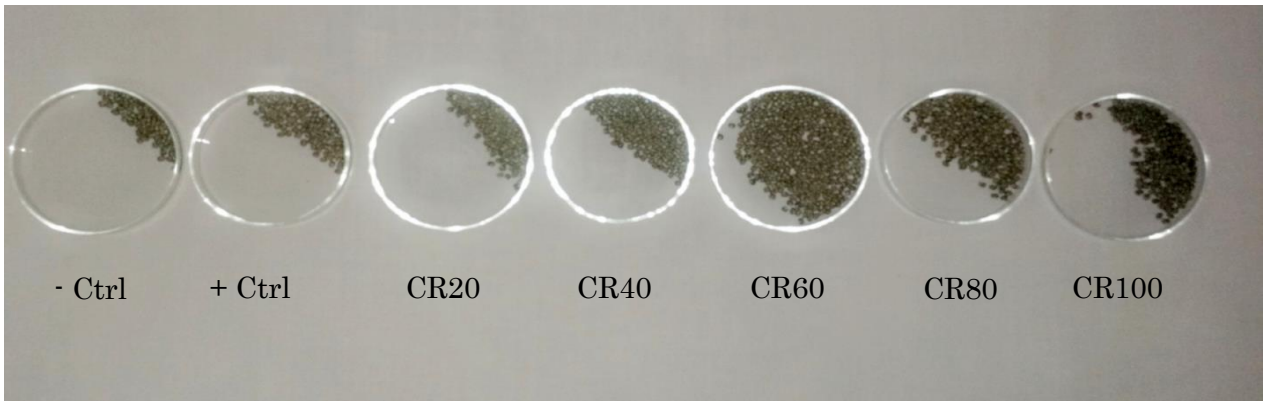
Fertigation System of Chilli Plant

PHILIPPINES

PGP Guide Sheet	
Fill-in　Date yy/mm/dd	2014/03/17
Country	Philippines
Name of Plant	Mungbean
Species	*Vigna radiata* (L .) Wilczek (Pag-asa 21)
Planting time, period, season	August (wet season)
Harvest time, period, season	November
Location (Temperature, Humidity etc.)	not recorded
Ground conditions, Climate, etc.	clay loam, tropical climate
Product information	
Material	oligo-kappa carrageenan
Molecular weight (kDa)	oligo-kappa carrageenan (ppm) used with average molecular weight　7 kDa
Measurement method	GPC
Initial Concentration (ppm)	10000 ppm
other additive	None
Methodology	

Cultivations	Pot experiment under screen-house conditions was conducted using different concentrations of oligo-kappa carrageenan (20, 40, 60, 80, and 100 ppm) with tap water serving as the negative control (0 ppm) and a mungbean inoculant (commercial preparation of *Rhizobium* spp.) served as positive control. Results showed that soaking the seeds in 60 ppm oligo-kappa carrageenan and spraying the same to resulting plants proved best as the tallest plant 62 days after sowing and at harvest time, earliest to flower, longest pod (fruit) length, and highest yield (in terms of number and weight of seeds) were obtained. Generally, plants treated with oligo-carrageenan were better than the control plants (both untreated and those treated with legume inoculant) except for the 100-seed weight where there is no difference among treatments.
Recommend applications	seed treatment prior to sowing
Germination	seeds are soaked in 60 ppm oligo-kappa carrageenan for 30 minutes before sowing
Timing	
Foliar spray (Germination stage)	two-weeks after sowing and every 2 weeks after each foliar spray until plant maturity
Concentration (ppm)	60
Foliar spray (Field stage)	

Concentration (ppm)	
Effects of seed treatment and foliar spray on plant growth and yield	Application protocol for rice field 1. Dilution oligochitosan 30,000 ppm with 1000 times by water to 30 ppm. 2. Spraying oligochitosan 4 times on day 10, 20, 30 and 40 after 10 days of planting. 400-500 L of diluted oligochitosan applies for 1 hectare which means about 0.5L of original oligochitosan with concentration 30,000 ppm/ha/spraying time.



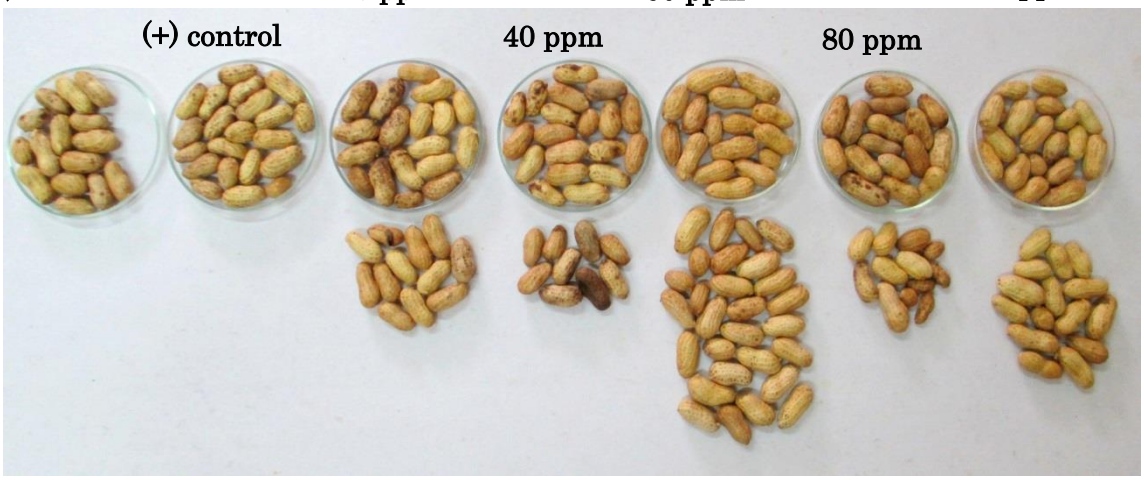
PGP Guide Sheet	
Fill-in Date yy/mm/dd	2014/03/17
Country	Philippines
Name of Plant	Peanut
Species	*Arachis hypogaea* L. (Namnama)
Planting time, period, season	October
Harvest time, period, season	January
Location (Temperature, Humidity etc.)	not recorded
Ground conditions, Climate, etc.	clay loam, tropical climate
Product information	
Material	oligo-kappa carrageenan
Molecular weight (kDa)	oligo-kappa carrageenan (ppm) used with average molecular weight 7 kDa
Measurement method	GPC
Initial Concentration (ppm)	10000 ppm
other additive	None
Methodology	

Cultivations	Pot experiment under open field conditions was conducted using different concentrations of oligo-kappa carrageenan (20, 40, 60, 80, and 100 ppm) with tap water serving as the negative control (0 ppm) and a peanut inoculant (commercial preparation of Rhizobium spp.) served as positive control. Results showed that seeds soaked in radiation-modified kappa carrageenan germinated earlier than the controls. The tallest peanut seedlings 16 days after sowing averaged 21.28 mm for 60 ppm oligo-kappa carrageenan but it was not significantly different from plants treated with other concentrations of oligo-kappa carrageenan. At maturity, it was the tallest at 624.00 mm and significantly different from other treatments. The negative control (0 ppm) and positive control (0 ppm but with commercial inoculant) were short at 6.62 and 8.00 mm, respectively, 16 days after sowing and at 409.00 mm and 442.67 mm, respectively, at harvest time. The values are significantly different from those treated with radiation-modified kappa carrageenan. The plants flowered as early as 29 days after sowing (for 60 ppm) compared to 50.25 days from sowing (for negative control) and 41.5 days from sowing (positive control). An experiment using the peanut variety Biyaya 6 that have been treated with 60 ppm oligo-kappa carrageenan produced flowers as early as 29 days from sowing. With regards to yield, 60 ppm oligo-kappa carrageenan produced 36 pegs and 17 pods per plant. Likewise, the length of pod, 100-seed weight, and yield per plant were significantly the greatest at 60 ppm oligo-kappa carrageenan. Number of seeds per pod did not vary among treatments.

Recommend applications	seed treatment prior to sowing
Germination	seeds are soaked in 60 ppm oligo-kappa carrageenan for 30 minutes before sowing
Timing	
Foliar spray (Germination stage)	two-weeks after sowing and every 2 weeks after each foliar spray until plant maturity
Concentration (ppm)	60
Foliar spray (Field stage)	
Concentration (ppm)	
Effects of seed treatment and foliar spray on plant growth and yield	Application protocol for rice germination 1. Rice seed treatment, dilution of 30,000 ppm oligochitosan solution with 300 times water (1/300). Rice seeds are dipped in oligochitosan solution with concentration of 100 ppm about 24 hours and continue with 24 hours dripping. 2. Transfer seeds on seedling field. 3. After 12 for seedling growth, transfer seedlings to rice field. The germination study on rice seed found that rice seed soaked with 100 ppm oligochitosan overnight the germination increased from 88.0% to 94.4% for control and treated rice seeds, respectively. In addition, fresh biomass of rice seedlings increased from 1.18 g/seedling to 1.7 g/seedling for control and treated rice seeds, respectively.

	Application protocol for rice field 1. Dilution oligochitosan 30,000 ppm with 1000 times by water to 30 ppm. 2. Spraying oligochitosan 4 times on day 10, 20, 30 and 40 after 10 days of planting. 400-500 L of diluted oligochitosan applies for 1 hectare which means about 0.5 L of original oligochitosan with concentration 30,000 ppm/ha/spraying time.

(-) control (+) control 20 ppm 40 ppm 60 ppm 80 ppm 100 ppm

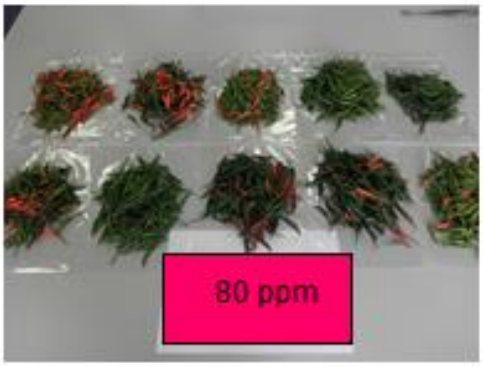


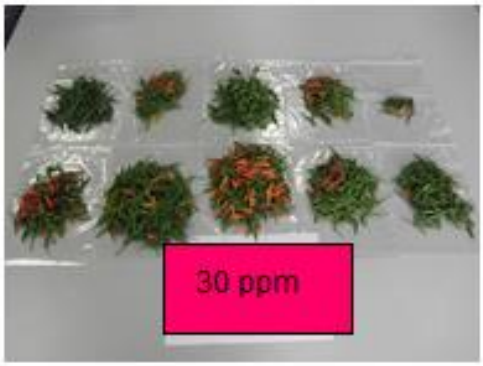
THAILAND

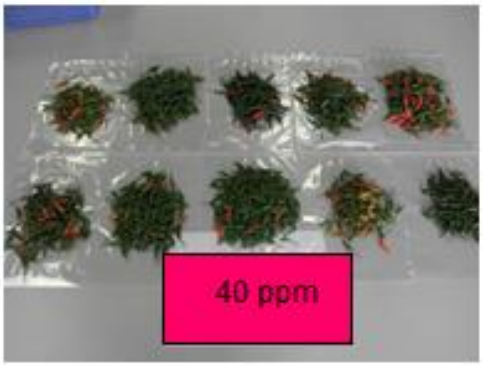
PGP Guide Sheet	
Fill-in Date yy/mm/dd	April 28,2014
Country	Thailand
Name of Plant	Chilli
Species	Capsicum annuum L.
Planting time, season	August-November
Harvest time, season	November-December
Location (Temperature, Humidity etc.)	Temp (30 -35 C), >80%RH(25C)
Ground conditions, Climate, etc.	loamy soil, tropical climate
Product information	
Material	Chitosan flake from shrimp shell
Molecular weight (kDa)	Oligochitosan 20,000 ppm used with average molecular weight 11.6 kDa
Measurement method	GPC
Initial Concentration (ppm)	20,000
other additive	no
Methodology	
Cultivations	The application of oligochitosan at 80 ppm showed significant effects, statistically, not only on chilli's growth but also on chilli's productivity. The oligochitosan also displayed ability to protect aphid inflection in chilli plants. Additionally,

	oligochitosan can also shorten the harvest time of chilli plants for three weeks. The treatment of Thai chilli plants with 80 ppm oligochitosan showed positive effects on Thai chilli's growth and productivity, compared with the untreated chilli plants. It was found that there was 34% increase in total weight for the chilli plants treated with oligochitosan as compared to the untreated plants.
Recommend applications	transplanting method
Germination	Chilli seeds were implanted in Petri dishes for three days, and then the seedlings were transplanted into clay plots. All chilli plants were treated with the same amount of fertilizer (16-16-16) via soil treatment at the beginning of the experiment.
Timing	
Foliar spray (Germination stage)	no
Concentration (ppm)	no
Foliar spray (Field stage)	The foliar spraying of oligochitosan was applied once a week.
Concentration (ppm)	80 ppm
Effect of foliar spray frequency on Plant Growth & Elicitor （crop yields. length, weight, etc. of Sprayed and Control)	The chilli plants treated with 80 ppm of oligochitosan showed significant differences, statistically, in terms of height, over the control plants. It is quite obvious that the productivity of chilli plants, in terms of the numbers of chillies, increased with increasing concentration of oligochitosan. The total weight of chilli, total number of chillies and weight per chilli increased significantly for the chilli plants treated with 80 ppm oligochitosan compared to the control group.

	In addition to enhancement of growth and productivity, the chilli plants treated with oligochitosan also displayed an ability to protect aphid infection.
	Application protocol for chilli Dilution of 20,000 ppm oligochitosan solution with water. Chilli pot test: Spraying 80 ppm oligochitosan once a week.







VIETNAM

PGP Guide Sheet	
Fill-in Date yy/mm/dd	2014/1/13
Country	Vietnam
Name of Plant	Rice
Species	Japonica
Planting time, period, season	April ~ May
Harvest time, period, season	Sep. ~ Oct.
Location (Temperature, Humidity etc.)	Temp (25~28 C), 80%RH(25C)
Ground conditions, Climate, etc.	Paddy red soil, subtoropical,
Product information	
Material	Chitosan flake from Crab and/or shrimp shells
Molecular weight (kDa)	Oligochitosan 30,000 ppm used with average molecular weight 10 kDa
Measurement method	GPC
Initial Concentration (ppm)	30,000 ppm
other additive	
Methodology	

Cultivations	Field test of oligochitosan on rice was performed in collaboration with Mekong Delta Rice Research Institute, Can tho City. The germination study on rice seed found that rice seed soaked with 100 ppm oligochitosan and sprayed 4 times with 30 ppm oligochitosan on rice plant in field with 10-15 days for one spraying time interval. Result of field test, rice yield increases up from 10 to 20% compared to control without oligochitosan. Results also indicated that oligochitosan can be used as plant elicitor and growth promoter for rice	
Recommend applications	direct seeding method	transplanting method
Germination	Rice seed treatment, dilution of 30,000 ppm oligochitosan solution with 300 times water (1/300). Rice seeds are dipped in oligochitosan solution with concentration of 100 ppm about 24 hours and continue with 24 hours dripping.	1. Rice seed treatment, dilution of 30,000 ppm oligochitosan solution with 300 times water (1/300). Rice seeds are dipped in oligochitosan solution with concentration of 100 ppm about 24 hours and continue with 24 hours dripping. 2. Transfer seeds on seedling field. 3. After 12 for seedling growth, transfer seedlings to rice field.
Timing		
Foliar spray (Germination stage)		
Concentration (ppm)		

Foliar spray (Field stage)	Spray on day 15, 30, 45 and 60	Rice field: Spray time of 30 ppm oligochitosan with 10-15 days for one spraying time interval
Concentration (ppm)	30	30
Effect of foliar spray frequency on Plant Growth & Elicitor （crop yields. length, weight, etc. of Sprayed and Control ）	Application protocol for rice germination 1. Rice seed treatment, dilution of 30,000 ppm oligochitosan solution with 300 times water (1/300). Rice seeds are dipped in oligochitosan solution with concentration of 100 ppm about 24 hours and continue with 24 hours dripping. 2. Transfer seeds on seedling field. 3. After 12 for seedling growth, transfer seedlings to rice field. The germination study on rice seed found that rice seed soaked with 100 ppm oligochitosan overnight the germination increased from 88.0 to 94.4% for control and treated rice seeds, respectively. In addition, fresh biomass of rice seedlings increased from 1.18 g/seedling to 1.7 g/seedling for control and treated rice seeds, respectively.	
	 Fig.1. Increase of germination rate of rice seeds treated with 100 mg/L oligochitosan	

	 Đối chứng Oligochitosan Fig.2. Increase of fresh biomass of rice seedlings when treated rice seed with 100 mg/L oligochitosan
	Application protocol for rice field 1. Dilution oligochitosan 30,000 ppm with 1000 times by water to 30 ppm. 2. Spraying oligochitosan 4 times on day 10, 20, 30 and 40 after 10 days of planting. 400-500 L of diluted oligochitosan applies for 1 hectare which means about 0.5 L of original oligochitosan with concentration 30,000 ppm/ha/spraying time.