EFFICIENCY OF CHITOSAN FOR CONTROLLING DIRTY PANICLE DISEASE IN RICE PLANTS

Chaweewan Boonreung and Suchada Boonlertnirun
Faculty of Agricultural Technology and Agro-industry, Rajamangala University of Technology Suvarnnabhumi, Phranakhon Si Ayutthaya Province, Thailand
E-Mail: suchada.b@rmutsb.ac.th

ABSTRACT

Currently, chitosan has been popularly used to trigger immune system in many crop species for plant disease protection. This study aimed to determine chitosan effect on controlling dirty panicle disease and affecting on rice yield. The experimental design was a split-plot in Randomized complete block with 2 conditions (inoculated and non inoculated rice plant) as main plot and 3 application methods (chemical fertilizer in combination with chitosan, chemical fertilizer alone and no both chemical fertilizer and chitosan) as sub plot. Pot experiment was conducted in an open greenhouse during March to June 2012. The results were revealed that all studied traits of inoculated and non inoculated rice plants applied with various application methods were not significantly different. Application of chemical fertilizer in combination with chitosan did not significantly differ from application of chemical fertilizer alone on leaf greenness, plant height, dry matter, grain yield and panicle numbers but significantly differed from those unapplied both chemical fertilizer and chitosan. However, seeds of dirty panicle disease were significantly affected by various application methods, the lowest numbers were obtained from application of chemical fertilizer in combination with chitosan whereas no application of both chemical fertilizer and chitosan showed negative effect on controlling dirty panicle disease in both inoculated and non inoculated rice plant. From this present study it might be explained that it is possibly to spray chitosan to rice plants for controlling dirty panicle disease caused by fungi pathogens.

Keywords: chitosan, inoculated rice plant, dirty panicle disease.

INTRODUCTION

Rice is very important cereal crop in many countries. Especially, in Thailand it is very important economic crop because the main income of our country comes from rice export. In 2011, rice yields were exported about 10,706 million- tons with the values of 632 million US dollar (Thai Rice Exporters Association, 2012). However, there are many serious problems negatively affecting rice yield resulted in yield losses. One of them is dirty panicle disease caused by fungi and bacteria. This disease damages rice yield and also reduces seed quality such as germination percentage (Thavong, 2002) and usually infects rice plants before and after harvest. The severity is depended on environmental surrounding and pathogen race. Some fungi pathogens caused dirty panicle diseases are *Helminthosporium oryzae*, *Curvularia lunata* and *Fusarium moniliformae* etc. Therefore, finding the effective method to solve this problem should be considerably focused. Chitosan, a natural polymer, has been reported to use to stimulate immune system involved in plant resistance to pathogen infection. Furthermore, it can induce the accumulation of phytoalexins resuting in antifungal responses and also enhances protection of further infection (Uthairatanakij *et al*., 2007). In addition, chitosan has been widely used as growth stimulator, germination acceleration and yield enhancement in many crop species such as in orchid (Nge *et al*., 2006), faba bean (El-sawy *et al*., 2010), cucumber (Shehata *et al*., 2012) and corn (Boonlertnirun *et al*., 2011; Lizárraga-Paulín *et al.*, 2011). Due to this reason, our objectives is to investigate chitosan effect on controlling dirty panicle disease and affecting on rice yield.

MATERIALS AND METHOD

Experiment design

Split plot in Randomized complete block design with 2 conditions (inoculated and non inoculated rice plant) as main plot and 3 application methods (chemical fertilizer in combination with chitosan, chemical fertilizer alone and no both chemical fertilizer and chitosan) as sub plot. The details of treatment are as the following:

Tr1. Mixed chemical fertilizer application between formula 46-0-0 and 16-20-0 at the rate of 312.5 kg/ha in combination with chitosan at the concentration of 40 mg/l.
Tr2. Mixed chemical fertilizer application between formula 46-0-0 and 16-20-0 at the rate of 312.5 kg/ha
Tr3. No application both chemical fertilizer and chitosan

Inoculum and plant preparation

Isolation of fungi. Three fungi pathogens caused of dirty panicle disease were isolated from dirty panicle disease seeds by Tissue transplanting method. Dirty panicle disease seeds were surface-disinfected with 70% ethanol for 1 minute, rinsed with sterilized distilled water, and then air-dried on a clean bench. Dried samples were placed on potato dextrose agar (PDA) and incubated at 25°C. After 5 days of incubation, growing mycelium tips were placed on new PDA medium and incubated at 25°C to obtain pure culture of the fungi. Conidial suspension ($1x10^5$ conidia/ml) and mycelial dishes (Ø 5 mm) prepared from 7 days old culture of three fungi pathogens: *Helminthosporium oryzae*, *Curvularia lunata* and *Fusarium moniliformae*.

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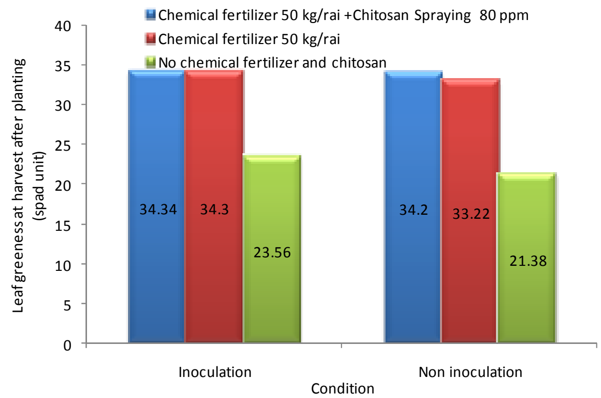
Rice seeds cv. Pathum Thanee 1 was planted in 60-cm diameter cement tank containing clay soil which was analyzed chemical properties: (pH= 7, % OM = 2.35 (moderate), aval. P = 88 mg/kg (very high), exch. K = 230 mg/kg (very high)). Only six healthy seedlings per treatment per replication were selected to use as representative for data recording. When rice plant age was 25-30 days after planting, the first chitosan spraying was done, after that chitosan was again sprayed at 7-10 days intervals for four times through crop season. Chemical fertilizer at the rate as mentioned above was divided to apply 3 times at 15, 35 and 55 days after planting. When age of rice plants was 65 days, all rice plants were divided into 2 sections (inoculation and non inoculation section), conidial suspension ($1x10^5$ conidia/ml) and mycelial plugs (Ø 5 mm) of *Helminthosporium oryzae*, *Curvularia lunata* and *Fusarium moniliformae* were inoculated on wound rice leaves of inoculation section, and then water was immediately sprayed all over rice plants and covered by plastic bag for 24 hrs. for maintaining moisture content within canopy, after that plastic bag was withdrawn from rice plants. This experiment was carried out at Rajamangala University of Technology Suvarnabhumi during March to June 2012. All data were subjected to ANOVA by MSTAT and treatment mean comparison was done by the use of Least Significant Difference (LSD).

RESULTS AND DISCUSSIONS

Leaf greenness

Leaf greenness of inoculated and non inoculated rice plants was not significantly different after application of various methods. Leaf greenness of rice plants applied with chemical fertilizer had darker green than those unapplied fertilizer, however it did not significantly differ from those applied chemical fertilizer in combination with chitosan. Application of chemical fertilizer in combination with chitosan tended to promote leaf greenness in both inoculated and non inoculated rice plants greater than unapplied fertilizer (Figure-1). It may be explained that the main components of chitosan are comprised of nitrogen molecules which contributes to more chlorophyll synthesis resulting in darker green leaves. Limpanavech *et al.* (2008) reported that chitosan O-80 significantly increased the chloroplast diameter of Dendrobium 'Eiskul' in young leaves, when the plants were treated with 10 and 50 ppm of chitosan O-80. At concentration of 50 ppm, chitosan O-80 also significantly caused chloroplast enlargement in the old leaves.

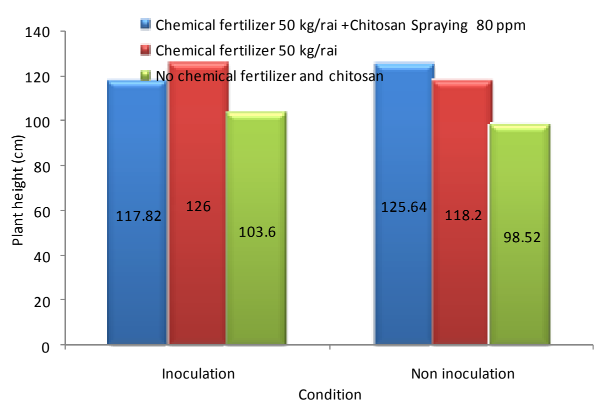


Leaf greenness
LSD.05 (condition, M) = ns
LSD.05 (application method, S) = 2.142
LSD.05 (M*S) = ns CV (%) = 7.49

Figure-1. Leaf greenness of inoculated and non inoculated rice plants after application of various methods.

Plant height

No application of both chemical fertilizer and foliar chitosan significantly reduced height of inoculated and non inoculated rice plants. Plant height between rice applied with chemical fertilizer alone and in combination with chitosan was not significantly but differed from those unapplied both chemical fertilizer and chitosan. In this present result chitosan did not clearly show positive effect on height of both inoculated and non inoculated rice plants (Figure-2). This is in accord with work of Khan *et al.* (2002) who found that foliar application of oligomeric chitosan did not affect soybean height. The same result in rice cv. Pathum Thanee 1 applied with oligomeric chitosan in combination with chemical fertilizer was also reported (Boonlertnirun *et al*., 2012).



Plant height
LSD.05 (condition, M) = ns
LSD.05 (application method, S) = 6.375
LSD.05 (M*S) = ns CV (%) = 5.85

Figure-2. Plant height of inoculated and non inoculated rice plants after application of various methods.

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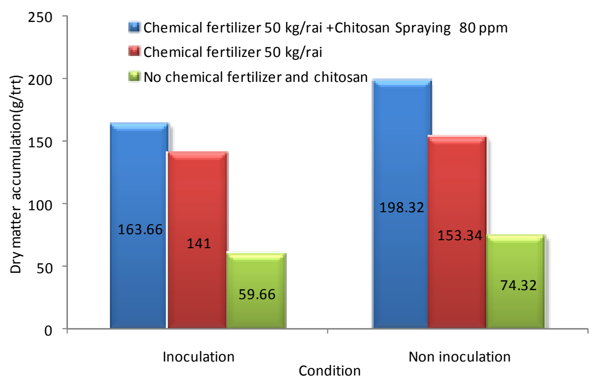
Dry weight

Dry matter of inoculated and non inoculated rice plants was not significantly different among various application methods. Focusing on application method, it indicated that dry matter of rice plants did not differ between application of chemical fertilizer alone and in combination with chitosan but significantly differed from no application of both chemical fertilizer and chitosan. However, application of chemical fertilizer in combination with chitosan in non inoculated rice plant tended to promote dry matter over than the other treatments (Figure-3). Total dry mass per plant of okra was increased with increasing concentration of chitosan until 25 ppm (Mondal *et al.*, 2012). The similar result studied in cucumber reported by Shehata *et al.* (2012) found that foliar application with chitosan at rates of 4 mlL^{-1} recorded the best treatment to obtain the highest vegetative growth.

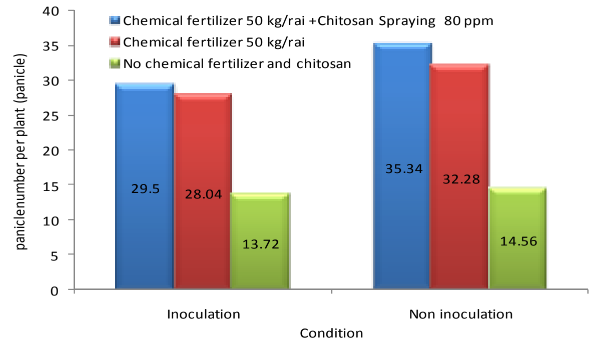


Dry matter accumulation
LSD.05 (condition, M) = ns
LSD.05 (application method, S) = 28.51
LSD.05 (M*S) = ns CV (%) = 12.83

Figure-3. Dry matter accumulation of inoculated and non inoculated rice plants after application of various methods.

Panicle number per plant

No significant difference between inoculated and non inoculated rice plant was observed in terms of panicle number per plant after application of various methods. Similar result was reported by Boonlertnirun *et al*. (2008) in rice plant cv. Suphan Buri 3. The lowest panicle number was obtained from treatment without fertilizer and chitosan whereas application of fertilizer alone or in combination with chitosan did not show any significant difference on panicle numbers. However, application of fertilizer in combination with chitosan had positive trends on panicle numbers of both inoculated and non inoculated rice plant (Figure-4). Chitosan application improved yield components (number and weight) of strawberry plants (Abdel-Mawgoud *et al*., 2010).

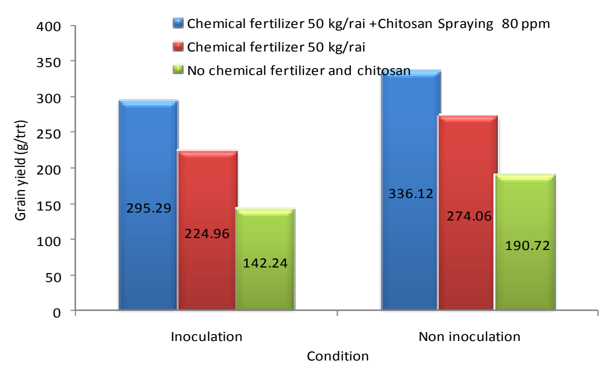


Panicle number per plant
LSD.05 (condition, M) = ns
LSD.05 (application method, S) = 6.35
LSD.05 (M*S) = ns CV (%) = 14.61

Figure-4. Panicle number per plant of inoculated and non inoculated rice plants after application of various methods.

Grain yield

Yield of non inoculated rice plants was rather higher than that of inoculated ones; however it did not show any significant difference. The highest grain yield tended to obtain from applying fertilizer in combination with chitosan. Similar result revealed by El-Tantawy (2009) found that application of chitosan in combination with manure could increase yield of tomato over than applying manure alone. Soybean yield was increased about 20% over the control when applied chitosan at the concentration of 5% (Zeng *et al*., 2012). Whereas the lowest grain yield was observed in unapplied both chemical fertilizer and chitosan (Figure-5). From this finding it could be explained that chitosan had potential to be used to control fungi pathogen because yield of inoculated rice plants were not significantly different from those of non inoculated ones. There were many previous studies reported about chitosan properties on plant disease control (Rodriguez *et al*., 2007; Rakwal *et al.* 2002; Ben-Shalom *et al*., 2003; Liu *et al*., 2007; Win *et al*., 2007).

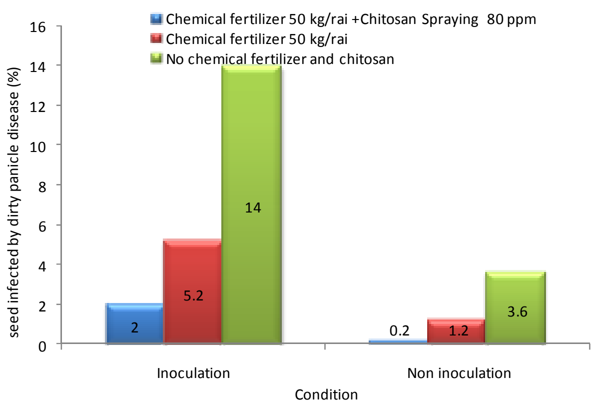


Grain yield
LSD.05 (condition, M) = ns
LSD.05 (application method, S) = 73.37
LSD.05 (M*S) = ns CV (%) = 11.73

Figure-5. Grain yield of inoculated and non inoculated rice plants after application of various methods.

Percentage of seed infected by dirty panicle disease

Percentage of seeds infected by dirty panicle disease from non inoculated rice plants was higher than those from inoculated ones and showed obviously significant difference. Application of chitosan in combination with chemical fertilizer achieved to reduce seeds infected by dirty panicle disease in both inoculated and non inoculated rice plants and significantly differed from the other treatments. The maximum seeds infected by dirty panicle disease were obtained from unapplied both chemical fertilizer and chitosan (Figure-6). This result implied that it might be possible to reduce seeds infected by dirty panicle disease by spraying chitosan during cropping season. Several chitosan studies about controlling plant disease were positively achieved such as in horticultural commodities (Bautista-Banos *et al.*, 2006), groundnut (*Arachis hypogaea;*) (Sathiyabama and Balasubramanian, 1998), pear fruit (Meng *et al.*, 2010) and tobacco plants (Falcon-Rodriguez *et al*., 2011).



Seeds infected by dirty panicle disease
LSD.05 (condition, M) = 1.341
LSD.05 (application method, S) = 1.896
LSD.05 (M*S) = ns CV (%) = 22.39

Figure-6. Seeds infected by dirty panicle disease percentage of inoculated and non inoculated rice plants after application of various methods.

CONCLUSIONS

According to this result it can be recommended that foliar application of chitosan four to five times throughout rice crop season should be added in rice growing for controlling dirty panicle disease caused by fungi pathogen which was the serious problem in rice production; however an advanced rice disease study will be focused for the further work.

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