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STUDY ON GROWTH-PROMOTION OF PADDY PLANTS TREATED WITH OLIGOCHITOSAN

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Abstract

Chitosan has been degraded to produced oligochitosan with different molecular weight using gamma ray irradiation from a Co-60 source in solid state (powder form) and liquid state (aqueous solution). Study on growth promotion of paddy plants was done using oligochitosan and conventional plant growth promoter as a comparison. Oligochitosan was used with different molecular weight and different concentrations. Smaller molecular weight of oligochitosan with smaller concentration showed better result than bigger molecular weight of oligochitosan as a plant growth promoter. This study also showed that conventional growth promoter can be replaced with oligochitosan as it is more effective as plant growth promoter as well as more environmental friendly.

Abstrak

Kitosan telah diurai untuk menghasilkan oligokitosan dengan berat molekul yang berbeza menggunakan radiasi sinaran gamma daripada sumber Co-60 dan disinarkan dalam bentuk serbuk dan larutan. Kajian kadar pertumbuhan ke atas padi telah dijalankan menggunakan oligokitosan dan sumber yang sedia ada di pasaran. Oligokitosan yang digunakan adalah dengan berat molekul dan kepekatan yang berbeza. Berat molekul oligokitosan dengan kepekatan yang rendah menunjukkan keputusan yang lebih baik berbanding berat molekul oligokitosan yang lebih besar. Kajian ini juga telah menunjukkan bahawa sumber sedia ada boleh diganti dengan oligochitosan memandangkan ianya lebih efektif dari segi kadar pertumbuhan di samping lebih bersifat mesra alam.

Keywords/Katakunci: Chitosan, Oligochitosan, Radiation degradation, Plant growth-promotion

INTRODUCTION

Oligochitosan is also known as chitosan oligomers which are obtained from the partial degradation of chitosan. Chitosan is the N-deacetylated derivative of chitin which is a natural polysaccharide that occurs mainly in invertebrates, fungi and yeasts (Knaul et al., 1998). Due to its unique properties such as biocompatibility, biodegradability, bioactivity and non-toxic, chitosan and its derivative are extensively studied and widely utilized in food industry, pharmaceuticals, cosmetics, agriculture, and water treatment(Hai et al., 2003). Chitosan, a (1→4)-linked 2-amido-2-deoxy-β-D-glucan can be depolymerizing into series of oligochitosan either by the chemical, physical or enzymatic process (Prashanth and Tharanathan, 2007). Degradation of chitosan through the chemical treatment is very common, low cost and fast method to produce oligochitosan, but through this method concentrated of acidic waste will dispose to the environment. In the case of enzymatic process, it requires multiple steps, particularly, enzyme preparation and purification of the product. This is inconvenient for the large scale production (Hai et al., 2003). Radiation is one of the most popular physical methods which can produce high yield of oligochitosan in certain period depends on the dose applied without any waste to the environment (Choi et al., 2002). Natural polymer such as chitosan can be degraded due to the scission of glycosidic bond by radiation. The degraded chitosan induces various kinds of biological activities on plants such as promotion of plant growth, antibacterial activity, phytoalexins and suppression of environmental stress on plants (Nagasawa et al., 2001). Study

carried out by Kume et al. in 2002 claimed that degraded polysaccharides such as chitosan, alginate or carrageenan can increase tea, carrot or cabbage productivity by 15-40%. In 2007, paper published by Chmielewski et al. in 2007 also reported that degraded chitosan promotes about 11-13% growth of roots diameter and 51-65% growth of roots mass of rape plant. The experiment was carried out in greenhouse condition in comparison to the control.

The ultimate objective of the research was to study the effect of oligochitosan compare to the conventional plant regulator, Vitacambah on the growth-promotion of paddy seed. This product is produced locally in order to increase the percentage of germinated paddy seeds as well as it supplies extra oxygen to enhance the growth of germinated seeds. It contains nitrous acid and other chemicals which are very soluble in water and very acidic with pH value below 4. As another aim was to determine the best treatment which gives optimum result to the growth of paddy.

MATERIALS

Paddy seed from variety of MR 219, supplied by FELCRA Berhad was used for the experiment. The MR 219 was developed from a cross between MR 137 and MR 151. This variety has short maturation period, high resistance to bacterial leaf blight and brown plant hopper, long grade grains and low amylase content. The rice husk ash used as the planting media instead of cotton. It is believed that rice husk ash can provide natural fertilizer to the growth of seeds. Hence, FELCRA uses the rice husk ash as the planting media for the commercially transplant method. Transplant and direct seeding are two methods commonly practiced by the local farmers. However, transplant is more costly than direct seeding. The conventional plant growth promoter, Vitacambah also supplied by FELCRA Berhad. Chitosan (degree of deacetylation = 90%) with 10% moisture content was bought from China. Lactic acid 90% was purchased from the local company, OFT Chemicals Sdn. Bhd., Shah Alam. All main materials such as chitosan, lactic acid and ethanol were from industrial grade.

METHODOLOGY

A. Preparation of oligochitosan and Analytical Procedures

Chitosan powder with estimated molecular weight,Mw of 200 kDa was irradiated at Sinagamma at 75kGy using gamma rays from Co-60 source. The irradiated chitosan powder later was dissolved in the lactic acid solution and kept overnight for the complete hydration of chitosan. Hydrogen peroxide was added in the solution just before the irradiation process took place. Hydrogen peroxide causes rapid decrease of chitosan molecular weight by breaking of 1,4-β-D-glucoside bonds (Chmielewski et al., 2007). The first batch of chitosan solution was irradiated at Raymintex at 12kGy and the second batch at 24kGy using gamma rays from Co-60 source. The first and second batch product was labeled as Oligochitosan 1 and Oligochitosan 2, respectively. Both products were added with sodium hydroxide solution to increase the pH value to pH 5. Ethanol was added at the final stage of the process to inhibit bacterial growth as well as to prolong the shelf life of the product. Molecular weight of purified oligochitosan was measured by Ubbe lohde Viscometer AVS 440 (Schott Instrument). The instrument was equipped with Capillary: 531 10/I and elution at 25 $\pm$ 0.1°C with buffer solution; 0.25M acetic acid/0.25M sodium acetate (Knaul et al., 1998).

B. Germination Test of Paddy Seed

The aim of the germination test was to determine the impact of oligochitosan on growth of paddy seed compared to conventional plant regulator, Vitacambah. Total of germinated seed for each treatment were counted from day 1 to day 3 to determine the optimum treatment which gives good result. The seeds were treated with different molecular weight of oligochitosan and different concentration. The paddy seeds were soaked in 5 different concentrations of oligochitosan 1 and oligochitosan 2. The concentrated oligochitosans were diluted with filtered water to 20ppm, 40ppm, 80ppm, 100ppm and 200ppm. The concentration of Vitacambah applied was 3.85% which was copied from the conventional procedure. Each treatment was done in 3 replicates. The seeds were packed in 100 pieces in a tea

bag with tied and labeled. Later the tea bags were soaked and emerged in beakers each contained different treatment solutions for 24 hours.

After soaking, the tea bags were taking out from the beaker and left for dripping. The seeds were then place on the wet media contained in the plastic container. The seeds were watered for thrice a day and left with exposed to the sunlight at the room temperature. The germinated seeds were counted on the next day as result on Day 1. The observation and counting continue until Day 3.

In the second step of germination test, the oligochitosan which gives better result to the growth of paddy seeds subsequently mixed with Vitacambah in order to observe the plant growth promotion effect. The compositions of oligochitosan to Vitacambah were 9:1, 3:7, 5:5 and 7:3. The dilution of oligochitosan and Vitacambah were kept at the same dose as in previous test. The same test steps were copied from the previous test for this experiment. The data was collected from day 1 until day 3.

RESULTS AND DISCUSSION

Effect of radiation on chitosan

Effect of radiation to the chitosan was investigated. The irradiation of chitosan in solid state and liquid state led to the reduction in molecular weight. A wide variety of lower molecular weight, shorter chain polymers or oligomers could be produced, depending on the radiation dose applied (Hai et al. 2003). Chitosan in the solid state as well as in liquid state will only be degraded and that crosslinking is negligible if exposed to the radiation. In this study, the oligochitosan produced from the radiation degradation of chitosan was purified using ammonium hydroxide. The average molecular weight purified oligochitosans were determined using viscometer ubbe lohde. The average molecular weight of oligochitosan 1 and oligochitosan 2 were 10kDa and 3kDa, respectively. The molecular weight of chitosan reduced from 200kDa to 10kDa and 3kDa after being irradiated at 75kGy in solid state and 12kGy and 24kGy in liquid state, respectively. From the results obtained, it can be concluded that the chitosan molecule was depolymerized, resulting in the reduction of its molecular weight.

Effect of oligochitosan on growth of paddy seeds

Study done by Nagasawa et al. reported in 2001 claimed that degraded chitosan obtained from high dose shows a stronger effect on barley growth. This means, oligochitosan with lower molecular weight give better result on growth of plant compared to higher molecular weight oligochitosan. The growth of paddy seeds at various concentrations of oligochitosan 1, oligochitosan 2 and Vitacambah is shown in Fig. 1. The result shows oligochitosan 2 which has average molecular weight 3kDa is more effective than oligochitosan 1, 10kDa. The result shows total of germinated seeds treated with oligochitosan 1 decreased with the increasing of concentration in day 1. Treatment of oligochitosan 2, 200ppm can only gave less than 65% of germinated seeds. But the results obtained from the oligochitosan 2 treatment are insignificant between the concentrations. Treatment of paddy seeds with oligochitosan 2 with 80ppm shows same effect as Vitacambah on growth promotion. On day 1, oligochitosan 2, 80ppm gave more than 85% of germinated seeds. This shows that oligochitosan 2 with only 80ppm in concentration was enough to give good effect as Vitacambah in promoting seeds growth.

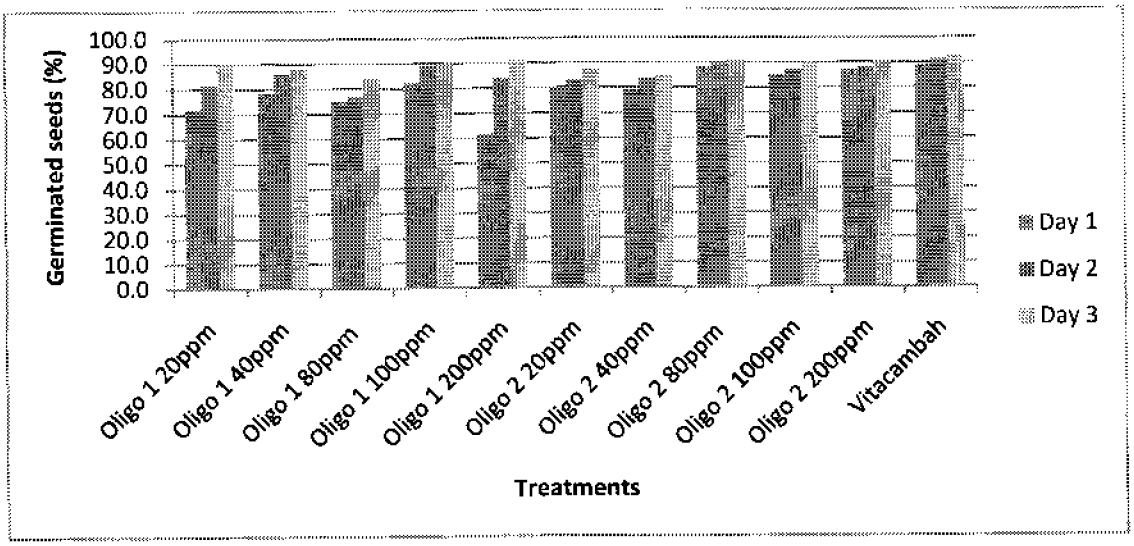

Fig 1. Total (%) of germinated paddy seeds after treated with oligochitosan 1, oligochitosan 2and Vitacambah from day 1 until day 3

The oligochitosan 2 with concentration 20ppm, 40ppm, 80ppm and 200ppm were mixed with Vitacambah with the composition of oligochitosan 2 to Vitacambah 1:9, 3:7, 5:5, 7:3 and 9:1. Observations were done from day 1 until day 3. The results in Fig 2 show total of germinated seeds are less than 80% on day 1 for all treatments. All treatments of oligochitosan 2 with concentration of 200ppm with combination of Vitacambah showed the worst effect on the growth of seeds. Mixture of oligochitosan 2, 80ppm with Vitacambah in composition of 9 to 1 gave the significant result from day 1 to day 3. It was 61% of germinated seeds on day 1 and increased up to over 90%. On the other hand, reatment of oligochitosan 2, 20ppm with composition of 3 to 7 of Vitacambah, gave the highest number of germinated seeds on day 1 but on the day 3 the number of germinated seeds was among the lowest. If compare to the previous experiment, total of germinated seeds was higher if treated with lower molecular weight oligochitosan itself than treated with mixture of oligochitosan and Vitacambah. Majority of the treatment in previous experiment gave almost 80% of number of germinated seeds in day 1. But, most of the mixture treatments only contribute below 70% of germinated seeds in day 1.

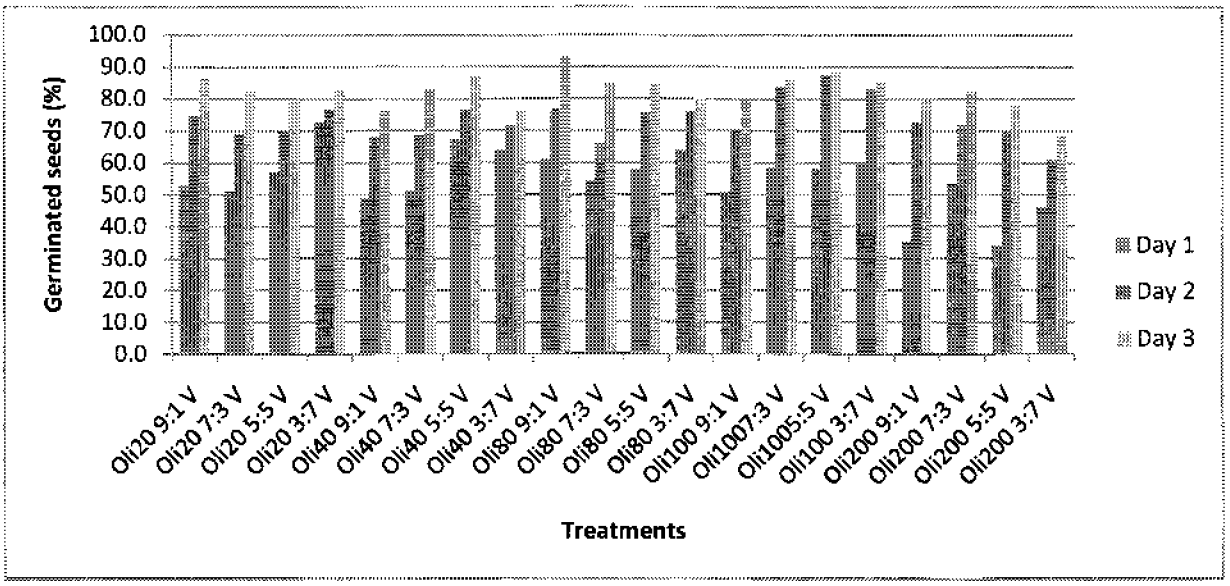

Fig 2. Total (%) of germinated seeds after treated with mixture of oligochitosan 2 and Vitacambah from day 1 until day 3

CONCLUSION

It is concluded that irradiation of chitosan under dry condition can produce low molecular weight of chitosan. Irradiation of chitosan in liquid state with addition of hydrogen peroxide enhances the reduction of molecular weight of chitosan to the lower chain of chitosan. Degraded chitosan also known as oligochitosan with lower molecular weight shows good effect in promoting plant growth. The impact of oligochitosan was as good as commercial plant regulator, Vitacambah. From the results, it is suggested that oligochitosan can replace Vitacambah. The suitable concentration of oligochitosan 2 for paddy seed treatment is 80ppm. Combination formulation of oligochitosan 2 and Vitacambah didn't give significant impact on seeds growth.

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