รายงานการวิจัย

ประสิทธิภาพของไคโตซานต่อการควบคุมโรคใบร่วงของต้นกล้ายางพารา

Efficacy of Chitosan in Controlling Phytopthora Leaf Fall Disease of Para Rubber Seedlings

โดย

อนุรักษ์ สันป่าเป้า

ภาควิชาการจัดการศัตรูพืช มหาวิทยาลัยสงขลานครินทร์

คณะทรัพยากรธรรมชาติ วิทยาเขตหาดใหญ่ จังหวัดสงขลา

กิตติกรรมประกาศ

ขอขอบคุณมหาวิทยาลัยสงขลานครินทร์ สำหรับทุนวิจัยจากเงินรายได้มหาวิทยาลัย ประเภททั่วไป ที่ได้ให้การสนับสนุนทุนวิจัย และขอขอบคุณ ภาควิชาการจัดการศัตรูพืช คณะทรัพยากรธรรมชาติ มหาวิทยาลัยสงขลานครินทร์ วิทยาเขตหาดใหญ่ จังหวัดสงขลา สำหรับสถานที่ทดลอง อุปกรณ์ เครื่องมือ เพื่อให้ดำเนินการวิจัยได้สำเร็จลุล่วงด้วยดี

อนุรักษ์ สันป่าเป้า

มิถุนายน 2557

ประสิทธิภาพของไคโตซานต่อการควบคุมโรคใบร่วงของต้นกล้ายางพารา

บทคัดย่อ

โรคใบร่วงของยางพาราเป็นโรคที่ก่อให้เกิดความเสียหายอย่างมากต่อต้นกล้ายางพารา โรคนี้มีเชื้อสาเหตุที่พบบ่อยคือเชื้อ *Phytophthora botryosa* (Chee) และ *P. palmivora* (Butler) การใช้วัสดุออกฤทธิ์ทางธรรมชาติไคโตซานต่อการควบคุมเชื้อสาเหตุโรคใบร่วงถูกศึกษาในการทดลองทั้งในห้องปฏิบัติการและในสภาพโรงเรือน จากการทดลองวัดอัตราการเจริญของเชื้อสาเหตุทั้งสองในอาหาร potato dextrose agar ที่ผสมไคโตซานแต่ละความเข้มข้น พบว่าไคโตซานที่ความเข้มข้น 0.5 มิลลิกรัม/มิลลิลิตร สามารถยับยั้งการเจริญของเชื้อทั้งสองได้ดี (87%) จากการทดสอบโดยวิธี detached leaves ไคซานสามารถลดการพัฒนาของเนื้อเยื่อตาย (necrosis) ที่มีสาเหตุมาจากเชื้อ *Phytopthora* ในช่วงระยะแรกของการติดเชื้อได้ แต่ไม่สามารถยับยั้งการลุกลามของเชื้อบนใบยางพาราได้แบบสมบูรณ์ นอกจากนี้การให้ไคโตซานกับต้นกล้ายางพาราทั้งแบบรดกับฉีดพ่นสามารถลดการลุกลามของเนื้อเยื่อตายจากการปลูกเชื้อ *Phytophthora* บนใบยางพาราอย่างไม่มีนัยสำคัญ

คำสำคัญ: การควบคุมโดยชีววิธี ไคโตซาน โรคใบร่วง ยางพารา *Phytophthora*

Efficacy of Chitosan in Controlling *Phytophthora* Leaf Fall Disease of Para Rubber Seedling

Abstract

The most destructive disease of Para rubber seedling is a leaf fall caused by *Phytophthora botryosa* (Chee) and *P. palmivora* (Butler). The bioactivity of chitosan against *P. botryosa* and *P. palmivora* infection was studied *in vitro* and *in vivo*. The growth rates of *P. botryosa* and *P. palmivora* in potato dextrose agar-were determined with different concentrations of chitosan. The chitosan concentration of 0.5 mg/ml was sufficient to cause clear growth inhibition (about 87%) of both *P. botryosa* and *P. palmivora*. Effect of chitosan on necrotic progression of both *Phytophthora* was observed by detached leaves method. The expansion of necrotic lesion caused by both *Phytophthora* was restricted in the early infection period but was not completely limit the expansion of the pathogen. Furthermore, the treatment in green house was not significantly differed between treated and untreated chitosan in restricting the disease expansion.

Keywords: Biological control, chitosan, leaf fall, Para rubber, *Phytophthora*

Table of Contents

INTRODUCTION

Para rubber is one of the most important economic crops in Thailand. The major rubber-production areas are in southwest and northeast of Thailand. Like most other cultivated crops, rubber is facing serious problems from several diseases: after long periods of high rainfall, the leaf fall disease becomes a problem. Three species of *Phytophthora* common in Thailand are *Phytophthora palmivora* Butler (Tsao *et al*., 1976), *P*. *botryosa* Chee (Suzuki *et al*., 1979), and *P. nicotianae* var. *parasitica* Dastur (Rubber Research Institute of Thailand, 2010). These microorganisms are belonging to *Phytiaceae* Family and *Phytophthora* Genus.

Phytophthora Taxonomy

Domain Eukaryota
Kingdom Straminipila
Phylum Oomycota
Class Oomycetes
Order Peronosporales
Family Phytiaceae
Genus *Phytophthora*

The infection process of a soil borne pathogen, *Phytophthora* starts at immature pod resulting to pod rot and become source of inoculum (Chantarapratin *et al.*, 2001). Zoospores of the pathogen are spread by rain splash from the infected leaves to the trapping panel (Johnston, 1989). In Thailand, leaf fall epidemics occur during June to December (Chantarapratin *et al.*, 2001). An obvious sign caused by *Phytophthora* leaf fall is the coagulated latex in the center of lesion of the petiole (Fig.1). However, the lesions can occur anywhere along the length of the petioles. Heavy defoliation may lead to dieback of terminal branches (Chee, 1968; Turner, 1969; Johnston, 1989).

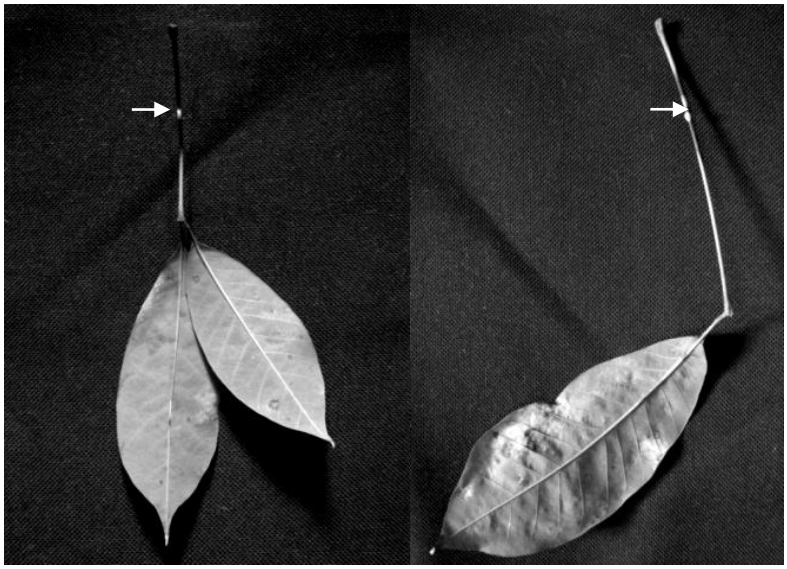

Figure 1. Para rubber leaf fall symptoms. The white arrows indicate coagulated latex in the centers of lesions on petioles.

Application of fungicides, such as metalaxyl and fosetyl-Al, is the most effective method to control *Phytophthora* disease. However, chemical control may select for fungicide-resistant strains of these pathogens, and is under review in many countries also due to human health concerns. Bioactive substances are actively pursued for an alternative approach. An application of chitosan and derivative chitosan has been shown to be an effective biological control against several fungi in several hosts (Allan and Hadwiger, 1979; El Ghaouth *et al*., 1999; Hirano and Nagao, 1989; Kendra *et al.*, 1989; Stossel and Leuba, 1984). The application of chitosan has been demonstrated to reduce disease severity and incidence of *Puccinia pimpinellae* (Saber *et al.*, 2009). The inhibitory effects of chitosan were also demonstrated with soil borne phytopathogenic fungi (Stossel and Leuba, 1984; Hernandez-Lauzardo *et al.*, 2011).

OBJECTIVE

1. To test chitosan against *Phytophthora in vitro* and *in vivo*

2. To test effect of chitosan in Para rubber growth

MATERIALS AND METHODS

Culture and growth condition of *Phytophthora*

Both *P. botryosa* and *P. palmivora* were provided from Asst. Prof. S. Chuenchit (Department of Pest Management, Faculty of Natural Resources, Prince of Songkla University). The fungi were stored in potato dextrose agar (PDA) (HiMedia, Mumbai, India) slants at 4°C. The fungal mycelia were routinely maintained on PDA made of 200 g potato, 20 g dextrose and 15 g agar at room temperature for 5 days, prior to testing with chitosan.

Preparation of chitosan

Low molecular weight (10253 kDa) chitosan was purchased from Aldrich, China. Purified chitosan was prepared as described by Benhamou *et al.* (1998). The stock solution (1%, w/v) of chitosan was prepared by dissolving purified chitosan in 0.5% (v/v) glacial acetic acid under continuous stirring, and the pH was adjusted to 5.6 using 1N NaOH (Du *et al*., 1998). The chitosan solution was autoclaved (120^{o}C, 20 min) prior to use in assays.

Effect of chitosan on radial growth

To evaluate the effect of chitosan on *Phytophthora* growth, PDA plates were amended with chitosan at different concentrations (0.125, 0.25, 0.5, 1 and 2 mg/ml) as in Laflamme *et al.* (2000). Unamended PDA plates with 0.05% final concentration of acetic acid (pH 5.6) served as negative controls. Comparison to these controls shows the activity of chitosan and excludes the effects of acidic conditions. The most prevalent Para rubber pathogenic microorganisms, *P. botryosa* and *P. palmivora*, were assessed for its growth inhibition. Five replicate plates at each chitosan concentration were inoculated in the center with a plug (5 mm diameter) from the edge of a 3–5 day–old–colony of *P. botryosa* and *P.palmivora*. The colony radii were measured 7 days after the inoculations. The experiments were repeated twice. Percent inhibition of diameter growth (PIDG) was calculated using the following formula:

$$\text{Percent inhibition of diameter growth (PIDG)} = \frac{A-B}{A} \times 100$$

Where: A = Diameter of control colonies, B = Diameter of treated colonies

Effect of chitosan *in vivo*

In this study, Para rubber RRIM 600 cultivar on seedling stage (3 months old) was used as a plant model to be infected with *Phytophthora*. The fully expanded leaves of Para rubber were separated into 2 parts. The upper parts were immersed into 0.5, 1 and 2 mg/ml of chitosan solution for 1 hr, while the lower parts were immersed with 0.05% acetic acid (served as control). The treated Para rubber leaves were cleaned with sterilized DW. The plug (5 mm diameter) from the edge of a 3–5 day–old–colony of *P. botryosa* and *P. palmivora* was inoculated into chitosan treated Para rubber leaves, incubated in moist chamber for 24, 48 and 72 hr. The necrotic lesions from *Phytophthora* penetration in leaves were measured. Percent

inhibition was calculated using the formula which showed previously. A completed randomize design (CRD) was repeated twice for each experiment. Statistical analyses were run with SPSS software. The differences between means from chitosan treatment and controls were tested for statistical significance by Duncan's Multiple Range Test (DMRT) for multiple comparisons. The graph of necrotic lesion development was plotted.

Effect of chitosan on restricting of necrotic local lesion

The optimized chitosan concentration 0.5 mg/ml was treated onto Para rubber seedling pods with Para rubber 3 months old. The treatments were included spray onto Para rubber leaves and water onto seedling pod with 50 ml chitosan solution for one and two months. All treatments are as followed:

Treatment 1: Water chitosan (50 ml) once per week, every week for 1 month

Treatment 2: Water chitosan (50 ml) once per week, every week for 2 month

Treatment 3: Spray chitosan (50 ml) once per week, every week for 1 month

Treatment 4: Spray chitosan (50 ml) once per week, every week for 2 month

Control: Water or spray DW

Each treatments were four replicates, the experiments were repeated twice. Para rubber leaves were collected and subjected to inoculation methods as described previously. Development of necrotic lesions was measured compared with control (distilled water).

Statistical analyses

A complete randomized design (CRD) was repeated twice for each experiment. Statistical analyses were run with SPSS software. Prior to such analyses, the growth effects of chitosan treatments were normalized to percentages relative to control. The differences between means from chitosan treatments and controls were tested for statistical significance by Duncan's multiple range test (DMRT) for multiple comparisons.

RESULTS AND DISCUSSION

Effect of chitosan on radial growth of *P. botryosa* and *P. palmivora*

Chitosan solutions in five concentrations (0.125, 0.25, 0.5, 1, and 2 mg/ml) were tested for their inhibitory effect of *P. botryosa* and *P. palmivora* linear growth. The antimicrobial activity of chitosan against *P. botryosa* and *P. palmivora* is shown in Figure 2, 3 and Table 1. All concentrations reduced the growth of both *P. botryosa* and *P. palmivora* at 7 days post inoculation, and the growth inhibition consistently increased with concentration. Effective inhibition was obtained with 0.5, 1 and 2 mg/ml (87.5, 90.6, and 91.3 % linear growth reduction, respectively) for *P. botryosa*, while inhibition rate of *P. palmivora* was 87.6, 90.3 and 93.2 % linear growth. When the concentration was 0.5 mg/ml, the antimicrobial activity of chitosan was already at an acceptable level. The PDA amended with 0.05% acetic acid served as control. No growth differences were observed between this control and plain medium without acetic acid (data not shown).

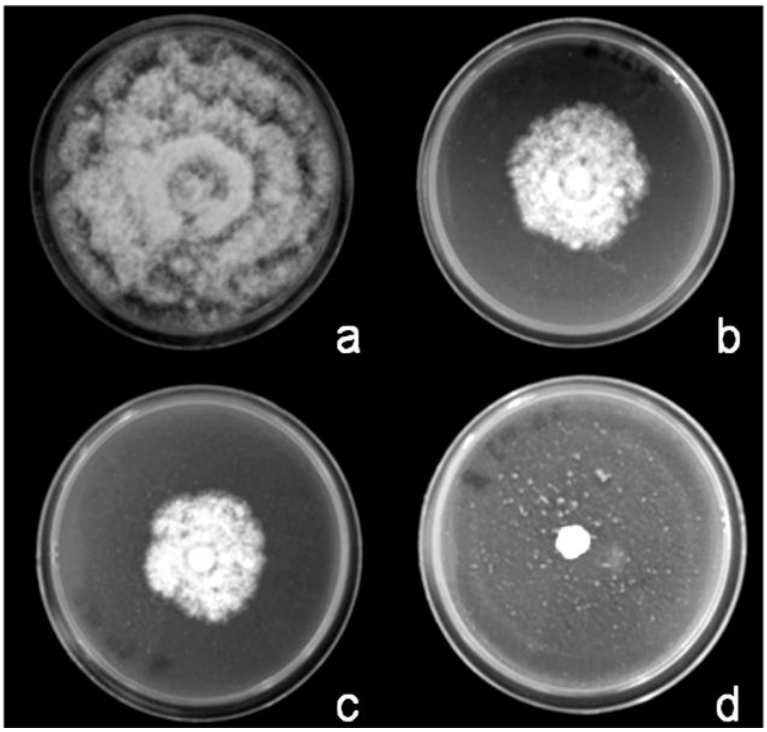

Figure 2. Effects of chitosan on radial growth of *P. botryosa*. PDA amended with 0.05% acetic acid served as control (a), for comparison with chitosan solutions at 0.125 (b), 0.25 (c) and 0.5 (d).

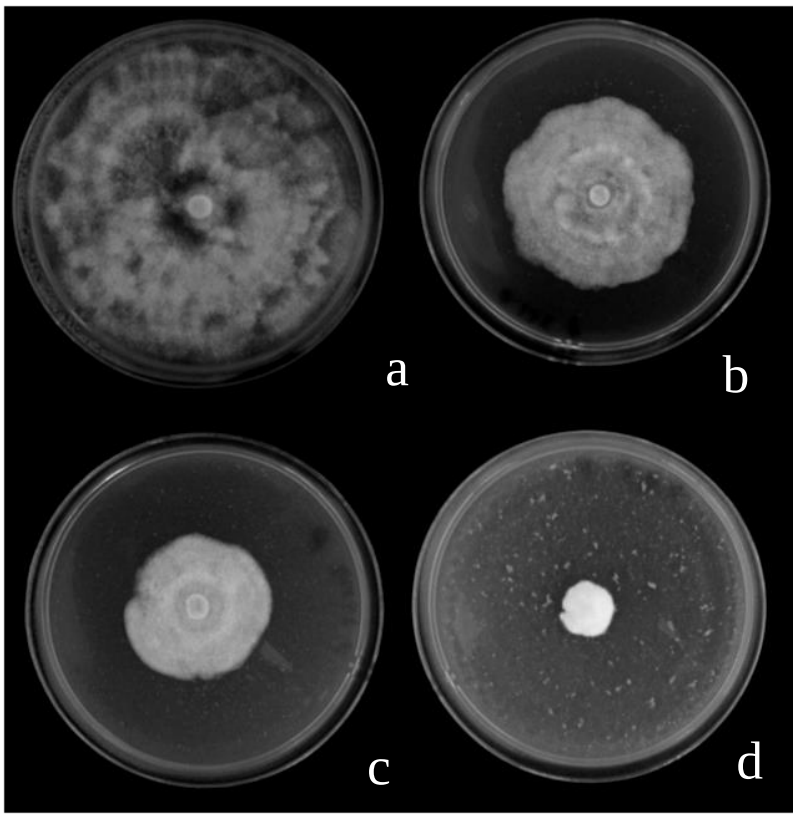

Figure 3. Effects of chitosan on radial growth of *P. palmivora*. PDA amended with 0.05% acetic acid served as control (a), for comparison with chitosan solutions at 0.125 (b), 0.25 (c) and 0.5 (d).

Table 1. Effect of chitosan concentration on growth rate of *P. botryosa* and *P. palmivora*

Chitosan concentration (mg/ml)	Inhibition rate (%)	
	P. botryosa	*P. palmivora*
0.125	47.77±6.09^{c}	45.65±4.56^{d}
0.25	60.94±5.48^{b}	57.64±3.63^{c}
0.5	87.53±1.06^{a}	87.65±1.18^{b}
1.0	90.59±0.00^{a}	90.35±0.53^{ab}
2.0	91.29±0.64^{a}	93.24±0.59^{a}

% inhibition rate relative to corresponding control rate. Superscripts on the right show differences between fungi growing under different conditions. Values with different letters are significantly different ($P<0.01$)

Statistically analysis of the effect of chitosan concentration on the antifungal activity against *P. botryosa* and *P. palmivora* was shown in Table 1. With the increase of chitosan concentration, the antifungal activity is raised up. With relation to concentration, the higher the chitosan concentration, the effective was antifungal activity. All data indicated that there were significantly different ($P<0.01$) antifungal rates for chitosan and concentration treatment. The plant pathogenic fungus-like organism *P. botryosa* and *P. palmivora* was most sensitive to chitosan. These *in vitro* results suggest that chitosan may be an effective growth inhibitor of both *P. botryosa* and *P. palmivora*.

Effect of chitosan *in vivo*

After treatments with chitosan concentrations 0.5, 1 and 2 mg/ml, *P. palmivora* and *P. botryosa* were inoculated on Para rubber leaves. Necrotic lesions would indicate that *Phytophthora* had invaded the plant tissue during the infection period (Fig. 4). The necrotic lesion diameters were measured at 24, 48 and 72 hours post inoculation (hpi), and the growth curves are shown in Figure 5. The lesions consistently grew over time, regardless of treatment group (Fig. 5), but the growth curves for actual treatments are below that for control (Fig. 5). There were four replicates of each treatment in an experiment, and the experiments were repeated twice. The growth inhibition effects were statistically significant, as detailed in Table 2 and 3.

Table 2. Effect of chitosan concentration on development of necrotic lesions and percent inhibition of *P. botryosa* on Para rubber leaves.

Chitosan	Necrotic lesion (mm)			% inhibition		
(mg/ml)	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr
Control	0.500[a]	0.733[a]	0.783[a]	-	-	-
0.5	0.400[a]	0.400[b]	0.425[b]	25.00[a]	36.90	38.54
1	0.275[b]	0.475[b]	0.525[b]	45.00[ab]	45.83	44.72
2	0.225[b]	0.325[b]	0.500[b]	56.67[b]	51.79	28.57

Within the column, different superscripts indicates statistically significant difference for necrotic lesion ($p<0.01$), and for percent inhibition ($p<0.05$).

Table 3. Effect of chitosan concentration on development of necrotic lesions and percent inhibition of *P. palmivora* on Para rubber leaves.

Chitosan	Necrotic lesion (cm)			% inhibition		
(mg/ml)	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr
control	0.58[a]	1.27[a]	1.83[a]	-	-	-
0.5	0.25[b]	0.70[b]	0.80[c]	52.50[a]	47.56[ab]	48.50
1	0.20[c]	0.68[b]	1.23[b]	65.00[b]	41.96[a]	37.48
2	0.20[c]	0.53[b]	0.75[c]	67.86[b]	58.84[b]	60.58

Within the column, different superscripts indicates statistically significant difference for necrotic lesion ($p<0.01$), and for percent inhibition ($p<0.05$).

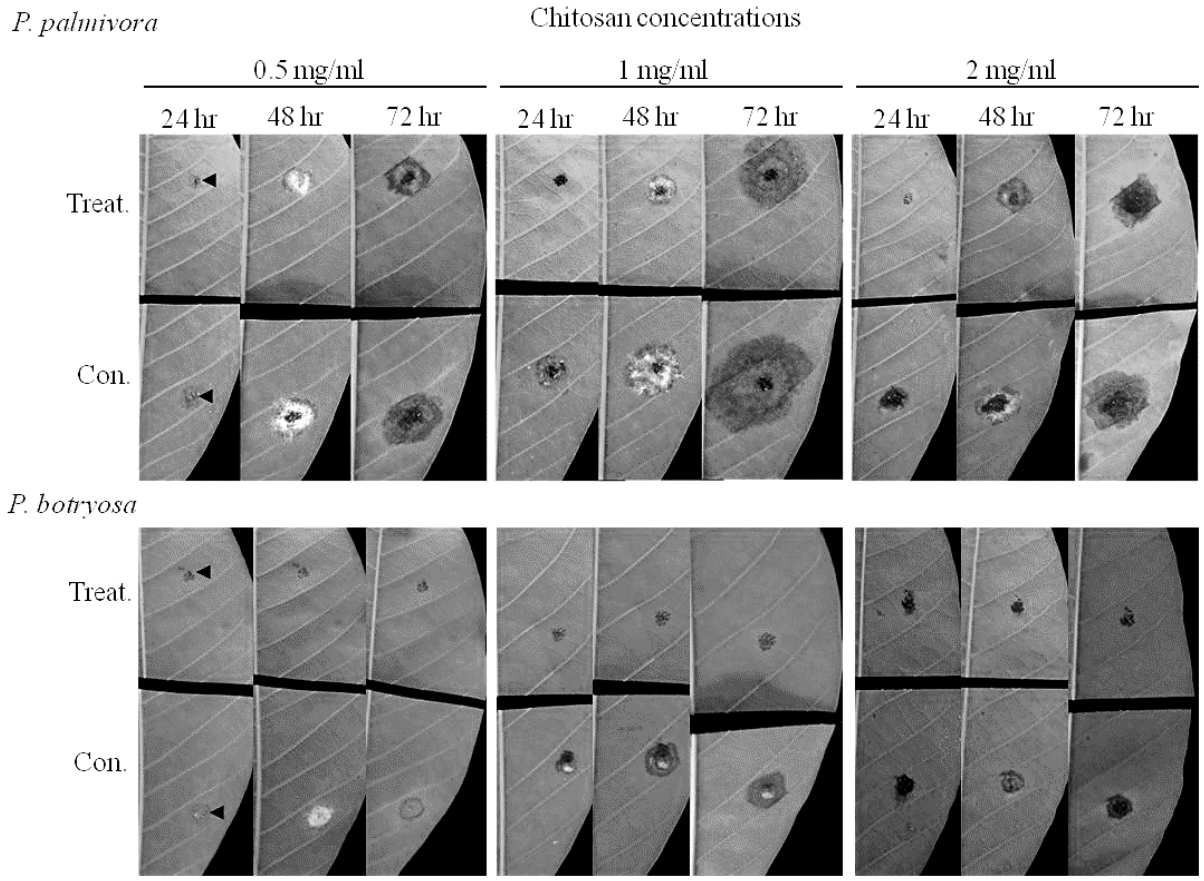

Figure 4. Effect of chitosan concentration on necrotic lesion development caused by *P. palmivora* (upper panel) and *P. botryosa* (lower panel). Arrows indicate the inoculation points.

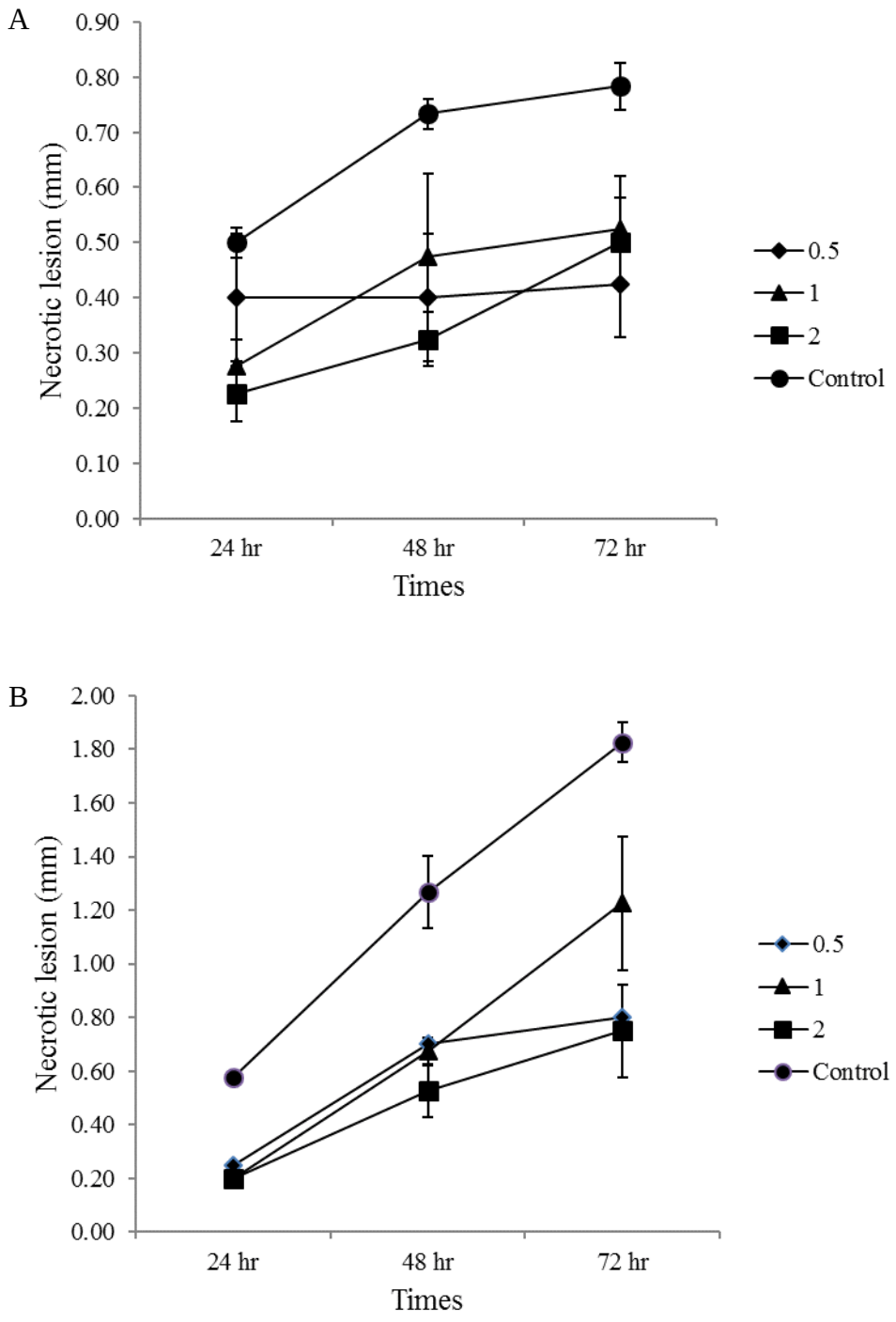

Figure 5. The growth curves of *P. botryosa* (A) and *P. palmivora* (B) after inoculation onto Para rubber leaves treated and un-treated with chitosan solution.

Effect of chitosan on Para rubber growth

The optimized chitosan solution was treated by sprayed on Para rubber leaves and watered on to Para rubber seedlings for 2 months. The length of seedling was measured. After treatments with chitosan solution (chitosan concentration 0.5 and 1.0 mg/ml) compared with control (distilled water, DW) for 2 months, the length of Para rubber seedling was measured to determine the growth rate. Morphological change was observed for toxicity of chitosan on Para rubber seedling. The results showed that the treatments with both concentrations on Para rubber seedling does not significantly increased the growth rate of seedlings compared with control during the treatments (Table 4). According to no morphological change during the treatment periods, chitosan at concentration 0.5 and 1.0 mg/ml showed no toxic to Para rubber seedlings (Table 4).

Table. 4 Effect of chitosan on Para rubber growth and toxicity.

Treatments	Growth rate (cm.) (length ± SD)	Toxicity
Spray chitosan (mg/ml)		
0.5	3.16±0.76	N[1]
1.0	3.00±1.15	N[1]
DW	2.35±0.37	N[1]
Water chitosan (mg/ml)		
0.5	2.83±0.50	N[1]
1.0	2.60±0.64	N[1]
DW	2.48±0.33	N[1]
F-test	ns	

[1] = no different between treated and untreated

ns=non significantly different ($p>0.05$)

Effect of chitosan on restriction of *Phytophthora* infection

The chitosan solution at concentration 0.5 mg/ml was treated onto Para rubber seedling for 1 and 2 months. Distilled water was served as negative control for each treatment. Treatments were included sprayed and watered to Para rubber seedling. After that, Para rubber leaves were collected and subjected to inoculation by both species of *Phytophthora*. The necrotic lesion caused by *Phytophthora* was considered as the sign for pathogen colonization in plant tissues. Based on the result on Table 5, the necrotic lesion caused by *P. botryosa* and *P. palmivora* were not significantly different among the treatments. This phenomenon may due to the various factors including, genetic factor of plants, chitosan stabilization on Para rubber leaves and in soils.

Table. 5 Effect of chitosan on expansion of necrotic lesion caused by *Phytophthora*

Treatments (chitosan 0.5 mg/ml)	Necrotic lesion (cm)	
	P. botryosa	*P. palmivora*
Spray 1 month	1.45±0.19	1.53±0.13
Spray 2 month	1.45±0.13	1.60±0.16
Water 1 month	1.63±0.13	1.58±0.15
Water 2 month	1.53±0.23	1.48±0.17
DW	1.66±0.11	1.67±0.16
F-test	ns	ns

ns=non significantly different ($p>0.05$)

Chitosan has been reported as an effective biocompound against several bacterial and fungal strains (Liman *et al.,* 2011). The results of the present study demonstrate that the plant pathogenic fungus-like organism *P. botryosa* and *P. palmivora* is also sensitive to chitosan. Chitosan has great potential as a biodegradable substance. Recent studies have shown that chitosan is not only effective in inhibiting the growth of the pathogen, but also in eliciting activities (Benhamou, 1996; El Ghaouth *et al.,* 1999; Barka *et al.,* 2004). It has been shown that mycelia growth of fungi is inhibited by chitosan. The level of inhibition was highly correlated with chitosan concentration (in the range 0.75-6.0 mg/ml), decreasing the radial growth of *Alternaria alternata*, *Botrytis cinerea*, *Colletotrichum gloeosporioides* and *Rhizopus stolonifer* (El Ghaouth *et al.,* 1992b). The same effect was found on *Rhizoctonia solani* (Elmer and LaMondia, 1994), and *Sclerotium sclerotiorum* (Cheah *et al.,* 1997). Furthermore, seed coating with modified chitosan has inhibited *Sphacelotheca reiliana,* a causal agent of head smut of corn (Zeng *et al.,* 2010). The result indicates that the radial growth of *P. botryosa* and *P. palmivora* was fully inhibited when cultured in 0.5% chitosan-amended PDA, is similar to these prior observations.

In this study, we investigated the potential of chitosan in controlling leaf fall disease of Para rubber seedlings, both *in vitro* and *in vivo*. The growth rate of *P. botryosa* and *P. palmivora* was reduced in PDA plates amended with chitosan at 0.5, 1 and 2 mg/ml concentrations. The expansion of necrotic lesions on chitosan treated Para rubber leaves was less than in control, up to 24 h post inoculation, while after that the growth rates in Figure 4 appear similar. Nevertheless, necrotic lesions still spread to cover most of Para rubber leaves, demonstrating that chitosan has potential to inhibit the growth of both *Phytophthora* and slow the initial infection expand, but does not prevent or limit extent of leaf fall disease in Para rubber seedlings.

Recently, Sunpapao and Pornsuriya (2013) reported that chitosan directly inhibits the growth of *P. botryosa,* one causal agent of Para rubber leaf fall disease, and withers its mycelia and oospores. The authors hypothesized that chitosan may have potential for practical disease management of leaf fall in nursery stage. The effects of chitosan against bacterial and fungal strains have been reported (Liman *et al*., 2011), and the inhibition correlates with chitosan concentration (El-Ghaouth *et al*., 1992b). Chitosan has been widely used to reduce the growth of *Rhizoctonia fragariae* (Elmer and LaMondia, 1994), *Sclerotium sclerotiorum* (Cheah *et al*., 1997) and *P. botryosa* (Sunpapao and Pornsuriya 2013). Furthermore, chitosan changes the pathogen morphologies of *Fusarium oxysporum* f.sp. *radis-lycopersici*, *R. stolonifer* and *S. sclerotiorum,* to abnormal shapes and sizes (Benhamou and Theriault, 1992; El-Ghaouth *et al*., 1992a, b; Cheah *et al*., 1997). However, most of these studies have focused on the efficacy of chitosan in directly inhibiting pathogen growth, not on its potential in controlling phytopathogenic fungi in plants. Therefore, we also examined *in vivo* the potential of chitosan in controlling the leaf fall disease of Para rubber seedlings, caused by both *Phytophthora*.

The chitosan concentration for the *in vivo* study was selected by tests *in vitro*, so it was effective in controlling the radial growth of *phytopthora* in agar plates. The chitosan was applied on Para rubber leaves prior to inoculation with *P. botryosa and P. palmivora*. The necrotic lesions caused by *Phytophthora* was not restricted by the treatments (Fig. 4), but the treatment effects match a delay in early infection by about one day. Given time, *Phytophthora* could invade most parts of a leaf, so the treatments did not limit or restrict the extent of infection symptoms. Therefore, chitosan only reduced *Phytopthora* activity in early infection, but could not control leaf fall disease of Para rubber seedlings. In contrast to our study, Boonreung and Boonlertnirun (2013) applied chitosan to control dirty panicle disease of rice, caused by

Helminthosporium oryzae, *Curvularia lunata* and *Fusarium moniliforme* in plantation fields. They suggested it is possible to control dirty panicle disease by spraying chitosan onto rice plants (Boonreung and Boonlertnirun, 2013).

Conclusion

As a summary, chitosan inhibits the growth of *Phytophthora*, and slightly delays the initial onset of infection in leaves, but does not prevent or limit the extent of leaf fall disease in Para rubber seedlings. Currently, chitosan is known to control plant diseases caused by several fungi, mostly based on *in vitro* studies. The efficacy in practical application of chitosan *in vivo*, in green houses and nurseries or in the fields, would further depend on environmental factors that impact disease management in field conditions. The current study attempted to bridge between purely *in vitro* studies and practical conditions, by including a laboratory scale *in vivo* study. For the specific pathogen in this study, the *in vivo* results were clearly discouraging, and relying solely on *in vitro* results would have been misleading. This suggests that an *in vivo* component in laboratory studies of similar nature is a useful low cost screen on seeking alternative pest control treatments. In the greenhouse test, chitosan showed no significantly differed between control. Therefore, application of chitosan in greenhouse and field nurseries for Para rubber seedling is still need to be concerned the cost and the coming results too.

REFERENCES

Allan, C.R. and L.A. Hadwiger. 1979. The fungicidal effect of chitosan on fungi of varying cell wall composition. Exp. Mycol., 3, 285-287.

Barka, E., Eullaffroy, P., Clement, C. and Vernet, G. 2004. Chitosan improves development, and protects *Vitis vinifera* L. against *Botrytis cinerea*. Plant Cell Rep., 22, 608-614.

Benhamou, N., 1996. Elicitor-induced plant defense pathways. Trends Plant Sci., 1, 233-240.

Benhamou, N. and Theriault, G. 1992. Treament with chitosan enhances resistance of tomato plants to the crown and root rot pathogen, *Fusarium oxysporum* f. sp. *radicis-lycopersici*. Physiol. Mol. Plant Pathol., 41, 33-52.

Benhamou, N., Kloepper J.W. and Tuzun, S. 1998. Induction of resistance against *Fusarium* wilt of tomato by combination of chitosan with endophytic bacteria strain: ultrastructure and cytochemistry of the host response. Planta 204, 153-168.

Boonreung, C. and Boonlertnirun, S. 2013. Efficacy of chitosan for controlling dirty panicle disease in rice plants. ARPN J. Agri. Biol. Sci., 8(5), 380-384.

Chantarapratin, U., Pattanakul, P., Changreung, N., Rojanasujit, A., Romreunsukarom, P. and Ramlee, A. 2001. Rubber disease survey on large scale clone trail. Research Report, Rubber Research Institute Thailand, pp: 1-52.

Cheah, L.H., Page B.B.C. and Shepherd, R. 1997. Chitosan coating for inhibition of sclerotinia rot of carrots. N Z J Crop Hort. Sci., 25, 89-92.

Chee, K.H., 1968. *Phytophthora* leaf disease in Malaysia. J. Rubber Res. Inst. Malaya 21, 79-86.

Du, J., Gemma, H. and Iwahori, S. 1998. Effects of chitosan coating on the storability and on the ultrastructural change of Jonagold apple fruit in storage. Food Pres. Sci., 24, 23-29.

El Ghaouth, A., Arul, J., Grenier J. and Asselin, A. 1992a. Antifungal activity of chitosan on two postharvest pathogens of strawberry fruits. Phytopathol., 82, 938-402.

El Ghaouth, A., Arul, J. Asselin A. and Benhamou, N. 1992b. Antifungal activity of chitosan on post-harvest pathogens: Induction of morphological and cytological alterations in *Rhizopus stolonifer*. Mycol. Res., 96, 769-779.

El Ghaouth, A., Smilanick, J.L. Brown, G.E. Ippolito, A. Wisniewski M. and Wilson, C.L. 1999. Application of *Candida saitoana* and glycolchitosan for the control of postharvest diseases of apple and citrus fruit under semi-commercial conditions. Plant Dis., 84, 243-248.

Elmer, W.H. and LaMondia, J.A. 1994. Chitosan inhibits *Rhizoctonia fragariae* but not strawberry black root rot. Adv. Strawberry Res., 13, 26-31.

Hernandez-Lauzardo, A.N., Velazquez-del Valle M.G. and Guerra-Sanchez, M.G. 2011. Current status of action mode and effect of chitosan against phytopathogens fungi. Afr. J. Microbiol. Res., 5, 4243-4247.

Hirano, A. and Nagao, N. 1989. Effect of chitosan, pectic acid, lysozyme and chitinase on the growth of several phytopathogens. Agric. Biol. Chem., 53, 3065-3066.

Johnston, A., 1989. Diseases and Pests. In: Rubber, Webster, C.C. and W.J.I. Baulkwil (Eds.). Longman Scientific and Technical, New York, USA., pp: 415-458.

Kendra, D.F., Christian D. and Hadwiger, L.A. 1989. Chitosan oligomers from *Fusarium solani*/pea interactions, chitinase/β-glucanase digestion of sporelings and from fungal wall chitin actively inhibit fungal growth and enhance disease resistance. Physiol. Mol. Plant Pahtol., 35, 215-230.

Laflamme, P., Benhamou, N. Bussieres N. and Dessureault, M. 2000. Differential effect of chitosan on root rot fungal pathogens in forest nurseries. Can. J. Bot., 77, 1460-1468.

Liman, Z., Selmi, S. Sadok S. and El Abed, A. 2011. Extraction and characterization of chitin and chitosan from crustacean by-products: Biological and physicochemical properties. Afr. J. Biotechnol., 10, 640-647.

Rubber Research Institute of Thailand. 2010. Thailand rubber statistics. (Annual report). Ministry of Agriculture Bangkok, Thailand, 39(2), 29.

Saber, W.I.A., Ghoneem, K.M.. El-Metwally M.M and Elwakil, M.A. 2009. Identification of *Puccinia pimpinellae* on Anise Plant in Egypt and its control. Plant Pathol. J., 8, 32 -41.

Stossel, P. and Leuba, J.L. 1984. Effect of chitosan, chitin and some aminosugars on growth of various soilborne phytopathogenic fungi. J. Phytopathol., 111, 82-90.

Sunpapao, A. and Pornsuriya, C. 2013. Chitosan inhibits the growth of *Phytophthora* botryose: The causal agent of Para rubber leaf fall diseases. Plant Pathol. J., 12(2), 92-97.

Suzuki, T.J., Kueprakone, U. and Kamphangridthrong, T. 1979. *Phytophthora* spp. Isolated from some economic plants in Thailand. Technical Bulletin Tropical Agricultural Research Center, Japan., 12, 32-41.

Tsao, P.H., Tummakate, A. and Bhavakul, K. 1976. Recovery of *Phytopthora* species from old, badly decayed, infected tissues of *Hevea brasiliensis*. Transac. British Myco. Soc., 66, 557-558.

Turner, P.D., 1969. Diseases of Hevea rubber in Thailand: With particular reference to those associated with *Phytophthora* species. Report No. FAO-AGP--THA/64/515, Rubber Research Center Thailand, pp: 1-86.

Zeng, D., Mei X. Wu, and J. 2010. Effects of an environmentally friendly seed coating agent on combating head smut of corn caused by *Sphacelotheca reiliana* and corn growth. J. Agric. Biotechnol. Sustainable Dev., 2, 108-112.

APPENDIX

Original articles cited PSU grant no. NAT550131S

Sunpapao, A. and Pornsuriya, C. 2013. Chitosan inhibits the growth of *Phytopthora botryosa*: the causal agent of Para rubber leaf fall disease. Plant Pathology Journal 12(2), 92-97. (Indexed in CABI and AGRIS data base)

Manuscript draft

Sunpapao, A. and Pornsuriya, C. Effect of chitosan treatments on Para rubber leaf fall disease caused by *Phytophthpra palmivora*, a laboratory study. Songklanakarin Journal of Science and Technology (Accepted)
(Indexed in SCOPUS)