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Full Length Research Paper

Effect of benzo-thiadiazole-7-carbothioic acid S-methyl ester (BTH) treatment on the resistant substance in postharvest mango fruits of different varieties

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Nang klangwan **and** ***Tainong*** **mango fruits were treated with benzo-thiadiazole-7-carbothioic acid S-methyl ester (BTH). The effect of BTH treatment on the resistant substance in postharvest fruits was evaluated to explore the enhanced disease-resistance mechanisms of mango fruits by BTH from different varieties. Results indicate that the main resistant substance such as total phenolic, flavonoid, ligin and hydroxyproline-rich glycoprotein (HRGP) content were obviously increased by BTH treatment in different varieties. However, the increase in resistant substances of** ***Tainong*** **mango fruit was higher than that in** ***Nang klangwan*** **fruit. At the same time,** ***Tainong*** **fruit without BTH treatment had relatively higher level of these resistant substances than** ***Nang klangwan*** **mango fruit, especially in the contents of phenolic compounds and lignin.**

Key words: Mango, total phenolic, flavonoid, ligin, hydroxyproline-rich glycoprotein (HRGP).

INTRODUCTION

Postharvest decay in fruits and vegetables is a serious problem in the world. Chemical fungicides are the major weapon against postharvest diseases. However, fungicides are becoming less effective because of the development of pathogen resistance, along with consumer concerns about possible risks associated with the use of chemicals (Wilson et al., 1994). A number of new strategies are being investigated to control postharvest decay without the pollution of the environment and risk to public health. As such, induced resistance in harvested crops is promising (Wilson et al., 1994; Terry and Joyce, 2004). This phenomenon is also known as systemic acquired resistance (SAR). SAR can be activated by exogenous treatments with chemical inducers such as benzo-thiadiazole-7-carbothioic acid S-methyl ester (BTH) (Gorlach et al., 1996).

Most studies have shown the effectiveness of BTH in protecting different plant species against diseases caused by viral, bacterial and fungal pathogens (Gorlach et al., 1996; Benhanou and Belanger, 1998; Cole, 1999). Most studies have used vegetative tissues as materials to elucidate how BTH may affect disease resistance, and little work has been done in horticultural products such as fruit and vegetables (Liu et al., 2005). But some researches have shown that BTH is also effective to induce resistance in some fruits and vegetables such as beet (Burketová et al., 2003), tomato (Anfoka et al., 2000), cucumber (Li et al., 2005), peach (Liu et al., 2005), banana (Wang, 2005; Zhu et al., 2007), strawberry (Terry et al., 2000) loquat (Zhang et al., 2009) and so on. In the previous study, we found that BTH treatment can also reduce the incidence of postharvest mango fruit anthracnose (Liu et al., 2009) but the effective concentration of BTH is different according to the mango varieties. The most effective concentration of BTH treatment

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was 200 and 50 mg/L for the *Nang klangwan* fruit and *Tainong* fruit, respectively (Liu et al., 2009).

Although BTH is highly effective in inducing enhanced disease resistance, its mechanism of action and cellular targets are less known (Liu et al., 2005), especially in fruits and vegetables. In addition, many studies have focused on the effect of BTH treatment on the the defense-related enzymes peroxidase (POD), catalase (CAT), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), β-1,3-glucanase, and chitinase. However, researches about the effect of BTH treatment on the resistant substance are generally lacking. In addition, are there some correlations between different concentration of BTH, different mango varieties and resistance substance? Therefore, *Nang klangwan* and *Tainong* mango fruits were used as the experimental fruits to study the effects of BTH on the resistance substance in different mango varieties.

MATERIALS AND METHODS

Plant material

Nang klangwan and *Tainong* mango fruits were harvested from a commercial orchard in the Guangba farm, Dongfang city, Hainan Province, China, at the earliest stage of commercial ripening (green mature). Fruit were transported to the laboratory, selected for uniformity of size, ripeness and absence of defects.

BTH treatment

BTH (50% wettable granule formulation, Bion®, Novartis Ltd., Basel, Switzerland) solution was prepared with sterile deionized water plus 0.05% Tween 80.

Nang klangwan and *Tainong* mango fruits were soaked in 200 and 50 mg/L BTH solution for 10 min, respectively. The control fruit including *Nang klangwan* and *Tainong* mango fruits were soaked in sterile deionized water plus 0.05% Tween 80 for 10 min. After the treatment, both BTH-treated and control fruit were kept in trays covered with plastic film and incubated at 15°C, and 75 to 85% RH. Each treatment had 60 fruit with three replicates.

Inoculation and infection

Colletotichum gloeosporioides were obtained from the Environment and Plant Protection Institute Chinese Academy of Tropical Agricultural Sciences, Hainan Province, China, and then cocultured on potato dextrose agar (PDA) for eight days; the cultural temperature was 28°C.

The inoculations were carried out according to Liu et al. (2005). 72 h after the BTH treatment, both the BTH-treated and control fruit were sterilized with 70% ethanol, and then wounded with a syringe at three points (1 mm deep × 3 mm wide) on the equator of each fruit. Thereafter, agar disk containing *C. gloeosporioides* (φ1.5 mm) was stucked to the wounding site following 24 h moisturization with cotton, and the fruit wer incubated at 15°C, and 75 to 85% RH.

Disease incidence (the percentage of fruit with visible disease development) and lesion diameter on each fruit were recorded daily. When the visible rot zone beyond the wounded area on each fruit was more than 1 mm wide, it was counted as an affected fruit. Tissue samples outside the wounded and infected pulp were collected on the days as indicated in the results and stored at −20°C.

Measurements of phenolic compounds, flavonoid, lignine and hydroxyproline-rich glycoprotein (HRGP)

Phenolic compounds and flavonoid were measured according to the methods of Pirie and Mullins (1976). 1 g of frozen tissue was homogenized with 5 ml ice-cold 1% HCl–methanol solution and then extracted at room temperature for 2 h, and then filtered with filter paper. The filtrate was diluted 10 times with 1% HCl–methanol solution, and absorbance was measured at 280 nm and 325 nm using 1% HCl–methanol solution as a reference. The content of phenolic compounds was calculated with the standard curve obtained on the basis of content of gallic acid expressed as μg/g FW. Flavonoid content was expressed as OD_{325} nm/g FW.

Lignin content was determined according to Morrison (1972). 1 g of frozen tissue was homogenized with 5 ml ice-cold 95% alcohol solution and then centrifuged at 4°C for 10 min at 12,000× *g*. Extraction was repeated twice, but the second was extracted with alcohol; n-hexane = 1:2 (V/V), precipitation was collected, then heated at 60°C for 2 h, and then dissolved in 1 ml 25% brominated acetyl glacial acetic acid solution, and put in a 70°C water bath for 30 min. Furthermore, 1 ml of 2 mol/L NaOH was added to stop the reaction. 2 ml glacial acetic acid and 0.4 ml of 2 mol/L oxammonium hydrochloride were added and then centrifuged at 4°C for 10 min at 12,000× *g*. 5 ml of glacial acetic acid was put in 0.1 ml supernatant, and the optical density at 280 nm was measured. The contents of lignin content were calculated according to calibration curves of sinapyl alcohol, and expressed as μg/g FW.

HRGP content is positively related to hydroxyproline content; thus, the Hyp content of the cell wall is to represent the relative content of the HRGP.

1 g of frozen tissue was homogenized with 5 ml of 0.1 mol/L phosplate buffer (pH 7.2) solution and then centrifuged at 4°C for 10 min at 12,000× *g*. Precipitation was centrifuged, cleaned three times in the same buffer, one time with TritonX-100 (0.5%), two times with water, one time with 1 mol/L NaCl, following three times with water, and finally washed one time with acetone. The residue was dried and the cell wall product was obtained (Hu et al., 1999).

The hydroxyproline content was then assayed according to Kivirikko et al. (1967). 20 mg cell wall product and 5 ml of 6 mol/L HCl were placed in a screw-capped tube and the tube was heated at 110°C for 18 h. After the hydrolysis, 2 ml of the hydrolyzed supernatant solution was placed in another screw-capped tube and the sample was adjusted to pH 7.0. The sample was then oxidized by the addition of exactly 1 ml of 0.8 mol/L boric acid and 1.0 ml of the 0.2 mol/L chloramine T solution, and the sample was well mixed immediately. The tube was allowed to stand at room temperature for 25 min with occasional mixing, and 2.0 ml of 3.6 mol/L sodium thiosulfate was added. 2.5 g KCl was added in order to allow complete saturation of the solution. Furthermore, 3 ml of toluene was added to the tube, the tube was then shaken for 5 min and allow to stand in order to separate the toluene layer. The toluene layer was removed with suction. The tube was tightly capped and placed in a briskly boiling water bath for 30 min, then cooled with running tap water, and exactly 3.5 ml of toluene was added. After enough immingle and extraction, exactly 2 ml of the toluene extract was placed in a clean test tube and 1.0 ml of the Ehrlich's reagent was added, and rapidly mixed with the sample. After 20 min of standing at room temperature, the absorbance at 560 nm was determined.

Statistical analysis

All statistical analyses were performed with SPSS 10.0. Data were

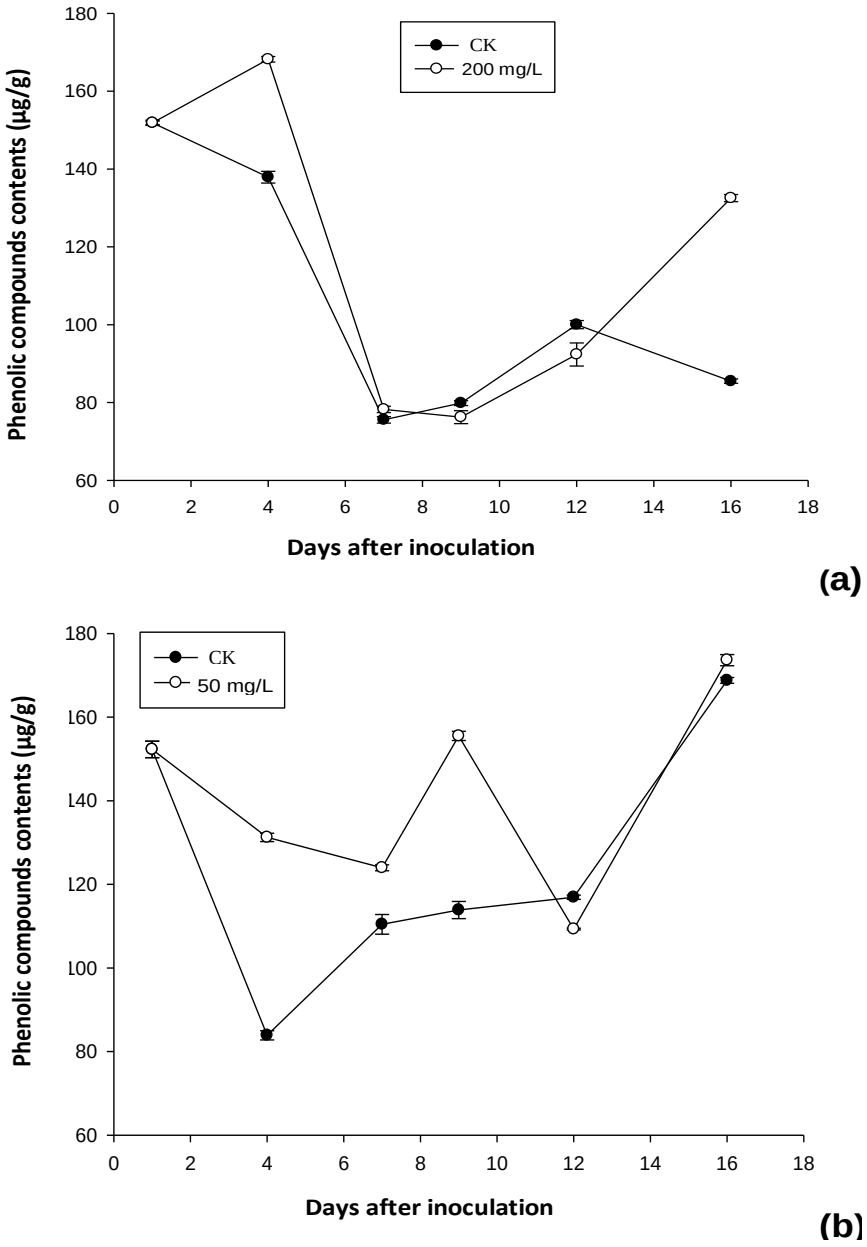

Figure 1. Effects on phenolic compounds contents in (a) "*Nang klangwan*" mango and (b) "*Tainong*" mango by BTH treatment during damnifying inoculation.

analyzed by one-way analysis of variance (ANOVA). Mean separations were performed using the least significant difference method (LSD test). Each experiment had three replicates and all experiments were run three times with similar results. Measurements from all the replicates were combined and treatment effects were analyzed.

RESULTS

Effect of BTH treatment on phenolic content

Phenolic compounds are inherent constituent of many plants themselves and are also the plant secondary metabolites. They play an important role in plants resistant mechanism. Phenolic content of *Nang klangwan* and *Tainong* mango fruits decreased at first and then increased after *C. gloeosporioides* was inoculated (Figure 1). The phenols content that was decreased could be due to the improvement of polyphenol oxidase activity in fruits which catalyzed polyphenols oxidization to quinonoids, etc (antifungal substances) to resist further expansion of the pathogen. Along with the enhancement of the secondary metabolism, the phenols content continuously increased. Changes in phenol of fruits treated with BTH

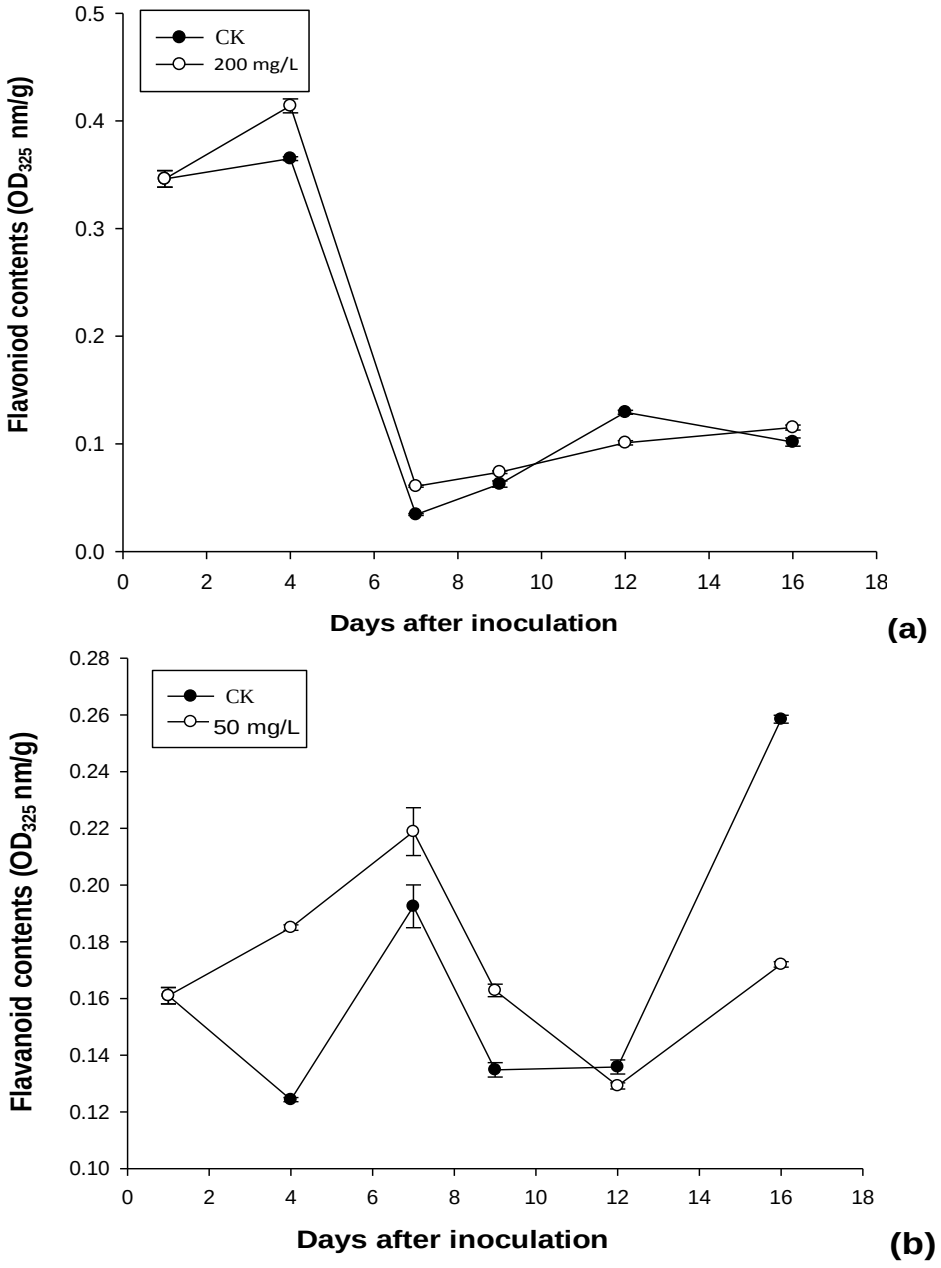

Figure 2.. Effects on flavanoid contents in (a) "*Nang klangwan*" and (b)"*Tainong*" by BTH treatment during damnifying inoculation.

had similar trend as that of the control. However, phenol content of BTH treated fruits was higher and phenol content *of Tainong* mango fruits increased more significantly especially in the early time of storage. The results show that BTH treatment promoted the accumulation of total phenol content in fruits. Meanwhile, it could be seen that phenol content of *Tainong* mango fruits was higher than *Nang klangwan* fruits without BTH treatment. It may be one of the reasons why *Tainong* mango fruits had lower incidence than *Nang klangwan* storage prophase showed the tendency of increase first, fruits.

Effect of BTH treatment on flavonoids content

Many studies indicate that host cell has plenty of flavonoids accumulation after the invasion of pathogens, so the flavonoids could be used as a biochemical marker of resistance to pathogen (Yu et al., 2001). As shown in Figure 2, the flavonoids content except ascending and decease later during storage for *Nang klangwan* and

Tainong mango fruits. BTH treatment enhanced the levels of flavonoids in the fruits. Flavonoids accumulation speed in *Tainong* mango fruits was obviously higher than those in *Nang klangwan* mango fruits. Also, the peak values appearance of flavonoids in *Nang klangwan* mango fruits was earlier than that in *Tainong* mango fruits. This was probably because the fruits were earlier attacked by pathogen and the induced flavonoids were quickly synthesized to prevent the expansion of pathogen. Flavonoids decrease was depleted to prevent the expansion of pathogen during storage.

Effect of BTH treatment on lignin content

Lignin is the cross-linking molecules of many phenylpropane which together always combine with other saccharides on cellular wall. Lignin is easily deposited in the cell walls to form cork which can stop pathogen infection and spread (Yang et al., 2003). The lignin content in both control and BTH-treated fruit showed a decrease trend. However, the contents of lignin *in Tainong* fruit were obviously higher (about 2 times) than that in *Nang klangwan* mango fruit (Figure 3), which was helpful to strengthen the disease resistance of *Tainong* fruit. The lignin content of fruits treated with BTH maintained higher level throughout the storage especially in *Tainong* fruit. It proved that BTH treatment had more evident effect to induce lignin synthesis in *Tainong* fruit.

Effect of BTH treatment on HRGP content

HRGP is the structural proteins in plants which may play certain roles on resistant diseases. The resistance mechanism of HRGP is due to the effect of clusterin. HRGP could combine with pathogenic bacteria, and have effect of barrier on cell wall. So it can prevent pathogenic bacteria from invading and spreading between cells. As shown in Figure 4, HRGP content increased both in fruits inoculated with *P. expansum* of the control fruit and of BTH-treated fruit during storage whereas, the HRGP content increased rapidly during the latter storage period (Figure 4a). Treatment with BTH significantly increased the HRGP content either in *Nang klangwan* mango fruit and *Tainong* fruit. It indicates that BTH treatment is helpful in increasing the accumulation of HRGP and decreasing the disease.

DISCUSSION

Phenol compounds are not only toxic to pathogen but also can be oxidized to more harmful quinonoids *in vivo*. Thus, they play an important role in plants defense response. In addition, phenols compounds are the precursor of the disease-resistant material such as lignin and phytoalexin. In general, lots of phenolics were rapidly synthesized and lignified (Yuan et al., 1995). Flavonoids are the most important class of phenolic substances which have very strong antibacterial action in plant and pathogen interactions. Flavonoids can be used as a kind of self-defense substances that can provide protein to reduce or remove excess free radicals produced from stress condition (Wang, 2007).

One defense mechanism against the pathogen infection in plants is through the synthesis of lignin, leading to tissue lignification. Ligin accumulation in host plants is a biological active response to pathogens in a number of plant–pathogen interactions. It is a common phenomenon that lignin content increases in plants after infection by pathogens. Hydroxy-proline-rich glycoprotein is the main structure protein in plants cell wall. Some researches showed that HRGP in plants can be induced and accumulated because of pathogen infection, and plays some part in plant resistance. It is considered that HRGP is related with plant resistance mechanism (Fan et al., 2005; Cheng et al., 2006). It was also found that HRGP and lignin content increased significantly in muskmelons by preharvest BTH spraying, and enhanced the disease resistance of fruits (Zhang et al., 2006). This was also confirmed in muskmelon cultivar Yindi (Li et al., 2005).

Our results indicate that BTH significantly increased the total phenolic, flavonoid, ligin and HRGP content of mango fruits. Therefore, it enhanced the fruit resistance and reduced the incidence of a disease and the expansion of disease spot in fruits. The same results were obtained for peach (Liu, 2004), Pear (Cao, 2005) and banana (Ma et al., 2006) fruits treated with BTH. These results indicate that the antibacterial substance played a very important role in the induction resistance of fruits. Our results also show that accumulation of total phenolic, flavonoid and ligin in *Nang klangwan* mango fruit were much slower than that in *Tainong* fruit. The contents of total phenolic, flavonoid and ligin in *Tainong* fruit were significantly higher than those in *Nang klangwan* mango fruit both in the control and treated fruits. Thus, *Tainong* fruit had much more antibacterial substance than *Nang klangwan* fruit. *Tainong* fruit had stronger resistance during storage. At the same time, this might be the reason why lower BTH concentrations can induce the efficient disease-resistant *of Tainong* fruits.

Conclusion

BTH treatment increased the content of the main resistant substances such as total phenolic, flavonoid, ligin and HRGP both in *Nang klangwan* and *Tainong* mango fruit. However, the increase in resistant substance contents of *Tainong* mango fruit was higher than that of *Nang klangwan* fruit. At the same time, these resistant substances especially phenolic compounds and lignin of

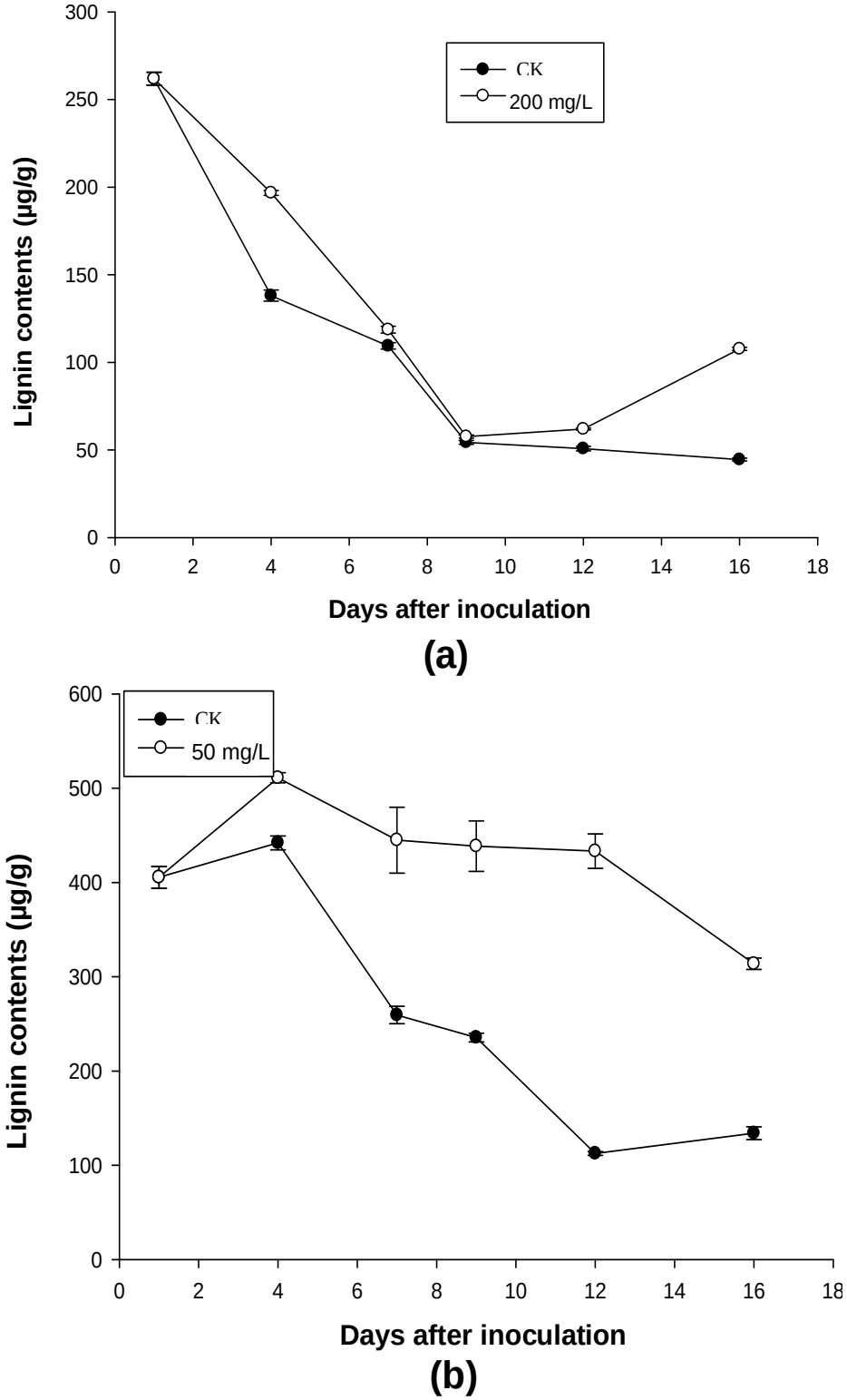

Figure 3. Effects on lignin contents in (a) "*Nang klangwan*" mango and (b) "*Tainong*" mango by BTH treatment during damnifying inoculation.

Tainong fruit without BTH treatment were relatively higher than that of *Nang klangwan* mango fruit. It indicate that, *Tainong* fruits had stronger resistance to disease than *Nang klangwan* fruits.

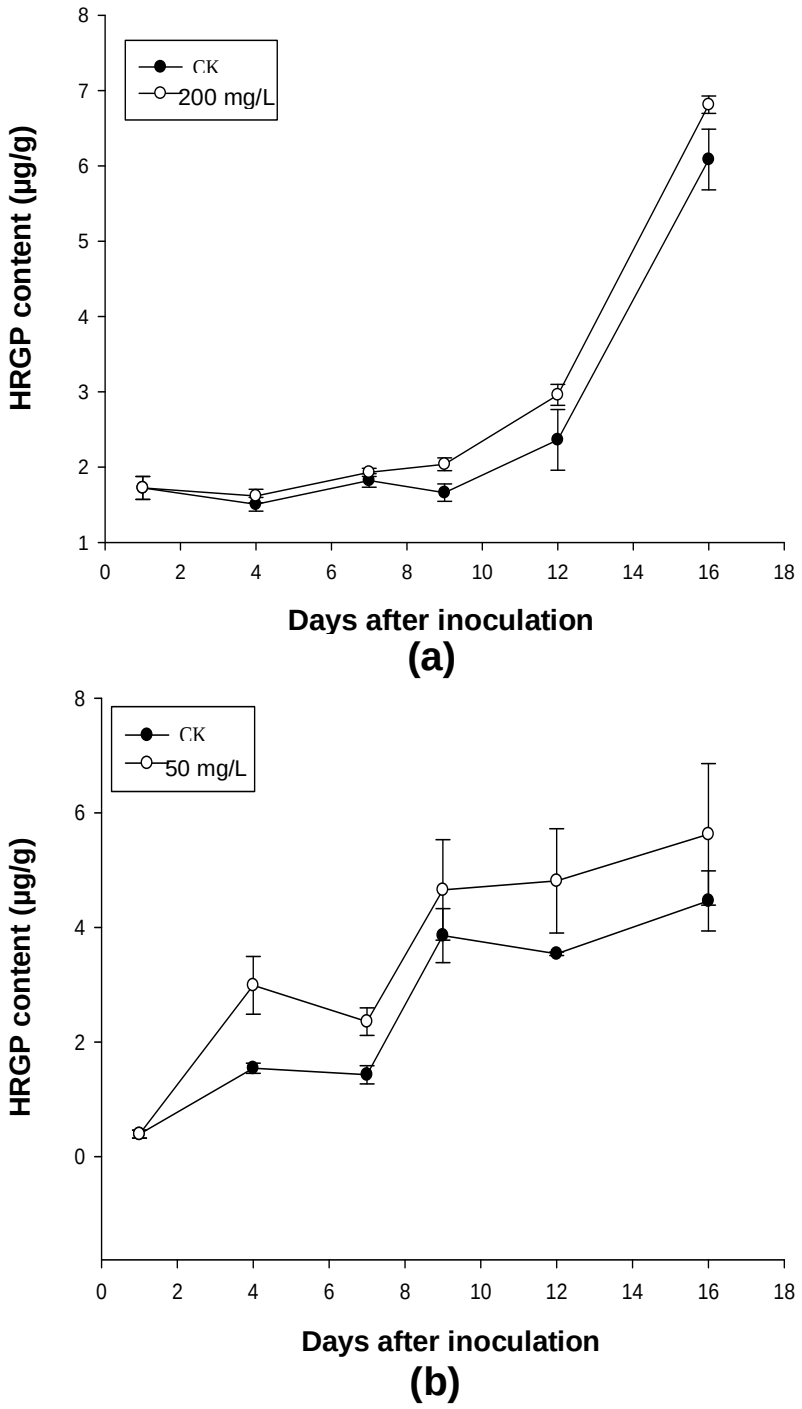

Figure 4. Effects on hydroxyl-proline-rich glycoprotein (HRGP) content in (a) "*Nang klangwan*" mango and (b) "*Tainong*" mango by BTH treatment during damnifying inoculation.

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