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Effects of Molecular Weight of Chitosan on Yield Potential of Rice Cultivar Suphan Buri 1

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ABSTRACT

Chitosan is a natural polymer extracted from chitin. It can be applied to plants in order to increase yield. The aims of this study were to determine the effective type of chitosan and the appropriate method for increasing rice yield. The experimental design was 3×3 factorial in RCB with one control and replicated four times. The factor A was seed soaking, seed soaking + foliar spraying and foliar spray; the factor B was polymeric chitosan, oligomeric chitosan and monomeric chitosan. The mixed chemical fertilizer between 46-0-0 and 16-20-0 (ratio 1:1w/w) at the rate of 50 kg/rai was applied to the rice plants of all treatments, but without chitosan in the control treatment. This study was conducted in pot experiment in a greenhouse at Rajamangala University of Technology Suvarnabhumi during January to April 2005. The results indicated that application of polymeric chitosan by seed soaking before planting followed by four foliar sprayings throughout cropping season significantly increased ($P<0.05$) the tiller numbers per plant and dry matter accumulation but decreased unfilled grains. However, the application of chitosan with different molecular weights and different application methods did not affect plant height, thousand-grain weight and filled grains. Focusing on the rice yield, there was a tendency to achieve higher yield when polymeric chitosan was applied by seed soaking before planting followed by foliar spraying but it did not show any significant difference from the other treatments.

Key words: chitosan, *Oryza sativa* L. , rice yield, molecular weight

INTRODUCTION

Chitosan is a natural biopolymer extracted from chitin, which is the main structural component of the shells of shrimps, crabs, squid pens and cell walls of some fungi. Chitin and chitosan are co-polymers found together in nature. They especially have an inherent property of being environmentally friendly and easily degradable. Thailand is a world-leading exporter of frozen shrimps, therefore, it has abundant raw materials for chitosan production, which is a way to create an added value to the materials. Chitosan has wide scope of application on various plants. With high affinity and being non-toxic, it does no harm to human beings or livestock. Chitosan regulates the immune system of plants, and induces the excretion of enzyme contributed to plant resistance. Therefore, chitosan not only activates the cells and stimulates the growth of plants, but also improves its disease and insect resistant ability (Doares *et al.*,1995). Chitosan has a great effect

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on agriculture including being carbon source for microbes in the soil, accelerating the transferring process of organic matter into inorganic matter and facilitaing the root system of plants to absorb more nutrients from the soil. Chitosan is absorbed by the roots after being decomposed by bacteria in the soil and chitin enzyme secreted by the roots (Somashekar and Ricard,1996, Brian *et al*., 2004). Plants with high content of chitin enzyme have better disease resistance (Khan *et al.*, 2003). Agriculture using chitosan, even without using chemical fertilizer, increases the microbes in soil by a large scale, and mineralized organic form of nutrients into inorganic forms, which can be easily absorbed by the plant roots, so that the more nutrients can be absorbed by the plant. High chitin enzyme level improves the durability and resistance of the plant, makes it not easily infected by germs, not proliferate even infected, and cures the disease by itself. It increases the yield, content of sugar and preservation (Synowiecki and Nadia, 2003). Using chitosan in agriculture with less use of chemical fertilizer increases the production, in different kinds of plant, by 15-20% (Hong *et al*., 1998). The objectives of this study were to determine the most effective chitosan type and appropriate application method for increasing rice yield.

MATERIALS AND METHODS

The experimental design was set up with 3 × 3 factorial in RCB with one control and replicated four times. The factor A was seed soaking, seed soaking+ foliar spraying and foliar spraying: the factor B was monomeric chitosan (degree of deacetylation = 99.74%, molecular weight ~216 KDa), oligomeric chitosan (degree of deacetylation = 95.01%, molecular weight ~10,000 KDa) and polymeric chitosan (degree of deacetylation = 96.62%, molecular weight ~ 100,000 KDa)). Chitosan used in this experiment was flake. It was dissolved in 2% of dilute acetic acid to be liquid chitosan before applying to rice plants. Rice seeds cv. Suphan Buri 1 were soaked in chitosan solution for 5 hours before planting and soaked in water for control treatment. All of these seeds were planted in the pots which contained eight kg/pot of paddy soil analysed chemical properties before planting: (pH = 4.8, % organic matter = 2.4, aval. P = 2 mg/kg, exch. K =195 mg/kg). Each treatment per replication consisted of eight pots,and each pot had eight plants. Four pots were used for dry matter accumulation data and the remainders were used for the data of yield and yield components. Chitosan was sprayed four times at seedling, tillering, panicle initiation and heading stage at 20 ppm concentration. Chemical fertilizer mixed between 46-0-0 and 16-20-0 (ratio:1:1w/w) at the rate of 50 kg/rai(3.1 g/pot)was applied in all treatments, without chitosan in control treatment. This experiment was conducted at Rajamangala University of Technology Suvarnabhumi during January to April 2005. Data were subject to analysis of variance (ANOVA) and least significant difference (LSD) was used to compare treatment mean differences.

RESULTS AND DISCUSSION

Plant height

Plant heights of rice are shown in Table 1. Application with different molecular weights of chitosan did not affect plant height. It was also found that plant height was not significantly different under various application methods. This result was similar to the report of Khan *et al.* (2002) which reported that foliar application of oligomeric chitosan did not affect plant height of soybean. On the other hand, Ouyang and Xu (2003) who studied the chinese cabbage (*Brassica campestris*) *cv.* Dwarf hybrid No. 1, found that seed dressing with 0.4-0.6 mg/g seed and leaf spraying 20-40 micro g/ml increased plant height and leaf area of chinese cabbage.

Table 1 Effects of chitosan with different molecular weights on plant height (cm) of rice *cv*. Suphan Buri 1.

Application method (M)	Type of chitosan (S)			Average
	Monomer	Oligomer	Polymer	
Soaking seed	99.75	96.81	97.06	97.87
Soaking seed+ foliar spraying	96.56	99.43	98.43	98.14
Foliar spraying	99.18	101.37	100.81	100.45
Average	98.50	99.20	98.77	98.82
Control (no chitosan)				95.75
	Control × Grand mean	M	S	M × S
LSD.05	ns	ns	ns	ns
CV.(%)	2.78			

Dry matter accumulation

Table 2 shows dry matter accumulation of rice affected by chitosan application with different molecular weights and various methods of application. Application by seed soaking before planting and folair sprays four times affected dry matter accumulation at panicle initiation stage but did not affect those of the other growth stages. Application of polymeric chitosan significantly increased ($P<0.05$) dry matter accumulation at harvesting stage. This result indicated that application of polymeric chitosan by seed soaking before planting followed by four folair sprayings remarkably increased dry matter accumulation. There were many reports that supported this result such as work of Ali *et al.* (1997) which revealed that dry matter accumulation of soybean *cv.* Akishirome increased 42 days after sowing with soils supplemented with 0.1% chitosan. Ouyang and Langlai (2003) also reported that seeds of non-heading chinese cabbage dressed with chitosan at the rate 0.4-0.6 mg/g seed and leaf spraying with 20-40 micro g/ml increased fresh weight. The results of Hidalgo *et al.* (1996) showed that tomato plants grown from seed coated with chitosan increased dry weight and stem thickness more than the untreated plants.

Grain yield

The effects of different molecular weights and application methods on rice yield are shown in Table 3. Application of chitosan with different molecular weights did not affect rice yield. In terms of methods of application, there were no significant differences in yield, however, the application of polymeric chitosan by seed soaking and four sprayings foliar later tended to obtain higher yield than the other treatments. This results supported the result of Krivtsov *et al.* (1996) which revealed that wheat plants treated with polymeric or oligomeric chitosan increased spike weight and grain yield. In contrast, the work of Kuznia *et al.* (1993) found that seeds of white lupine treated with chitosan did not consistently increase yields.

Yield components

Tiller numbers per plant

The effects of chitosan with different molecular weights on the tiller numbers per plant are given in Table 4. Tillers per plant significantly increased ($P<0.05$) with the increase in molecular weights of chitosan. Application of polymeric chitosan yielded greater tillers per plant than those of the others. For the various application methods, there were no significant differences in tillers per plant, however, seeds were soaked with polymeric

Table 2 Effects of chitosan with different molecular weights on dry matter accumulation of rice cv. Suphan Buri 1.

At panicle initiation stage (g/plant)				
Application method (M)	Type of chitosan (S)			Average
	Monomer	Oligomer	Polymer	
Soaking seed	15.84	12.69	16.11	14.88
Soaking seed+ foliar spraying	13.46	19.74	21.48	18.22
Foliar spraying	15.64	11.91	12.67	13.41
Average	14.85	14.78	16.75	15.50
Control (no chitosan)				9.48
	Control × Grand mean	M	S	M × S
LSD.05	*	1.59	ns	5.15
CV.(%)		22.77		

At harvesting stage (g/plant)				
Application method (M)	Type of chitosan (S)			Average
	Monomer	Oligomer	Polymer	
Soaking seed	22.5	21.09	24.45	22.68
Soaking seed+ foliar spraying	21.46	22.73	25.15	23.12
Foliar spraying	20.70	19.76	21.95	20.80
Average	21.56	21.19	23.85	29.26
Control (no chitosan)				19.60
	Control × Grand mean	M	S	M × S
LSD.05	*	ns	2.03	ns
CV.(%)		10.48		

chitosan before planting and then foliar spraying four times resulted in more tillers per plant over the control (no chitosan). A similar result was also reported by Harada *et al.* (1995) who revealed that soil application with chitosan increased branch and the node numbers per plant of soybean. Similarly, Lu *et al.* (2002) found that panicle numbers of rice increased when rice plant was watered with 0.4 g chitosan/50 cm^3 of water. Ohta *et al.* (2001) also reported that the application of a soil mix of chitosan 1% w/w at sowing remarkably increased flower numbers of *Eustoma grandiflorum.*

Filled and unfilled grain numbers

Table 5 shows the effects on numbers of filled and unfilled grains of rice by chitosan with different molecular weights and various application methods. Filled grains of rice were not affected by chitosan application. Application of polymeric chitosan significantly resulted in less number of unfilled grains than those of the others. The application of polymeric chitosan by seed soaking and four folair sprayings throughout cropping season reduced unfilled grains of rice. This meant that the quality of rice grains was improved by chitosan application. This result was supported by the work of Bhaskara Reddy *et al.* (2000) which studied the effect of pre-harvest sprays of chitosan on post-harvest quality of strawberries stored at 3 and 13°C, and found that chitosan sprays significantly maintained the quality of fruit compared with control. Utsunomiya *et al.* (1998) reported that the number of flowers and the harvested fruits of purple passion fruit increased with soil treated with oligomeric chitosan under high nitrogen conditions.

Table 3 Effects of chitosan with different molecular weights on rice yield (g/pot) cv. Suphan Buri 1.

Application method (M)	Type of chitosan (S)			Average
	Monomer	Oligomer	Polymer	
Soaking seed	131.02	141.56	131.47	134.68
Soaking seed+ foliar spraying	148.26	162.73	171.25	160.75
Foliar spraying	141.76	147.16	168.59	152.50
Average	140.35	150.48	157.10	149.31
Control (no chitosan)				111.72
	Control × Grand mean	M	S	M × S
LSD.05	*	ns	ns	ns
CV.(%)		22.63		

Table 4 Effects of chitosan with different molecular weights on tiller numbers per plant of rice cv. Suphan Buri 1.

Application method (M)	Type of chitosan (S)			Average
	Monomer	Oligomer	Polymer	
Soaking seed	5.82	6.25	6.67	6.25
Soaking seed+ foliar spraying	6.95	6.02	7.72	6.90
Foliar spraying	6.62	6.82	6.87	6.77
Average	6.46	6.36	7.09	6.64
Control (no chitosan)				4.42
	Control × Grand mean	M	S	M × S
LSD.05	*	ns	0.53	ns
CV.(%)		9.58		

Table 5 Effects of Chitosan with Different Molecular Weights on Filled and Unfilled Grain numbers of Rice cv. Suphan Buri 1.

Filled grain numbers (g/panicle)				
Application method (M)	Type of chitosan (S)			Average
	Monomer	Oligomer	Polymer	
Soaking seed	71.75	69372	74.90	72.15
Soaking seed+ foliar spraying	80.35	87.85	92.95	87.05
Foliar spraying	79.30	71.15	80.55	77.0
Average	77.13	76.24	82.80	78.73
Control (no chitosan)				73.75
	Control × Grand mean	M	S	M × S
LSD.05	ns	ns	ns	ns
CV.(%)		17.39		

Unfilled grain numbers (g/panicle)				
Application method (M)	Type of chitosan (S)			Average
	Monomer	Oligomer	Polymer	
Soaking seed	24.25	22.55	22.40	23.06
Soaking seed+ foliar spraying	28.75	23.05	14.30	20.03
Foliar spraying	24.60	20.65	23.55	22.93
Average	25.06	22.03	20.08	22.67
Control (no chitosan)				32.40
	Control × Grand mean	M	S	M × S
LSD.05	*	ns	3.82	6.62
CV.(%)		22.64		

Table 6 Effects of chitosan with different molecular weights on thousand grain weight (g) of rice cv. Suphan Buri 1.

Application method (M)	Type of chitosan (S)			Average
	Monomer	Oligomer	Polymer	
Soaking seed	25.55	24.57	26.65	25.59
Soaking seed+ foliar spraying	25.32	26.90	25.45	25.89
Foliar spraying	25.37	27.65	24.42	25.81
Average	25.41	26.37	25.50	25.76
Control (no chitosan)				26.47
	Control × Grand mean	M	S	M × S
LSD.05	ns	ns	ns	ns
CV.(%)		6.52		

Thousand-grain weight

Thousand grain weights of rice are given in Table 6. Application of chitosan with different molecular weights was not significantly different on thousand-grain weight. Chitosan application with different application methods did not increase thousand-grain weight. This result was contrary to the report of Krivtsov *et al.* (1996) which found that thousand-grain weight and spike weight of wheat plants increased in treatment with low concentrations of polymeric chitosan.

CONCLUSION

There was a tendency to stimulate growth and increase rice yields by soaking seeds before planting followed by four foliar sprayings with polymeric chitosan at the rate of 20 ppm throughout cropping season.

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