Evaluation of potential for chitosan to control TYLCV disease and promote the growth of Sridathip 3 tomato

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Abstract

Sridathip 3 tomato is a hot-tolerant tomato variety for summer production appropriate to grow in Thailand. Tomato yellow leaf curl virus disease (TYLCV) is the most devastating virus disease both in terms of quantitative and qualitative yield losses in tomato production in Thailand. Chitosan, an N-acetylated derivative of the polysaccharide chitin, is a natural compound for enhancing plant growth, inducing plant disease resistance and reducing damage from plant pathogens. This study aimed to evaluate potential use of chitosan to control TYLCV disease and promote a growth of Sridathip 3 tomato. Our results indicated that chitosan treatment improved seed germination. The application of 60 ppm chitosan was founded to enhance the height of Sridathip 3 tomato plants. All concentrations of chitosan retarded tomato yellow leaf curl disease symptoms and reduced TYLCV accumulation. Thus, chitosan has the potential to control TYLCV disease and enhance the growth of Sridathip 3 tomato.

Keywords: Chitosan, ELISA, Tomato Sridathip 3, Tomato yellow leaf curl virus

Introduction

Vegetables play a very important role in human diet and health as they provide minerals, vitamins, and antioxidants. Tomato (*Solanum lycopersicum* L.) is one of the most major and important crop of Solanaceae grown in Thailand which consumed in fresh or process forms such as ketchup, tomato soup or tomato juice. Most of tomato varieties introduced into the market are imported varieties. Thus, Thai scientists do create many new varieties to improving tomato production in Thailand. Tomato varieties developed by Tropical Vegetable research Centre, Kasetsart University, Kamphangsaen campus, Thailand, include Sridathip 1, Sridathip 2, Sridathip 3 and Sridathip 4. Sridathip 3 is a hot-tolerant tomato variety for summer production, fairly short growing time and also abundance of fruits at 22 fruits/plant. Tomato is often affected by many diseases leading to substantial losses in yield including fungal, bacterial, phytoplasmal infections and also a large number of viral diseases. Among tomato diseases, the Tomato yellow leaf curl virus disease is the most devastating virus disease both in terms of quantitative and qualitative yield losses in tomato production in Thailand (AVRDC, 2004). Tomato yellow leaf curl is a destructive viral disease of tomato caused by Tomato yellow leaf curl virus (TYLCV). In Thailand, the virus is a bipartite geminivirus, transmitted by the whitefly *Bemisia tabaci* whose severe population outbreaks are usually associated with high incidence of the disease (Tantiwanich *et al*., 1999).
Application of natural compound for enhancing plant growth and increase the ability to disease and pests is necessary. Among the approaches for inducing plant disease resistance and reducing damage from plant pathogens and also stimulate the immunity of plant is chitosan. Chitosan is a polysaccharide derived from chitin, a polysaccharide found in the

exoskeleton of shellfish such as shrimp, lobster, or crabs and cell walls of fungi (Wojdyla, 2001). It has been report that chitosan increased the growth of gerbera plants by enhancing the average values of flower stem-length, the number of growing leaves, including leaf width and length as well as the number of the flowers per bush (Wanichpongpan *et al*., 2001). Chitosan was reported to prolong storage life and control decay of several fruit (Bautista-Baños *et al*., 2006). Chitosan was able to control the seed-borne infection *Fusarirm graminearum* and increase the crop yield by 20 % after seed treatment of wheat (Bhaskara *et al*., 1999). The development and the possible mechanism of the chitosan-induced resistance to the potato virus X (PVX) infection were investigated in potato plants. The result showed that, a day after treatment, the plants acquired a resistance to viral infection and the chitosan treatment also significantly decreased the number of systemically infected plants as compared to the control (Chirkov *et al*., 2001). As the potential of chitosan reported to reduce damage from plant pathogens and enhance the growth of several plants, the objectives of this study were to determine the application of chitosan to control of TYLCV disease and promote a growth of tomato Thai variety, Sridathip 3.

Methodology

Raising Tomato seedlings
Tomato seeds (var., Sridathip 3) were sown on wet tissue paper in plastic box for 3 days in the dark room and transferred to trays filled with soil and farmyard manure. Twenty five days old seedlings were used for viral inoculation purposes and effect of chitosan application on vegetative growth.

Seed germination
Chitosan solutions (low molecular weight, Sigma-Aldrich, USA) of 0 ppm, 20 ppm, 10 ppm, 20 ppm, 40 ppm, 60 ppm and acetic acid were used for tomato seed (Sridathip 3) priming. Three replicates of 100 seeds each were used for each treatment. Treated seeds were place in plastic box and incubated at 28 ºC for 3 days.

Effect of chitosan application on vegetative growth
Chitosan solution was prepared by dissolving a proper amount of chitosan in 0.01 M acetic acid to obtain five concentrations at 0 ppm, 10 ppm, 20 ppm, 40 ppm and 60 ppm. One hundred milliliters of each concentration were applied to five tomato plants with one month intervals. The first application was carried out 8 weeks after transplanting. The data including plant height, the number of fruits and size of fruits were collected every week after application.

Chitosan treatment
Twenty five days old Sridathip 3 tomato plants were treated with chitosan solutions (Low molecular weight, Sigma-Aldrich, USA) at the various concentration including 0 ppm, 20 ppm, 10 ppm, 20 ppm, 40 ppm and 60 ppm. Five plants were used for each treatment. Treated plants were grown in the greenhouse without disease vectors, whitefly (*Bemisia tabaci*.)

The viral inoculation
The culture of TYLCV was kindly provided by Dr. Orawan Chatchawankarnpanich from Plant Genetic Engineering Unit, National Center for Genetic Engineering and Biotechnology. The healthy tomato plants treated with chitosan solutions at various concentrations were inoculated with TYLCV by grafting technique and the plants were maintained in the greenhouse throughout the period of study.

Monitoring of the disease

Symptom development was evaluated according to the symptom severity scale described by Friedmann *et al*. (1998) as follows: 0 = no visible symptoms, inoculated plants show same growth and development as non-inoculated plants; 1 = very slight yellowing of leaflet margins on apical leaf; 2 = some yellowing and minor curling of leaflet ends; 3 = a wide range of leaf yellowing, curling and cupping, with some reduction in size, yet plants continue to develop; and 4 = very severe plant stunting and yellowing, pronounced leaf cupping and curling, and plant growth stops.

Determination of the geminivirus by Sandwich ELISA using monoclonal antibody to TYLCV

Leaves (0.2 g) of geminivirus infected or healthy plants were homogenized in 500 µl of extraction buffer (0.05 M Tris-HCl, 0.06 M sodium sulphite, pH 8.5). The 96 well-plates were coated with rabbit polyclonal antibody to geminivirus causing yellow leaf puckering disease in pumpkin. After washing, plates were blocked by addition of 2% BSA in PBS-Tween 20. Plates were again washed. Plant extracts were added. After washing, monoclonal antibody M1 (MAb M1), which was specifically for TYLCV detection, was then applied to the wells. The plates were rewashed. Alkaline phosphatase-conjugated goat anti-mouse immunoglobulins were added. After washing, the wells were then incubated with substrate solution for alkaline phosphatase (p-nitrophenyl phosphate). The absorbance at 405 nm was measured using an automated microplate reader (Tecan, Switzerland).

Results

To determine an effect of chitosan on the germination of Sridathip 3 tomato seeds, various concentrations of chitosan including 0 ppm, 10 ppm, 20 ppm, 40 ppm and 60 ppm were used. The germination was checked 3 days after sowing by counting the number of sprouting seeds within each concentrations of chitosan experiment. The result showed that chitosan treatment of tomato seeds improved seed germination. The highest germination percentage (79.3 observed in 10 ppm chitosan while percentage of seed germination was 77 % at 20 ppm chitosan, 73.3% at 40 ppm chitosan and 73.2% at 60 ppm chitosan, respectively. The lowest percentage (69.3 %) was recorded for the control at 0 ppm chitosan treatment (Figure 1).

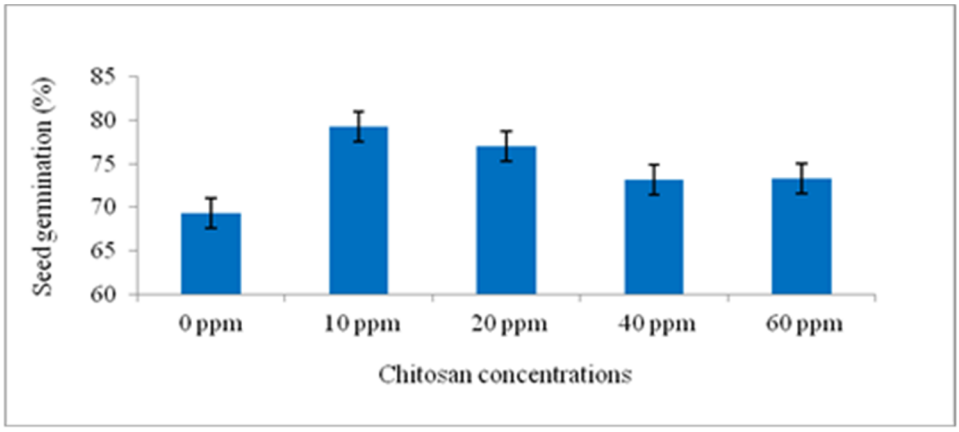

Figure 1: The effect of chitosan on the germination of Sridathip 3 tomato seeds.

A study on the effect of chitosan application on vegetative growth of Sridathip 3 tomato plants were done on five healthy tomato applied with various concentrations of low molecular weight chitosan including 0 ppm, 20 ppm, 10 ppm, 20 ppm, 40 ppm and 60 ppm. The result showed that concentrations of low molecular weight chitosan at 60 ppm enchanced the hight of Sridathip 3 tomato plants (Figure 2); however, there were no significant differences in the number and size of tomato fruits.

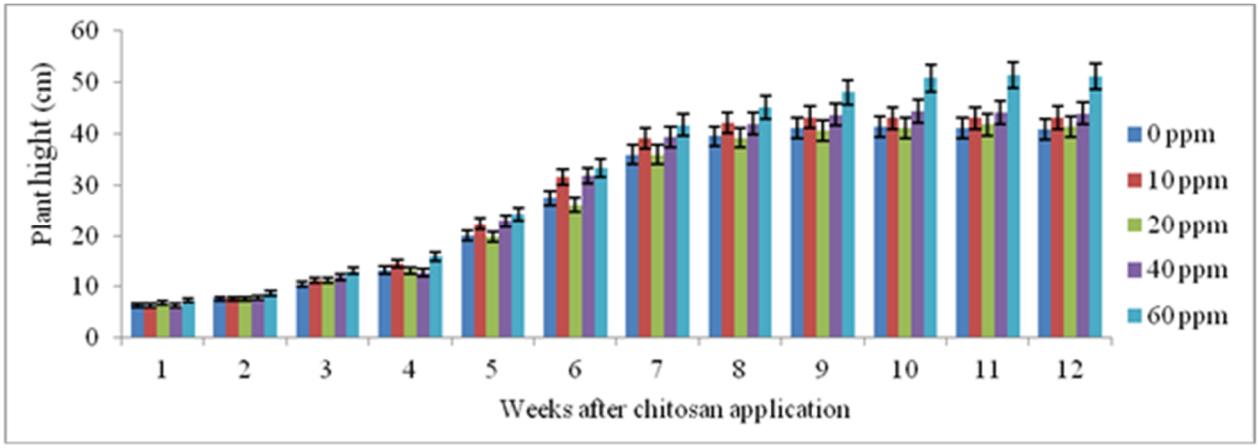

Figure 2: Effect of chitosan application on hight of Sridathip 3 tomato plants after chitosan application at various concentrations.

To determine the potential of chitosan to control TYLCV disease, twenty five days old Sridathip 3 tomato plants were treated with chitosan solutions and inoculated with TYLCV Thai isolate by grafting disease plants onto healthy tomato plants. Symptom development was evaluated according to the symptom severity scale. In this experiment, all concentrations of chitosan retarded tomato yellow leaf curl disease symptoms about 7-14 days compare to positive disease control plants (Table 1).

Table1 Tomato yellow leaf curl disease symptom development when apply with chitosan at various concentrations following inoculation of the virus.

Treatments	Days after inoculation								
	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42	Day 49	Day 56
Disease plants (Positive control)	0	0	1	2	3	3	4	4	4
Healthy plants (Negative control)	0	0	0	0	0	0	0	0	0
0 ppm Chitosan	0	0	1	2	2	3	3	3	4
10 ppm Chitosan	0	0	0	0	1	1	2	2	3
20 ppm Chitosan	0	0	0	1	1	2	2	2	3
40 ppm Chitosan	0	0	0	1	1	3	3	3	4
60 ppm Chitosan	0	0	0	1	1	2	3	4	4

Disease scores:

0 = no visible symptoms
1 = very slight yellowing of leaflet margins on apical leaf
2 = some yellowing and minor curling of leaflet ends
3 = a wide range of leaf yellowing, curling and cupping, with some reduction in size, yet plants continue to develop
4 = very severe plant stunting and yellowing, pronounced leaf cupping and curling, and plant growth stop

Determination of geminivirus by Sandwich ELISA using monoclonal antibody to TYLCV, 0.2 grams of disease leave, healthy leave and tomato leave applied with chitosan at various concentrations following inoculation of the virus were used. The result of ELISA assay showed that application of chitosan before viral inoculation retarded tomato yellow leaf curl disease as viral detection after 28 days after inoculation and reduced the viral accumulation (Table 2).

Table 2 Effect of chitosan on control of tomato yellow leaf curl disease.

Treatments	Days after inoculation								
	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42	Day 49	Day 56
Disease plants (Positive control)	3.178	4.144	3.710	2.700	2.400	1.856	1.860	2.059	2.297
Healthy plants (Negative control)	0.211	0.199	0.133	0.155	0.205	0.101	0.101	0.106	0.108
0 ppm Chitosan	0.236	0.196	0.488	1.357	1.237	1.350	1.439	1.713	2.435
10 ppm Chitosan	0.221	0.142	0.143	0.132	0.164	0.101	1.934	2.338	1.217
20 ppm Chitosan	0.207	0.171	0.138	0.338	0.228	0.300	0.494	0.738	0.993
40 ppm Chitosan	0.204	0.160	0.146	0.131	0.207	0.222	0.950	1.027	1.902
60 ppm Chitosan	0.200	0.141	0.140	0.176	0.421	0.408	1.324	2.205	1.660

Discussion

This study determined the effects of a chitosan in the control of TYLCV disease and on a growth of tomato Thai variety, Sridathip 3. Our results indicated that chitosan treatment of tomato seeds improved seed germination. Kurzawińska (2007) reported that percentage of germinated lettuce seeds treated with chitosan was higher than that of the control. In addition, chitosan treatment (2-8 mg/mL) of wheat seeds significantly improved seed germination. The germination was < 80% in the control and >85% in benomyl- and chitosan-treated seeds (Bhaskara *et al*., 1999). Mahdavi (2013) reported that chitosan treatments increased germination percentage and germination rate. Chitosan treatment of rice seeds induced tolerance to stress condition and improved seed germination and seedling growth (Ruan and Xue, 2002). A study on the effect of chitosan application on vegetative growth of Sridathip 3 tomato plants indicated that 60 ppm chitosan enchanced the hight of Sridathip 3 tomato plants; however, there were no significant differences in the number and size of tomato fruits. In previous, it has been report that chitosan increased the growth of gerbera plants by enhancing the average values of flower stem-length, the number of growing leaves, including leaf width and length as well as the number of the flowers per bush (Wanichpongpan *et al*., 2001). Furthermore, chitosan seed coating promoted the vegetative growth in pearl millet (Sharathchandra *et al*., 2004) and sweet basil (Kim *et al*., 2005). The application of chitosan in various crops such as soya bean sprouts (Lee *et al*., 2005) and sweet basil (Kim *et al*, 2005) were also reported to promote the growth. The mechanism of action of chitosan on growth is not clear. Uthairatanakij and co-workers (2007) suggested that chitosan may induce a signal to synthesize plant hormones such as gibberellins and enhance growth and development by some signaling pathway related to auxin biosynthesis. In this study, we also monitored the potential use of chitosan to control TYLCV disease. Our results showed that all concentrations of chitosan retarded tomato yellow leaf curl disease symptoms and reduced TYLCV accumulation. In previous experiments, it was observed that chitosan treatments reduced virus infection in various plants. On bean leaves, local infections produced by alfalfa mosaic virus (ALMV) were completely controlled with the highest chitosan concentration (0.1%) either sprayed or added to the inoculum (Pospiezny *et al*., 1991). Similar inhibition was reported on tomato leaves treated with chitosan at the same concentration and inoculated with potato spindle tuber viroid (Pospiezny, 1997). Chitosan antiviral activity investigated in the pathosystem *Phaseolus vulgaris* - tobacco necrosis virus (TNV). Chitosan application elicited both callose apposition and ABA accumulation in leaf tissues, at 12 and 24 h after treatment and induced a high level of resistance against TNV. The results indicated that the rise of ABA synthesis induced by chitosan plays an important role in enhancing callose deposition involving in virus spreading (Iriti and Faoro, 2008).

Conclusion

Determination of the effects of a chitosan in the control of TYLCV disease and on a growth of tomato Thai variety, Sridathip 3 indicated that chitosan treatment improved seed germination. 60 ppm chitosan enhanced the height of Sridathip 3 tomato plants; however, differences in the number and size of tomato fruits were not found. All concentrations of chitosan retarded tomato yellow leaf curl disease symptoms and reduced TYLCV accumulation. Due to our results, chitosan has the potential to control TYLCV disease and promote the growth of Sridathip 3 tomato.

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