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TITLE: Effects of Kaolin on Photosynthesis, Carbohydrate Content, Fruit Yield and Fruit Quality in Mango (*Mangifera indica* L.) cv. Nam Dok Mai

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THESIS

EFFECTS OF KAOLIN ON PHOTOSYNTHESIS, CARBOHYDRATE CONTENT, FRUIT YIELD AND FRUIT QUALITY IN MANGO (*Mangifera indica* L.) cv. NAM DOK MAI

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Mango (*Mangifera indica* L.) is a major exported fruit where the fruit yield and quality needed to be improved in order to increase the economic value. Kaolin application through the mango leaf coating can play an important role in the improvement of Thai mango production under the tropical climatic condition. The objectives of this study were to: 1) Compare the suspension, precipitation and light transmission properties of four coating materials—kaolin, bentonite, calcium carbonate and dolomite, as well as the leaf coating properties by measuring of photosynthesis after the application of materials. 2) Investigate the effects of kaolin coating on leaf gas exchange, carbohydrate and sugar contents, as well as the mango fruit yield and quality. The results showed that kaolin suspended well in water and showed the slowest rate of precipitation when compared to the other coating materials studied. In addition, kaolin was the most effective leaf coating material for reducing light transmission as it had the lowest photon transmittance through a glass plate. Mango leaves at 70 days of age treated with kaolin spraying twice a week had net photosynthesis (A) and stomatal conductance (g_s) values higher than those of the mango leaves in untreated control group. However, under low light intensity condition, kaolin sprayed mango leaves tended to have more adverse effects on photosynthetic rate than the untreated leaves at 90 days of leave age. At 110 days of leave age, mango leaves on once and twice a week kaolin leave sprayed group tended to have a higher g_s value than those on control unsprayed group, but there were no significantly different in A values among the mango leaves in all treatment. At 145 days of leaf age, the mango leaf treated with twice a week kaolin leave spraying showed a significantly higher ($P<0.05$) A value than those of once a week kaolin leave spraying, but no significantly differences from the untreated control group. Foliar application of kaolin twice a week at 70 days of leaf age tended to provide a higher leaf in the maximum rate of rubulose-1,5-bisphosphate (RuBP) carboxylation ($V_{c\ max}$) than those of the control by 80%, whereas, at 110 days of leaf age, kaolin application twice a week tended to provide a higher leaf in the balance between RuBP carboxylation and RuBP regeneration ($J_{max}/ V_{c\ max}$) than those of the control by 180%. There was no significantly difference in the triose phosphate use (TPU) among treatments throughout the study period, and there was no significance in chlorophyll content in mango leaves among the treatment. Foliar application of kaolin once or twice a week on mango leaves had higher sucrose content than those without kaolin treatment at all fruit ages. Glucose and fructose contents in both mango leaves and shoot tips tended to increase from the fruit age 2 weeks, attained the maximum at the fruit age 8 weeks and then decrease until the fruit age 13 weeks. However, glucose and fructose contents in mango shoot tips were higher than those in the leaves. At the fruit aged 13 weeks, the content of glucose, fructose and sucrose in both mango leaves and shoot tips were dramatically reduced while the total carbohydrate contents were maintained at a high level. Kaolin leave spraying of the mango tree can not only increase the total number and weight of the mango fruit but also reduce of severity of anthracnose and fruit rot during the post-harvest ripening period. This suggested that kaolin leaf coating of mango is an useful technology for the plant photosynthesis and the fruit quality improvement under a high temperature and excess solar radiation environment.

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LIST OF ABBREVIATIONS

A	=	Net photosynthesis rate
A:E	=	Ratio of net photosynthesis to leaf transpiration
C_a	=	Atmospheric CO_2concentration
C_i	=	Intercellular CO_2concentration
C_c	=	CO_2 concentration in the chloroplast stroma
C_i:C_a	=	Ratio of intercellular to air CO_2 concentration
E	=	Transpiration rate
g_m	=	Mesophyll conductance
g_s	=	Stomatal conductance
J_{max}	=	The maximum rate of electron transport driving RuBP regeneration
J_{max}/ $V_{c\ max}$	=	The ratio of the capacitie of ribulose-1,5-bisphosphate (RuBP) regeneration to RuBP carboxylation
PPF	=	Photosynthetic photon flux density
RH	=	Relative humidity
TA	=	Titratable acidity
T_{air}	=	Air temperature
T_{leaf}	=	Leaf temperature
TNC	=	Total non-structural carbohydrates
TSS	=	Total soluble solids
$V_{c\ max}$	=	The maximum rate of ribulose-1,5-bisphosphate carboxylation
VPD_{air}	=	The air vapor pressure deficit
$VPD_{leaf-air}$	=	Leaf to air vapor pressure deficit

EFFECTS OF KAOLIN ON PHOTOSYNTHESIS, CARBOHYDRATE CONTENT, FRUIT YIELD AND FRUIT QUALITY IN MANGO (*Mangifera indica* L.) cv. NAM DOK MAI

INTRODUCTION

Mango (*Mangifera indica* L.), a dicotyledonous plagnt in family Anacardiaceae, is one of the major economic fruits in Thailand and continues to be one of the major export crops in many tropical countries. Apart from being common tree in house gardens, mango is produced in medium and large-scale plantations all over Thailand under different agro-ecological conditions. Thailand is the major mango exporter in Southeast Asia. Fresh domestic consumption is the most important use of mango in Thailand while the rest is export to many countries i.e. Japan, Singapore, Malaysia and others. (Mendoza and Wills**,** 1984; Litz, 1997).

Khiew Sawoey, Nam Dok Mai, Rad, and Nang Klang Wun, and local autochthonous cultivars, are the most common grown mango varieties. Nam Dok Mai cultivar is mainly grown for domestic fresh market in all parts of Thailand. Most of mango fruits are harvested during the dry season from February to May. A fruit weights range from 340 to 540 g with a length of 17 to 19 cm, a wideness of 7.5 to 8.5 cm and a thickness of 6.5 to 7.5 cm. The fruit is long and slender with a sigmoid shape. Its peel is greenish to bright yellow with a medium thickness, tender touch and easy peeling. The flesh is yellow with low fiber content (Wangnai, 1986).

The major problems of mango production are frequently reported as low yield and poor quality due to a reduction in photosynthesis and increased diseases (Sangchote, 1987; Spreer *et al*., 2009). Many reports suggested that photosynthesis significantly affect most plants productions, the leaves are dependent on a high midday temperature, and do not always gain their full photosynthetic capacity under midday drier conditions (Yamada *et al*., 1996; Goldschmidt, 1999; Hirasawa and Hsiao, 1999). Severe climate change may result in high temperatures and severe drought, particularly during summer, which can result in a midday depression of

photosynthesis. The main physiological processes responsible for the midday depression of photosynthesis are stomatal closure or photosystem II (PSII) photoinhibition or both (Muraoka *et al*., 2000). Flexas and Medrano (2002) reported that under severe drought stress, C_3 plants had to close stomata indicating a dominant limitation on photosynthesis. In addition, ribulose-1,5-bisphophate carboxylase (RuBPCase) context was decreased from a progressive down-regulation or inhibition of metabolic processes. Furthermore, one of the constraints of mango export is a disease problem, especially anthracnose disease caused by *Colletotrichum gloeosporioides*. Disease expression usually started at the ripening stage after harvesting. 'Nam Dok Mai' exporting mango, is highly susceptible to this disease and can be infected as latent infection with high levels compared with other cultivars (Sangchote and Chana, 1983).

Reflective materials can be applied as a leaf or fruit particle film coating to reduce solar heat stress, especially in areas with hot or sunny weather for a substantial part of the year. Such coatings can reduce heat stress, the extent of solar-injured fruit and water stress, and are involved in pest control and the suppression of disease incidence (Glenn and Puterka, 2005). Some of the reflective materials that may be used as leaf coating material include kaolin, bentonite and calcium carbonate. Kaolin, white clay mineral, can disperse in water and be sprayed on the leaf or fruit surface to form a protective particle film. It also causes light reflection on leaf surface which contribute lower leaf temperature, and prolonger stomata opening during high VPD in the air (Jifon and Syvertsen, 2003). In addition, using kaolin particle film can contribute to high quality of fruit production (Glenn and Puterka, 2005). The efficiency of kaolin particle film to mitigate negative effects of heat stress has been reported in various circumstances i.e. increased net photosynthesis rate (A) , stomatal conductance (g_s) and water use efficiency in citrus (Jifon and Syvertsen, 2003), increased A and g_s in apple (Glenn *et al.*, 2002), increased whole canopy carbon assimilation rate under high air temperature (T_{air}) (Glenn *et al.*, 2003). However, there were some reports indicating that kaolin did not affect transpiration rate (E), g_s and A in pecan (Lombardini *et al*., 2005). Kaolin reduced leaf temperature (T_{leaf}) and leaf to air vapor pressure deficit ($VPD_{leaf\text{-}air}$) in walnut and almond, but did not affect g_s

(Rosati *et al*., 2006). In addition, the physical barrier formed by particle film prevents contact between pathogenic microorganisms and plant surfaces, thereby reduces infection and disease (Glenn *et al*., 1999; Puterka *et al*., 2000). Thus it is becoming popular to improve fruit yields and reduce fruit loss.

Thailand is one of the largest current producers of kaolin in Asia and most production of kaolin is mainly used in ceramic and paint industry (World Health Organization, 2005). As one of the major export fruits, mango fruit yield and quality should be improved in order to increase economic value. Kaolin application as mango leaf coating will play an important role in the improvement of Thai mango.

This research is planned to evaluate effects of kaolin particle film on fruit yield and fruit quality, as well as to underlying the biological and physiological characterization of photosynthesis in kaolin-treated mango.

OBJECTIVES

1. To evaluate the impact of kaolin coating on leaf gas exchange and growth of mango plants

2. To determine the effects of kaolin application on mango fruit yield and fruit quality

3. To determine the effects of kaolin application on carbohydrate contents and sugars in mango

LITERATURE REVIEW

Mango

Mango (*Mangifera indica* L.), a dicotyledonous plant in family Anacardiaceae, order Sapindales, is a native plant in India and Southeast Asia. Anacardiaceae family consists of 73 genera, mostly are trees or shrubs, often contain milky or acrid juice and some of which are even poisonous. Mature specimens can attain a height of 30 m. The leaves are simple, alternately arranged, 15-45 cm in length. Leaves are variable in shapes like oval-lanceolate, oblong or roundish-oblong. The apex ranges from acuminate to nearly round. The margin is usually entire, sometimes slightly undulated and wavy, rarely twisted or folded. The length and breadth varies from 12 to 45 cm and 2 to 12 cm, respectively, depending on variety and growth. The color of young leaves generally varies from variety to variety, generally being tan-red, pink, yellow-brown in color. As the leaf grows, its color changes from tan-red to green, passes through many different shades and becomes dark green at maturity. The inflorescence is generally an axillary or terminal panicle or spike bearing small and regular flowers. The panicle bear 500-6000 flowers of which 1-70% are bisexual, remainder are male depending on the cultivar and temperature during its development. Fruit is fleshy drupe type which color of the skin at maturity varies from dark green to different shades of pure yellow or flushed red (Mendoza and Wills, 1984; Litz, 1997).

Mango trees develop vegetative shoots when shoot initiation occurs in warm temperatures (30°C day/25°C night), whereas inflorescences develop when shoots initiate growth in cool temperature conditions (18°C day/10°C night; or 15°C day/10°C night) (Whiley *et al*., 1989).

Mango is one of the major economic fruits in Thailand and continues to be one of the major export crops in many tropical countries. Mango trees are cultivated in all parts of Thailand. Mango fruits are mainly exported to Japan, Hong Kong, Singapore, Malaysia, Brunei, Europe, and the Middle East (Mendoza and Wills, 1984).

'Nam Dok Mai' is a main cultivar for exporting. The fruits are long and slender with a sigmoid shape. The flesh is yellow with low fiber content. A fruit weight ranges from 340 to 540 g with a fruit length of 17 to 19 cm, a wideness of 7.5 to 8.5 cm and a thickness of 6.5 to 7.5 cm. Due to nice skin color, greenish to bright yellow, it is attractive to consumers. In addition, with medium thick skin, tender and easy peeling quality Nam Dok Mai is appropriate for exportation (Wangnai, 1986).

Anthracnose

Anthracnose, a severe disease of mangos, causes direct yield loss in the field and marketing issues. Anthracnose disease caused by *Colletotrichum gloeosporioides* is one of the major common diseases for pre- and post-harvested fruits. High rain fall and humidity are associated with infection and disease development (Fitzell and Peak, 1984; Jeffries *et al*., 1990). In the field, *C. gloeosporioides* produces conidia on lesions on leaves, twigs, panicles, and mummified fruit. This fungus infects both pre- and post-harvest fruits. Anthracnose affects leaves, flowers and fruits, and inoculums are present year-round throughout the canopy. In moist conditions, this disease causes serious problem in nurseries and orchards. Optimal temperature for *C.gloeosporioides*, the causal pathogen of this disease, is 24 to 32^{o}C and it can also survive on other hosts such as orange, lemon, tea tree, coffee, rubber and plant residues (Jeffries *et al*., 1990). Symptoms on fruits are the development of minute brown spots and fruit aborts if infection occurs at an early stage of fruit development. Infection of young fruit causes fruit drop. After fruit exceeds 4 to 5 cm in diameter, abortion is less common. Further disease development occurs after fruits mature and begin to ripen. Anthracnose for mango fruit appears as small, black circular spots on the fruit skin, these increases in sizes and later becomes sunken and collapsed forming larger spot (Sangchote, 1987).

Nam Dok Mai is the most susceptible cultivar among all planted mangos in Thailand (Visarathanonth, 1988). Chayasombat (1987) studied the latent infection of Anthracnose disease in five varieties of mango fruits and reported that Nam Dok Mai showed the highest percentage of anthracnose infection, while Ok-Rong was the least.

Furthermore, Sangchote and Chayasombat (1986) reported the relationship of Anthracnose diseases and physiological changes in Nam Dok Mai that the disease incidences were closely related to ripening stages and sugar contents of the fruits. The highest disease incidence was obtained in fully ripe mango fruits with highest sugar contents. Besides, the rate of respiration, deterioration and ethylene production in diseased fruits were accelerated faster than uninfected ones.

Relationship between biological model with photosynthesis in leaf

Photosynthesis was the process by plants use energy from sunlight to synthesize carbohydrates from carbon dioxide (CO_2) and water (H_2O). The biochemistry of photosynthesis was a very complex process. It consists of three separate processes: (1) light reactions which convert light energy into chemical energy, (2) dark reactions which use this chemical energy to reduce CO_2 to carbohydrates, and (3) diffusion in which stomata open to allow CO_2 to diffuse into leaves from the surrounding air (Bonan, 2008). For CO_2 fixation to occur in a leaf, CO_2 from atmosphere must diffuse into the intercellular airspace of the leaf and then into the site of the carboxylation in the chloroplasts. Methods to estimate the intercellular CO_2 partial pressure (C_i) have been well established, based on gas exchange measurements, whereas those to estimate the CO_2 partial pressure at Rubisco carboxylation sites (C_c) are less certain. From Fick's first law of diffusion, the relation between net photosynthesis rate or net CO_2 assimilation rate (A), C_i and C_c is expressed as

$$A = g_m (C_i - C_c) \quad (1)$$

Where g_m is the mesophyll diffusion conductance. Therefore, g_m affects the drawdown from C_i to C_c, which can be used to analyse photosynthetic limitation by mesophyll diffusion (Yin and Struik, 2009). Guard cells sense CO_2 concentrations and react by changing their turgor pressure. These changes mediate the closure of stomata. Stomata respond to intercellular rather than to leaf surface CO_2 concentrations, but the biochemical and physiological mechanisms involved in this response are not well understood (Ainsworth *et al*., 2007; Assmann, 1999).

Three factors can limit the speed of photosynthesis: light intensity, carbon dioxide concentration and temperature. Without enough light, a plant cannot photosynthesis every quickly, even if there is plenty of water and CO_2. Increasing the light intensity will boost the speed of photosynthesis. Sometimes photosynthesis is limited by the concentration of CO_2 in the air. Even if there is plenty of light, a plant cannot photosynthesise if there is insufficient CO_2.

Through the reactions of photosynthesis, plants are able to convert sunlight into usable chemical energy by a direct reduction of CO_2 into triose phosphates by the enzyme Rubisco in the Calvin cycle. The assimilated CO_2 is then utilized for sucrose biosynthesis which in turn is used for growth and development. Thus, it is clear that photosynthetic performance and biomass accumulation are closely related (Ensminger *et al*., 2006). However, the excess absorption of sunlight can lead to a phenomenon known as photoinhibition (Long *et al*., 1994; Osmond and Grace, 1995; Takahashi and Murata, 2008). This occurs when high or even moderate amounts of light decrease the rates of photosynthetic activity often causing damage of the photosynthetic apparatus. Photoinhibition occurs on a daily basis for many photosynthetic organisms and limits yields of many crop species. The effects are exacerbated when their growth is limited by environmental factors such as extremes of temperature, light, drought and salinity (Takahashi and Murata, 2008). Susceptibility to photoinhibition varies among species and cultivars but is dependent on the ability of the plant tomodulate photosynthetic reactions (Ensminger *et al*., 2006). An intricate balance must be maintained between photochemical processes and the utilization of their by-products (ATP and NADPH), primarily by the Calvin cycle for CO_2 assimilation (Huner *et al*., 1993; Ensminger *et al*., 2006).

The Farquhar's model is a mechanistic model for studying the temperature response of photosynthesis. This model is based on the kinetics of Rubisco and describes the main reaction in the biochemistry of photosynthesis. The Farquhar's photosynthesis model requires several critical parameters: the maximum ribulose 1·5-bisphosphate carboxylase/oxygenase (Rubisco) carboxylation rate ($V_{c\ max}$); the potential light saturated electron transport rate (J_{max}); leaf dark respiration in the light

(R_d); and mesophyll conductance (g_m). These parameters can be obtained from the leaf-level measurements of gas exchange, namely the net assimilation of CO_2–chloroplastic CO_2 concentration ($A–C_c$) or net assimilation of CO_2–intercellular CO_2 concentration ($A–C_i$) curves (Miao *et al*., 2009).

The lower rate of photosynthesis has been ascribed to a reduction in the capacity of RuBP carboxylation ($V_{c\ max}$) and/or RuBP regeneration (expressed as the maximum electron transport rate, J_{max}). Reduction in $V_{c\ max}$ is usually attributed to decreased amounts of Rubisco (RuBP carboxylase/oxygenase), and sometimes to a low activation state. The mechanism of lowering in J_{max}, on the other hand, is less clear, because the capacity of RuBP regeneration is determined through many biochemical steps in electron transport and the Calvin cycle (von Caemmerer and Farquhar, 1981; Onoda *et al.,* 2005).

Relation between carbohydrate on flower and fruit development

Carbohydrate and sugar regulations

Carbohydrates are organic compounds derived from plants through photosynthesis and that are used as a source of energy for cellular respiration. Moreover, it was retained as reserve food source for growth especially for flower bud and fruit development.

Carbohydrates in plants can be classified into two forms: structural and nonstructural. Structural carbohydrates such as cellulose are structural components in plant, which have agronomic important as sources of fiber for industrial purposes. While the total nonstructural carbohydrate (TNC) including starch and sugars reserved in different parts of fruit trees play important roles in growth and development (Kozlowski and Pallardy, 1997). During photosynthesis, starch and sucrose are the major plant storage products. Starch is synthesized from ADP-glucose in the chloroplast, sucrose from fructose 6-phosphate and UDP glucose in the leaf cytosol.

Sucrose and its derivatives represent the major transport forms of photosynthetically assimilated carbon in plants. Sucrose synthesized in green leaves is exported via the phloem, the long-distance distribution network for assimilates, to supply nonphotosynthetic organs with energy and carbon resources. Sucrose not only functions as a transport metabolite but also contributes to the osmotic driving force for phloem translocation (mass flow) and serves as a signal to activate or repress specific genes in a variety of different tissues (Lalonde *et al*., 2000).

Sugars, synthesized in the mesophyll cells, serve as the major exported photosynthetic product. To accommodate long-distance transport of sugars from source (net exporting) to sink (net importing) organs, a vascular network—the phloem—has evolved in land plants. The most abundant compound in the phloem sap of most plant species is the disaccharide sucrose (Zimmermann and Ziegler, 1975). For a minority of plant species, the principle translocated sugars fall into two main groups: the sugar alcohols (mannitol and sorbitol) and the raffinose series (raffinose, stachyose, and verbascose) (Zamski and Schnaffer, 1996; Lalonde *et al*., 1999). In most cases in which such sugars predominate, however, sucrose is also present.

From the point of sucrose synthesis in the mesophyll, the route to the SE involves several cell types: neighboring mesophyll cells, bundle-sheath cells, phloem parenchyma, and CCs. Cell-to-cell movement of sucrose is considered to occur via plasmodesmata from the point of synthesis up to the SE/CC complex which, in many species, is not well connected to the surrounding cells. Two principal routes for the delivery of sucrose into the SE/CC complex have been proposed: (1) transporter-mediated export from mesophyll cells, apoplasmic diffusion through the cell-wall continuum, and subsequent carrier-mediated transport across the SE/CC plasma membrane; and (2) direct symplasmic cell-to-cell diffusion via plasmodesmata (Gamalei, 1989; Lalonde *et al*., 1999).

In leaves, the first transport step must be release of sucrose into the cell wall directly from the mesophyll cell. Subsequently, at least one transporter is required for uptake into the phloem. These loading processes are required at various stages of

development, such as during germination for sugar export from leaves or for mobilization of stored carbon, events that might require distinct transporters. Reuptake of sucrose along the translocation pathway is necessary to allow solute exchange with the phloem, for example, in stems. In sink tissue, unloading can occur either by transmembrane export of sucrose or through plasmodesmata. Sucrose efflux transporters involved in phloem unloading have been postulated to function as facilitators or as proton antiporters. Sucrose in the apoplast of sink tissue can be taken up directly or through the hydrolysis of sucrose into glucose and fructose by invertase followed by hexose uptake (Lalonde *et al*., 1999). However, plant sugar regulation is mediated by diverse sugar signals, which are generated at different locations depending on environmental conditions and developmental stage. Sucrose transport and hydrolysis play key regulatory roles in sugar signal generation (Rolland *et al*., 2006).

Carbohydrate and development of mango flower and fruit

Corbesier *et al*. (1998) reported that sucrose is an early and essential component to stimulate flower buds. Studies with several physiological model plants indicate that the levels of sucrose in the phloem sap and/or soluble sugars in the apical bud increase early upon photoperiodic oral induction in both long-day (LD) and short-day (SD) plants.

At present, there are two major conceptual models to explain mango flowering, one regulated by carbohydrates and the other by hormones. The first model proposes that the yield is a result of the photoassimilate accumulation and redistribution during each annual production cycle. The second model proposes the role of phytohormones, such as auxins, cytokinins, and gibberellins, as instrumental metabolites involved in mango flowering (Santos-Villalobos *et al*., 2013).

In mango tree, however, Chacko (1986) indicated that carbohydrate reserves were directly correlated with flowering. Schaffer *et al*. (1994) noted that flowering was more likely to be problematical in tropical climates, where a dry period appeared

to be the main flowering trigger, than in the subtropics, where winter cold was the main environmental cue. Whiley *et al*. (1989) reported the accumulation of carbohydrate in resting shoots of mangos prior to flowering and found the decrease during flower emergence. This may be the major problem of fruit setting of mango during summer in Thailand.

Phavaphutanon *et al*. (2000) observed that current year mango shoots generally had greater fluctuation of total nonstructural carbohydrate (TNC) than last season shoots. Furthermore, decreased TNC in current year shoots was more closely related to the time of flowering than that of the last season shoots. Accumulation of TNC in current year shoots and last season shoots after an off-season and an on-season flowering period was probably related to low sink strength due to poor fruit set.

The development of the mango fruit can be divided into four stages (Singh, 1960): (i) the juvenile stage, up to 21 days from fertilization (rapid cellular growth); (ii) stage of maximum growth, 21-49 days from fertilization; (iii) maturation (respiration climateric and ripening, 49-77 days from fertilization; (iv) senescence. Preharvest development of fruit are processes of increase in dry matter, alcohol-insoluble solids, etc. inside a efficient barrier of cuticle-covered epidermis. Postharvest mangoes show a climacteric pattern of respiration whose dramatically physiological and chemical changes take place during ripening. Upon harvesting at maturity, hydrolytic processes are triggered, and the depletion of starch, the breakdown of insoluble pectin happened (Phan, 1987).

Some physiological response under environment stress

Environmental stresses such as a high midday temperature and high irradiance result in a midday depression of photosynthesis. It is well known that when leaves are usually exposed to high irradiance and a high air temperature (T_{air}), plants do not always gain their full photosynthetic capacity (Yamada *et al*., 1996; Goldschmidt, 1999; Hirasawa and Hsiao, 1999). Severe midday depression of photosynthesis under high photosynthetic photon flux density (PPF) and T_{air}, reduce the growth, fruit yield

and fruit quality in many plants such as citrus (Goldschmidt, 1999; Jifon and Syvertsen, 2001; Hu *et al*., 2007) and tomato (Leonardi *et al*., 2000; Adams *et al*., 2001). The depression might be due to stomatal and non-stomatal limitation (Yu *et al*., 2009). Stomatal closure is the major physiological factor responsible for this midday depression, which decreases the CO_2 concentration in the intercellular spaces (C_i) (Tenhunen *et al*., 1984; Xu and Shen, 2005). Moreover, the decrease in carboxylation efficiency, causing non-stomatal inhibition of photosynthesis, might be due to photodamage. The photodamage commonly occurs when absorbed light exceeds the amount required for electron transport and CO_2 assimilation (Müller *et al.* 2001). High PPF combined with environmental stress, intensifies photodamage of assimilatory apparatus, thereby inhibiting photosynthesis (Gamon and Pearcy 1990, Ohashi *et al*. 2006). Thus, the closure of stomata may contribute to the decrease in the net photosynthetic rate (A) by limiting the CO_2 supply. The stomatal factor is associated with decreasing leaf C_i caused by decreasing stomatal conductance (g_s) (Ishida *et al.*, 1999; Jones, 1992).

The temperature increase during the midday depression of photosynthesis impacts on the CO_2 and H_2O exchange by altering the leaf-to-air vapor pressure deficit ($VPD_{leaf-air}$). This causes particular sensitivity of the g_s of leaves to the change in VPD_{air}. Iio *et al*. (2004) reported that A, g_s and the transpiration rate (E) of *Fagus crenata* Blume. decreased more than 50% under a VPD_{air} value of about 2.5 kPa compared to the VPD_{air} value of about 1.0 kPa even under well-watered conditions. In addition, the midday depression of photosynthesis was reported to be the result of midday stomatal closure caused by high $VPD_{leaf-air}$ (Iio *et al*., 2004). Moreover, stomatal responses to VPD_{air} were mediated by E, and then affected the leaf water status or the gradient in the water potential between the guard cells and other epidermal cells (Gunasekera and Berkowitz, 1992). Nevertheless, this feedback mechanism was not consistent with observations of enhanced leaf water potential (Saliendra *et al*., 1995) and stomatal closure at high VPD_{air}, with concomitantly lower transpiration (Farquhar and Raschike, 1978). More recently, Hu *et al*. (2007) reported that decreased E and g_s at midday were interpreted as a combined feed-forward effect from the increased VPD_{air}.

Jifon and Syvertsen (2001, 2003) reported that leaf temperature (T_{leaf}), VPD $_{leaf-air}$, and photoinhibition during the midday period were reduced by moderate shade and particle film applications, and consequently g_s and A were increased in shaded leaves compared to sunlit leaves. In addition, the excess radiation and high temperatures of leaves caused water deficits and reduced the efficiency of light-use leading to redu0ced A, lower growth, less fruit yield and poorer fruit quality (Goldschmidt, 1999). Reducing the sink capacity by removing fruit could result in a greatly diminished rate of net photosynthesis in many species of fruit trees, such as apple and plum (Gucci *et al*., 1991, 1995). However, inconsistent effects resulting from the removal of reproductive sinks were observed on net photosynthesis (Nautiyal *et al*., 1999).

Effect of environment stress on pigments in plant

Chlorophylls are green pigments present in the thylakoid membrane or chloroplasts. Chlorophyll is a key biochemical component in the molecular apparatus that is responsible for photosynthesis, the critical process in which the energy from sunlight is used to produce life-sustaining oxygen. There are several kinds of chlorophylls namely chlorophyll a, b, c, d and e. Chlorophyll a and b are the two types widely distributed in higher plants. Chlorophyll a is present in all photosynthetic organisms and is the green photosynthetic pigment. Chlorophyll b is an accessory pigment and absorbs different pigments than a.

Chlorophyll loss is associated to environmental stress and the variation in total chlorophyll/carotenoids ratio may be a good indicator of stress in plants (Hendry and Price, 1993). In addition, excess absorbed energy can result in destruction of chlorophyll, causing an overall yellowing of the foliage. Species tolerant of high light are able to maintain high concentrations of chlorophyll when grown in a high light environment (Griffin *et al.* 2004). Reports on crops with a chlorophyll response in susceptible varieties resistant to drought or lack of water stress on chlorophyll concentration is presented. Seems to stress, partly due to a decrease in photosynthesis that is associated with decrease of chlorophyll concentrations (Jalalpoori, 2013).

Drought stress imposed during vegetative growth or anthesis significantly decreased chlorophyll a, chlorophyll b and total chlorophyll content. Photosynthesis, transpiration, stomatal conductance and yield were higher but sub-stomatal CO_2 concentration was lower under drought stress conditions than under control conditions. The results showed that mesophyll resistance is the basic determinate of rate of phototosynthesis under drought stress conditions (Mafakheri *et al.* 2011)

Kaolin

Kaolin or kaolinite is a white aluminosilicate clay with fine and porous, non-abrasive particles, disperses easily in water. This inert mineral is used in the paper, paint and plastic industries, depending on the processing method and characteristics of the particles (size, dimensions and brightness). It is used in pharmaceuticals drug due to its safety for ingestion (McBride, 2000). It is also a component of toothpaste, cosmetics and alimentary products (Glenn and Puterka 2005).

Kaolin particle film

Kaolin particle film is commercially available under the trade name Surround®WP crop protectant. This commercial product is composed of hydrophilic kaolin particles mixed with an oil based spreader-sticker (Glenn and Puterka, 2005). It is registered on the Organic Materials Review Institute (OMRI) list for use in organic agriculture in the U.S. and listed in the permitted products for organic production in Québec (Conseil des appellations agroalimentaires du Québec, 2007). In organic agriculture, kaolin particle film represents the first broad utility material that provides effective insect control and high product quality in organic fruits and vegetables. Its adoption by organic growers will further increase the growth of this expanding industry in the US and worldwide (Glenn and Puterka, 2005).

Kaolin application

Kaolin is sprayed onto tree foliages as liquid suspension, leaving kaolin a white porous protective powdery film on the surface of leaves and fruits after water evaporation. The physical properties of the kaolin film reduce damage from insects and plant pathogens, as well as enhancing photosynthesis, yield, and fruit quality, particularly in hot and dry climates. It also causes light reflection on the leaf surface which contributes to a lower leaf temperature, and prolonged stomatal opening during high VPD in the air (Jifon and Syvertsen, 2003). In addition, using kaolin particle film can contribute to high quality fruit production (Glenn and Puterka, 2005). According to Glenn and Puterka (2005), the mineral particle film must have the following properties: (1) chemically inert, (2) particle diameter <2 μm, (3) creation of a uniform film, (4) formation of a porous film allowing leaf gas exchange, (5) transmission of the photosynthetically active radiation (PAR) and reflection of the UV and the IR radiation, (6) interference with insect or pathogen behavior and (7) ability to be washed from the harvested products.

There have been many reports indicated that kaolin is an effective particle film to prevent insect infestation by coating plants with a physical barrier that deters insect infestation, and impedes insect movement, feeding and egg-laying (Glenn *et al*., 1999; Puterka *et al*., 2000). The physical barrier formed by particle film technology also acts to prevent contact between pathogenic microbial inocula and plant surfaces, thereby reducing infection and disease (Glenn *et al*., 1999; Puterka *et al*., 2000).

Moreover, it was also noted that the processed-kaolin film material was highly reflective to the UV wavelengths and this characteristic may be important in reducing solar injury to both fruit and leaves (Glenn *et al*., 2002). Kaolin particle film application transmits 90-98% of photosynthetically active radiation, while its reflective properties reduce heat build-up, ultraviolet radiation damage and heat stress in leaf canopies (Glenn and Puterka, 2005). For example, solar injury was suppressed with spray applications of a reflective, processed-kaolin particle film material on

apple [*Malus sylvestris* (L.) Mill var domestica (Borkh.) Mansf.]. In addition, kaolin has been recommended to lower the temperature of apple fruit, reduce sunburn and improve red fruit color in situations where temperatures are higher than an optimal condition (Werblow, 1999). Glenn *et al*. (2002) reported that fruit surface temperature was reduced by the application of reflective particles and the amount of temperature reduction was proportional to the amount of particle residue on the fruit surface. These properties ultimately produce more efficient photosynthesis, and increase the amount of assimilates available for fruit development (Glenn *et al*., 1999; Puterka *et al*., 2000).

The efficiency of kaolin particle film to mitigate the negative effects of heat stress has been reported in various circumstances—increased A, g_s and water use efficiency in citrus (Jifon and Syvertsen, 2003), increased A and g_s in apple (Glenn *et al.*, 2002) and an increased rate of whole canopy carbon assimilation under high T_{air} (Glenn *et al.*, 2003). However, Lombardini *et al*. (2005) reported that kaolin did not affect E, g_s and A in pecan. Kaolin reduced T_{leaf} and $VPD_{leaf\text{-}air}$ in walnut and almond, but did not affect g_s (Rosati *et al*., 2006). In addition, the physical barrier formed by the particle film prevents contact between pathogenic microorganisms and plant surfaces and thereby reduces infection and disease (Glenn *et al*., 1999; Puterka *et al*., 2000). Thus, it is becoming a popular way to improve fruit yields and reduce fruit loss.

Interestingly, the physical presence of the clay particles apparently did not inhibit leaf gas exchange, perhaps due to the porous nature of kaolin clay. This good quality leads to the increase of net carbon assimilation in kaolin-treated plant. It was indicated that an increasing of yield and fruit quality on kaolin-treated apple trees was caused by an increasing in whole-tree carbon assimilation under heat stress **(**Glenn *et al*., 2003).

MATERIALS AND METHODS

Materials

1. Experimental Plant

- Mango (*Mangifera indica* cv. Nam Dok Mai)

2. Chemical

2.1 Chemical for coating mango leaves

- Kaolin in size of 325 mesh (Bullazia Agrifluids Co. Ltd., Nonthaburi, Thailand)
- Bentonite in size of 240 mesh (Bullazia Agrifluids Co. Ltd., Nonthaburi, Thailand)
- Calcium carbonate in size of 240 mesh (Bullazia Agrifluids Co. Ltd., Nonthaburi, Thailand)
- Dolomite in size of 200 mesh (Bullazia Agrifluids Co. Ltd., Nonthaburi, Thailand)
- Kaolin in size of 200 mesh collected locally from a factory in Mae Tha, Lampang province

2.2 Chemical for soluble sugar analyses and starch quantification

- petroleum ether
- ethanol
- glucose
- fructose
- sucrose
- Total starch assay kit (Megazyme International Ireland Ltd., Bray, Republic of Ireland)

2.3 Chemical for fruit quality determination

- 0.1 N NaOH

3. Equipment

3.1 Equipment for field

- Spectrophotometer (Jenway Model 6400, United Kingdom)
- Portable photosynthesis system (LI-6400; LI-COR Inc.; Lincoln, NE, USA). with the light sensor (LI-250, Li-Cor) and light source (LI-6400-02 LED, Li-Cor)
- Quantum sensor and data-logger (Watch Dog model 1450, Spectrum technologies, Inc, USA)

3.2 Equipment for laboratory

- Small Hammer mill grinder (Janke & Kunkel IKA-Labortechnik, Germany)
- High Performance Liquid Chromatography (HPLC) (Shimadzu, Kyoto, Japan)
- Color difference meter (model CR-400, Konica Minolta, Osaka, Japan)
- Hand refractometer (Atago, Tokyo, Japan)

Methods

The selection of the most appropriate particle film for four coating leaf materials—bentonite, calcium carbonate, dolomite and kaolin clays—for developing a leaf coating material for mango were carried out at Kasetsart University, Bangkok, Thailand from October 2008 to March 2009.

The effects of kaolin on photosynthesis in field conditions were studied at commercial mango orchards in the province of Nakhon Pathom (14°8'27.34" N, 100°10'43.64" E), Thailand from March to July 2010. In addition, analysis of carbohydrate content, fruit yield and fruit qualities after spraying kaolin were studied at Kasetsart University, Bangkok, Thailand from November 2010 to September 2011.

Experiment 1 Selection of the most appropriate particle film for coating mango leaves

This experiment was a preliminary test to select the most appropriate particle film for coating mango leaves. The effectiveness for photosynthesis improvement was investigated using mango leaf coatings of kaolin, bentonite, calcium carbonate and dolomite with particle sizes of 325, 240, 240 and 200 mesh, respectively. Laboratory and field tests were performed at Kasetsart University, Bangkok, Thailand (13°50'50.39"N, 100°34'19.44"E). The suspendability, precipitation time of the precipitable particles, photon transmission through the particle film, leaf coating ability and gas exchange of the coated leaves were investigated. The following four experiments were performed.

1.1 Suspendability and precipitation time of tested substances

Suspendability in water and precipitation times of the dispersing particles were studied. According to previous work by the current authors (data not shown), the concentration of each material was varied from 50 to 70 $g.L^{-1}$. It was found that 60 $g.L^{-1}$ of each material was the optimum concentration to produce suspendability in water with the precipitation time of the dispersing particles being 2 h. Consequently, each material at 60 $g.L^{-1}$ was well stirred in water with the addition of 1–2 drops of sodium silicate to retard precipitation. The suspensions, after thorough stirring, were left undisturbed for 3 h to allow the precipitable particles to sink to the bottom and the precipitation times were recorded. The average precipitation time from three repetitions was taken for each material.

1.2 Photon transmission of coating films

Each leaf coating material was sprayed at 60 $g.L^{-1}$ as a coating onto a 12 × 20 cm glass plate and allowed to dry at ambient temperature. The glass plate was they placed under direct sunlight and the photosynthetic photon flux (PPF) was recorded continuously using a quantum light sensor (Watch Dog model 450; Spectrum Technologies, Inc.; Plainfield, IL, USA) placed 10 cm below the plate. The

mean percentage PPF indicating the photon transmittance of coated glass plate was determined as the percentage transmittance of photons through an uncoated glass plate.

1.3 Mango leaf coating ability and coated leaf gas exchange measurements

Kaolin and bentonite which previously had been found to form satisfactory films and produce satisfactory photon transmission were used in this experiment. Each of the materials was suspended well in water at 60 $g.L^{-1}$. The aqueous suspension was sprayed on each mango tree leaves twice a week using a hand-held sprayer. In total, 12 applications were made from 1 November to 16 December 2008 to maintain a uniform coating of the film on the leaves of treated trees throughout the study period.

Leaf gas exchange was measured with a portable photosynthetic system (LI-6400; LI-COR Inc.; Lincoln, NE, USA). The leaf sampled was clamped inside the leaf chamber of a portable photosynthesis system with the upper leaf surface inside the chamber fully exposed to direct sunlight. Leaf samples were chosen from the first mature leaves at exterior canopy positions after kaolin spraying. The measurements were performed on selected, clear, sunny days. Measurements of leaf gas exchange were made during the period from 1130 to 1330 hours. The net photosynthesis (A), stomatal conductance (g_s), transpiration rate (E), leaf temperature (T_{leaf}), leaf to air vapor pressure deficit ($VPD_{leaf-air}$), intercellular CO_2 concentration (C_i), air CO_2 concentration (C_a), ratio of intercellular to air CO_2 concentration (C_i:C_a), photosynthetic photon flux (PPF), air temperature (T_{air}) and relative humidity (RH) were measured under ambient conditions. All measurements were performed at the CO_2 concentration of 400 $\mu mol.mol^{-1}$. The air vapor pressure deficit (VPD_{air}) was calculated from T_{air} and RH, according to Goudriaan and van Laar (1994). VPD_{air} was calculated using Equation 1:

$$VPD_{air} = e^{0} - e \quad (1)$$

where e^0 is the saturation vapor pressure (kPa). e^0 can be calculated (LI-COR ,1990) using Equation 2:

$$e^0(T_{air}) = 0.61083 \times 10^{\frac{7.6448T}{242.62+T}} \quad (2)$$

where T is the dewpoint temperature (°C).

When e is the actual vapor pressure (kPa), e can be calculated using Equation 3:

$$e = e^0_{Tair} \times (1 - RH_{air}) \quad (3)$$

where T_{air} is the air temperature (°C) and RH_{air} is the percentage of the amount of water that the air can hold at a given temperature (%). RH and T_{air} must be taken in the same time period.

1.4 Light response curve of mango

Measurements of light response curves of photosynthetic characteristics were carried out on the same leaves that were evaluated for diurnal changes in gas exchange. A light response curve was obtained with PPF values of 2000, 1600, 1200, 1000, 800, 600, 400, 200, 100, 75, 50, 25 and 0 µmol PPF $m^{-2}.s^{-1}$, adjusted automatically by a red-blue light-emitting diode light source (LI-6400; LI-COR Inc.; Lincoln, NE, USA). The CO_2 concentration and temperature in the leaf chamber were maintained at 400 µmol CO_2 mol^{-1} and 37 °C, respectively. Leaves were allowed to stabilize at each light step for a minimum of 3 min and then data were locked after the steady-state condition was achieved. The data were fitted to a non-rectangular hyperbolic function (Thornley and Johnson, 1990) shown in Equation 4:

$$A = \frac{1}{2\theta}\left[\alpha I + P_m - \sqrt{(\alpha I + P_m)^2 - 4\theta\alpha I P_m}\right] - R_d \quad (4)$$

where A is the net photosynthesis (μmol CO_2 $m^{-2}.s^{-1}$), I is photosynthetic photon flux (μmol PPF $m^{-2}.s^{-1}$), P_m is the maximum net photosynthesis rate (μmol CO_2 $m^{-2}.s^{-1}$), R_d is the darkness respiration rate (μmol CO_2 $m^{-2}.s^{-1}$), α is the quantum or photochemical efficiency (mol CO_2 mol^{-1} PPF) and θ is the curvature factor.

Each parameter of the leaf photosynthetic response to PPF was recorded and the average determined from four leaves. The average values of each parameter were used in the model of the leaf photosynthetic response to PPF to describe the curve.

Experiment 2 Effects of kaolin on photosynthesis, carbohydrate content, fruit yield and fruit quality.

The study was carried out using 'Nam Dok Mai' cultivars, which were grown in commercial mango orchards in the province of Nakhon Pathom, Thailand. This experiment was performed twice—on first crop (April to October 2010) and continued through to the second crop (November 2010 to February 2011).

Microclimates

Climate data were recorded from a quantum sensor with connected data-logger (Watch Dog model 1450, Spectrum technologies, Inc, USA) located at the experiment field every 15 minutes throughout the study period. These data showed the photosynthetic photon flux (PPF), air temperature (T_{air}) and relative air humidity (RH). The air vapour pressure deficit (VPD_{air}) was then calculated from the records of air temperature and relative humidity, according to Goudriaan and van Laar (1994).

Plant Materials

Thirteen-year old mango trees with 2.5 m tall are used as plant materials and grown. Trees were planted with a distance of 2.5 m within-row and 3 m between-row and well-watered and fertilized. Before treatment, the mango leaves were dressed.

When the trees reached the mature-leaf stage with glossy, dark green leaves, they were sprayed with an aqueous suspension of kaolin 200 mesh from Mae Tha, Lampang province by using a power sprayer. Four trees were selected and were sprayed twice a week with 60 $g.L^{-1}$ of aqueous kaolin suspension by using power sprayer while the other four control trees were not sprayed. Each tree was sprayed with 0.7 L of aqueous kaolin suspension, in order to have all leaves of the entire canopy uniformly coated as observed by the white clay coating on all leaves. Leaves of the treated trees were sprayed onto with kaolin suspension once and twice a week during March 2010 until February 2011 to maintain a uniform film coating. The duration for kaolin application was after flowering with fruit set of 0.5 cm until fruit matured at week 2 before harvesting. The experiment is a completely randomize block design with 4 replicates per treatment.

Treatment 1 Trees were not sprayed (Control).

Treatment 2 Trees were sprayed onto with kaolin suspension once a week.

Treatment 3 Trees were sprayed onto with kaolin suspension twice a week.

In order to determine the effects of kaolin coating on photosynthesis, carbohydrate content, fruit yield and fruit quality under field conditions. The following three experiments were performed

2.1 Effects of kaolin on photosynthesis

2.1.1 Diurnal changes of gas exchange

Fully expanded, sun-acclimated leaves (approximately 45 days of leaf ages), positioned in exterior canopy are used for gas exchange measurements. Gas exchange measurements were performed four times on the 8 May (70 days of leaf ages), 29 May (90 days of leaf ages), 20 June (110 days of leaf ages) and 26 July 2010 (145 days of leaf ages) to compare physiological characteristics of the mango tree after kaolin treatment. The measurements are performed on selected clear days.

The photosynthetic measurement was performed every hour during 0800 to 1600 hours using a portable photosynthesis system (LI-6400; LI-COR Inc.; Lincoln, NE, USA) equipped with the light sensor (LI-250, Li-Cor). A mature expanded leaves per tree were randomized chosen from the exterior of canopy following sunshine direction and 4 representative trees of 70, 90, 110 and 145 days of leaf ages were employed. Each leaf was clamped inside the leaf chamber of the portable photosynthesis system with the upper leaf surface inside the chamber being fully exposed to natural PPF. The flow rate was controlled at 400 μmol mol^{-1}. The measurement parameters consisted of the photosynthetic photon flux (PPF), air temperature (T_{air}), relative humidity (RH), net photosynthesis (A), stomatal conductance (g_s), transpiration rate (E), leaf temperature (T_{leaf}), leaf to air vapor pressure deficit ($VPD_{leaf-air}$), and intercellular CO_2 concentration (C_i). The air vapour pressure deficit (VPD_{air}) was then calculated from the records of air temperature and relative humidity, according to Goudriaan and van Laar (1994).

2.1.2 CO_2 response (A/C_i) curves

Measurements of the leaf CO_2 assimilation responded to an increase of intercellular CO_2 concentrations (A/C_i) were carried out in the same leaves evaluated for diurnal changes of gas exchange. A/C_i curves were recorded under saturating PPF (1,600 μmol $m^{-2}.s^{-1}$) with a portable photosynthesis system equipped with light source (LI-6400-02 LED, Li-Cor). The intercellular CO_2 concentration (C_i) was controlled by varying CO_2 concentrations (C_a) from 50 to 2,000 μmol $m^{-2}.s^{-1}$ in 11 steps. First, C_a was set at 400 μmol mol^{-1}, and afterwards it was decreased until reaching 50 μmol $mol^{-2}.s^{-1}$. Next, C_a was again increased to 400 μmol mol^{-1}, and then to 2,000 μmol $m^{-2}.s^{-1}$. The measurement was recorded when net CO_2 assimilation rate and stomatal conductance were stable, after about 20–30 min.

The Sharkey *et al*. (2007) model was applied to these data. This model uses a non-linear curve fitting utility available in Excel and its outcome includes five parameters that were relevant for the description of photosynthesis (Figure 1). The parameters were: the maximum ribulose 1·5-bisphosphate carboxylase/oxygenase

(Rubisco) carboxylation rate ($V_{c\ max}$); the potential light saturated electron transport rate (J_{max}), use of triose phosphates or other carbon product generated in the Calvin cycle (TPU), day respiration (R_d) and mesophyll conductance (g_m). Using the model requires at least five pairs of data for A and C_i, as well as the estimation of the limiting factor by the user. The possible limiting factors indicate what is limiting A at certain C_i and were originally described in the Farquhar *et al*.(1980) model. According to A/C_i curves, three different situations can occur. At low [CO_2] (< 200 μmol mol^{-1}), the carboxylation rate of Rubisco is the limiting factor. In this stage, photosynthesis is limited by the capacity of Rubisco to fix CO_2.When CO_2 are higher (> 300 μmol mol^{-1}) the ratio carboxylation: oxygenation increases, and the regeneration of RuBP, which depends on electron transport, becomes limiting. It was also possible an intermediate situation that may represent different limitations occurring in different parts of the leaf. In the third and last situation the leaf cannot use all the products originated in the chloroplast; then, photosynthesis was TPU limited. In the current study, photosynthesis was considered Rubisco-limited when [CO_2] < 200 μmol $mol^{-1}$and RuBP-limited when [CO_2] >400 μmol mol^{-1}.Values laying in between were excluded.

These different states have a particular and characteristic photosynthetic response to the intercellular CO_2 concentrations, which gives important information about the involved biochemical mechanisms (Sharkey *et al*., 2007).

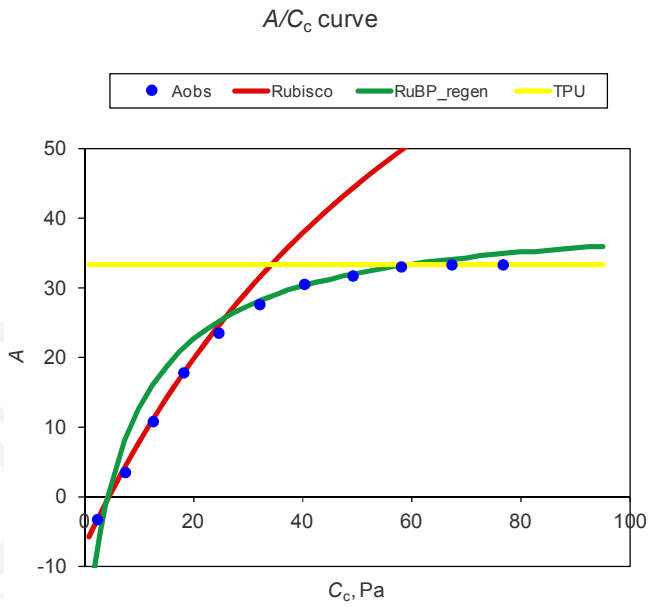

Figure 1 A-C_i curve obtained with the Sharkey *et al.* (2007) model.

2.1.3 Measurement of Chlorophyll Content

Three second leaves of each shoot at exterior canopy positions were harvested from each tree. For chlorophyll extraction, two discs were punched with a cork borer (0.64 cm^2) from the mid-laminar area of each leaf and soak in N, N dimethylformamide in the dark at 4°C for 24 hr. Chlorophyll absorbance was determined using a spectrophotometer (Jenway Model 6400, United Kingdom) at 647 and 664 nm. The chlorophyll a, b, and total chlorophyll were calculated from the equations of Moran (1982) as follow:

$$Chl_a = (-2.99\ A_{647} + 12.64A_{664}) \times Vol / (X \times Area \times 100) \quad (5)$$

$$Chl_b = (23.26\ A_{647} - 5.60A_{664}) \times Vol / (X \times Area \times 100) \quad (6)$$

$$Chl_{total} = (20.27\ A_{647} + 7.04A_{664}) \times Vol / (X \times Area \times 100) \quad (7)$$

Where Chl_a , Chl_b , and Chl_{total} are chlorophyll a, b, and total chlorophyll content (g/ m^2), respectively. A_{647} and A_{664} are light absorbance at 647 and 644 nm, respectively. Vol is the DMF volume (ml) that used for extracting chlorophyll. X is a diluted volume, in the case that the first extracted chlorophyll

gives higher reading concentration than 0.8. Area is the leaf area used for chlorophyll extraction (cm^2). 100 is number of multiply convert unit g cm^{-2} to g m^{-2}.

2.2 Effects of kaolin on carbohydrate content

Four shoots with matured leaves were selected and cut 30 cm from around the canopy of each tree from the second crop. The leaf samples were collected from the 1st and 2nd leaf positions from shoot apex. In order to evaluate the carbohydrate and sugars during fruit development, analyses were performed four times— at fruit's age 2, 5, 8, and 13 weeks. All samples were washed immediately in cold water and packed in bags. After that, they were dried at 70 °C for 1 week, ground into fine powder using Small Hammer mill grinder (Janke & Kunkel IKA-Labortechnik, Germany), and stored at room temperature (27°C) until the analysis of carbohydrate contents.

2.2.1 Soluble sugar analyses

The method according to Eshghi *et al*. (2007) was for sugar analysis. The 0.1 gram ground dried tissue was placed in a centrifuge tube, and 5 ml of 40–60°C petroleum ether was added. This mixture was centrifuged at 1109× g at 4°C for 5 min. The petroleum ether extract, containing lipids, chlorophyll and other contaminants, was carefully pipetted-off and discarded. Four milliliters of 80% (v/v) ethanol was added into the tube, and the mixture was mixed thoroughly on a vortex mixer. After that, the tube was incubated in a water-bath at 65 °C for 20 minutes. After centrifugation for 5 min at 1109 × g at 4 °C, the supernatant was transferred into another centrifuge tube by pipetting. The extraction of remaining plant material was repeated with 4 ml 80% (v/v) ethanol. After that, it was centrifuged and the supernatants were pooled. The supernatants were evaporated using a rotary evaporator. After evaporation the remaining solute were stored at -84^{o}C for soluble sugar analysis. Before injection onto the HPLC, samples were dissolved in 0.5 ml of ultra pure water and removed and filtered through a 0.45 mm membrane filter. Then, 20 µl of it was injected into the High Performance Liquid Chromatography (HPLC)

for sugars analysis. Each sample was assayed twice and the sugar concentrations were expressed as $mg.g^{-1}$ dry weight of tissue. The concentration of individual sugars in each sample was calculated using peak areas and the calibration curves.

2.2.2 Extraction and quantification of starch

Starch concentration in lyophilised samples was measured using a total starch assay kit (Megazyme International Ireland Ltd., Bray, Republic of Ireland) according to the manufacturer's instructions (AOAC method 996.11, AACC method 76.13, ICC standard method no. 168 (2006). The assay procedure was summarized as followings. The sample was ground and passed through a 0.5 mm screen. Approximately 100 mg (weighed accurately) of sample was added to a glass tube (16×120 mm). The tube was tabbed to ensure that all of the sample drops to the bottom of the tube. The 0.2 mL of aqueous ethanol (80% v/v) was added to wet the sample in order to aid sample dispersion, and the tube was stirred on a vortex mixer. The 3 mL of thermostable α-amylase (300 Units) in MOPS buffer (50 mM, pH 7.0) (Appendix A1) was immediately added and the tube was vigorously stirred on a vortex mixer. The tube was then incubated in a boiling water bath for 6 min (stir the tube vigorously after 2 min and 4 min). After that the tube was placed in a bath at 50°C, and 4 mL of sodium acetate buffer (200 mM, pH 4.5) (Appendix A1) was added, followed by 0.1 mL of amyloglucosidase (20 Units). The tube was stirred on a vortex mixer and incubated at 50°C for 30 min. The entire content in the test tube was transferred into a 100 mL volumetric flask. The tube content was rinsed thoroughly with a wash bottle, and the volume was adjusted with distilled water. The mixer was mixed thoroughly before it was subjected to the centrifugation at 3,000 rpm for 10 min. The duplicate aliquots (0.1 mL) of the diluted solution were transferred to the bottom of glass test tubes (16 × 100 mm). The 3.0 ml of GOPOD reagent was added to each tube (including the glucose controls and reagent blanks), and the tubes were incubated at 50°C for 20 min. The glucose controls consist of 0.1 mL of glucose standard solution (1 mg mL^{-1}) and 3.0 mL of GOPOD reagent. The reagent blank solutions consist of 0.1 mL of water and 3.0 mL of GOPOD reagent. The absorbance at 510 nm for each sample and the glucose control was read against the reagent blank.

The quantification of starch in each sample was calculated from the following formula in Appendix A2.

2.3 Effects of kaolin on fruit yield and fruit quality

In order to determine the effects of kaolin application on mango fruit yield and fruit quality. The following four experiments were performed.

2.3.1 Total weight and the total number of fruit products

Total numbers of fruit per tree were counted immediately after harvest and the average fruit weight was derived mathematically from the total weight and total number of fruit per tree. Fruit sizes were measured by using venire caliper from twenty representative fruits per tree at harvesting time.

2.3.2 Peel color characteristics

Fruit quality parameter was determined from twenty representative fruits per tree. After harvest, all fruits were stored to promote postharvest ripening at room temperature for 5 days. For peel brightness and the colors of fruit pericarps on both sides of twenty fruit samples were measured twice with color difference meter (model CR-400, Konica Minolta, Osaka, Japan) using the Hunter *L* (brightness), *a* (redness) and *b* (yellowness) system (Hunterlab, 2008).

2.3.3 Total soluble solids (TSS) and titratable acidity (TA)

Total soluble solids (TSS) and titratable acidity (TA) were analyzed using twenty fruits. TSS content in juice was determined by using a refractometer and expressed as °Brix at 20°C, while TA expressed as milliequivalent and measured by titration with a 0.1 N NaOH solution up to pH 8.1 endpoint, was reported as citric acid content (Appendix A3).

2.3.4 Severity of anthracnose and fruit rot

Visual observations of anthracnose and fruit rot symptoms on fruit surface were carried out before and after ripening induction. The levels of disease severity were numerically expressed as 0, 1, 2, 3 and 4 for, respectively, no disease spot (healthy fruit), 1-25%, 26-50%, 51-75% and 76-100% disease area.

RESULTS AND DISCUSSION

Results

Experiment 1 Selection of the most appropriate particle film for coating mango leaves

1.1 Suspendability and precipitability

Table 1 shows the average precipitation times of suspended particles of kaolin, bentonite, calcium carbonate and dolomite. The kaolin particles exhibited the longest precipitation times of 75.66 min followed by bentonite, calcium carbonate and dolomite particles with 48.33, 27.66 and 25.66 min, respectively.

Table 1 Average times of precipitation of suspended particles of kaolin, bentonite, calcium carbonate and dolomite.

Coating materials	Time of precipitation (min) [1/]
Kaolin	75.66 ± 2.33^{a} [2/]
Bentonite	48.33 ± 1.66^{b}
Calcium carbonate	27.66 ± 1.45^{c}
Dolomite	25.66 ± 0.66^{c}

[1/] Mean value of 4 replications (±S.E.)

[2/] Means followed by the same lowercase superscript letter within a column are not significantly different by Duncan's multiple range test at the $P \leq 0.05$ level.

1.2 Transmission of particle film coated

The results on transmission of photons through a suspended material coating on a glass plate (Figure 2 and Table 2) indicated that kaolin was the most effective coating material based on its lowest PPF values at 70.14%. The PPF values of light obtained from bentonite, calcium carbonate and dolomite were found to be higher at 93.50%**,** 92.28% and 86.78%, respectively**.**

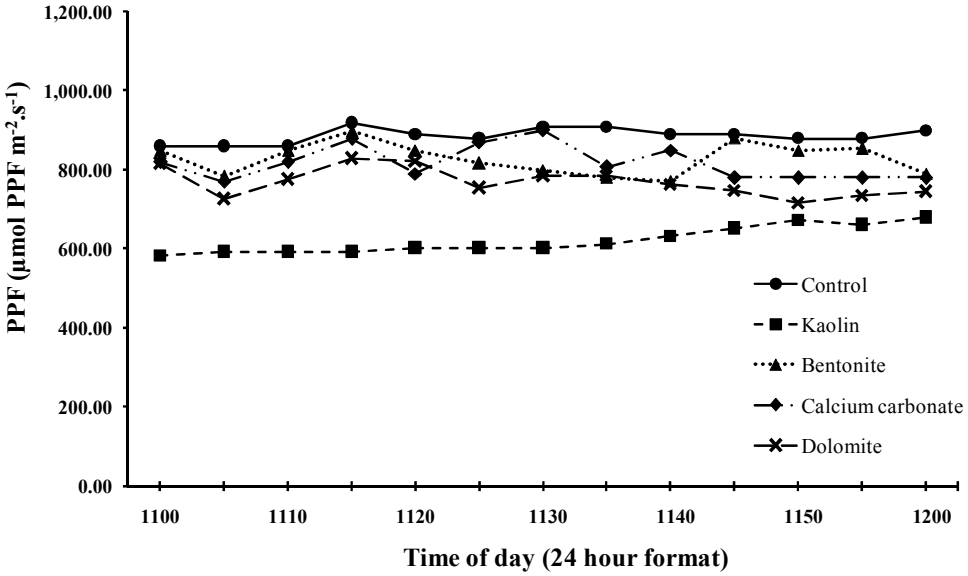

Figure 2 Photosynthetic photon flux (PPF) of light passing through glass coated by different coating materials.

Table 2 Average percentage of photosynthetic photon flux (PPF) through a glass plate coated with different materials.

Coating materials	PPF Transmittance (%)
Kaolin	70.14
Bentonite	93.50
Calcium carbonate	92.28
Dolomite	86.78

1.3 Leaf coating ability

The microclimate during the period of measurement was characterized as hot and dry**.** The average midday PPF was 1,462.67 µmol PPF $m^{-2}.s^{-1}$. The average T_{air} reached 41.49 °C, while the average RH was 20.78%. The VPD_{air} value was 6.34 kPa (Table 3).

The A values of the kaolin-sprayed leaves were greater than for the bentonite and untreated leaves throughout the measurement interval (Figure 3). The average A value in kaolin-sprayed leaves was significantly higher than in other treatments. Moreover, g_s and E values were similarly higher in kaolin-sprayed leaves than in bentonite and untreated leaves. The values of C_i and C_i:C_a in kaolin-treated leaves were higher than those in untreated leaves, but were lower than those of the bentonite treatment. Mango leaves treated with kaolin had lower average T_{leaf} and $VPD_{leaf\text{-}air}$ values than in the bentonite and untreated leaves which had average T_{leaf} and $VPD_{leaf\text{-}air}$ values that were not significantly different (Table 4).

On the contrary, the A values for the bentonite-sprayed leaves generally were lower than in the koalin and untreated leaves throughout the measurement interval (Figure 3). Furthermore, the A values in the bentonite-sprayed leaves were significantly the lowest (Table 4). In addition, the values of g_s and E in bentonite were significantly lower than those in the kaolin and untreated leaves. Nevertheless, the values of C_i and C_i:C_a in the bentonite treatment were significantly higher than those of the kaolin treatments (Table 4).

Table 3 Microclimatic parameters measured from 11:30 to 13:30 hours.

Parameters	Average[1/]
PPF (µmol PPF $m^{-2}.s^{-1}$)	1462.67 ± 25.67
T_{air} (°C)	41.49 ± 0.32
RH (%)	20.78 ± 0.47
VPD_{air} (kPa)	6.34 ± 0.14

[1/] Mean value of 4 replications (±S.E.)

PPF = Photosynthetic photon flux, T_{air} = Air temperature, RH = Relative humidity, VPD_{air} = The air vapor pressure deficit.

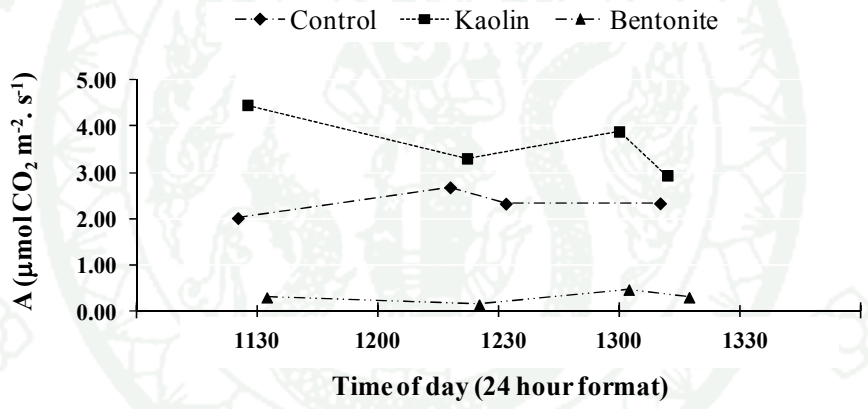

Figure 3 Net rate of photosynthesis (A) around midday for different leaf coatings.

Table 4 Physiological variables related to the photosynthesis of uncoated and coated mango leaves.

Treatment	Physiological variables*							
	T_{leaf} (°C)	$VPD_{leaf-air}$ (kPa)	A (µmol CO_2 $m^{-2}.s^{-1}$)	g_s (mmol H_2O $m^{-2}.s^{-1}$)	E (mmol H_2O $m^{-2}.s^{-1}$)	C_i (µmolCO_2 mol^{-1})	C_a (µmolCO_2 mol^{-1})	C_i:C_a
Control	41.51±0.41	6.04±0.16	2.34±1.34b	45.07±4.16b	2.81±0.32b	261.00±5.64c	382.90±0.46	0.68±0.01c[1/]
Kaolin	41.46±0.63	5.73±0.29	3.65±0.33a	91.97±7.75a	5.25±0.13a	281.75±2.65b	385.12±1.34	0.73±0.01b
Bentonite	41.48±0.74	6.21±0.31	0.31±0.06c	21.75±1.94c	1.40±0.13c	325.75±3.25a	385.99±2.43	0.84±0.01a

T_{leaf} = Leaf temperature, $VPD_{leaf-air}$ = Leaf to air vapor pressure deficit, A = net photosynthesis, g_s = Stomatal conductance, E = Transpiration rate, C_i = Intercellular CO_2 concentration, C_a = Air CO_2 concentration, C_i:C_a = Ratio of intercellular to air CO_2 concentration.

* = Mean value of 12 replications (±S.E.)

Means followed by the same lowercase superscript letter within a column are not significantly different by Duncan's multiple range test at the $P \leq 0.05$ level.

1.4 Light response curves of A

The light response curve of mango leaves exhibited a saturation phenomenon with the relationship between A and PPF. The A value increased rapidly as PPF increased to 200 µmol PPF $m^{-2}.s^{-1}$ and then increased slowly to the maximum, followed by a slow decrease as PPF increased to 2,000 µmol PPF $m^{-2}.s^{-1}$ (Figure 4). The value of A was found to be saturated at around 600 µmol PPF $m^{-2}.s^{-1}$. The maximum rate of photosynthesis (A_{max}) was determined at 3.5 µmol CO_2 $m^{-2}.s^{-1}$ (Figure 4). However, when the data were fitted to a non-rectangular hyperbola function (Thornley and Johnson, 1990), the light saturation point was calculated as 232.93. µmol PPF $m^{-2}.s^{-1}$.

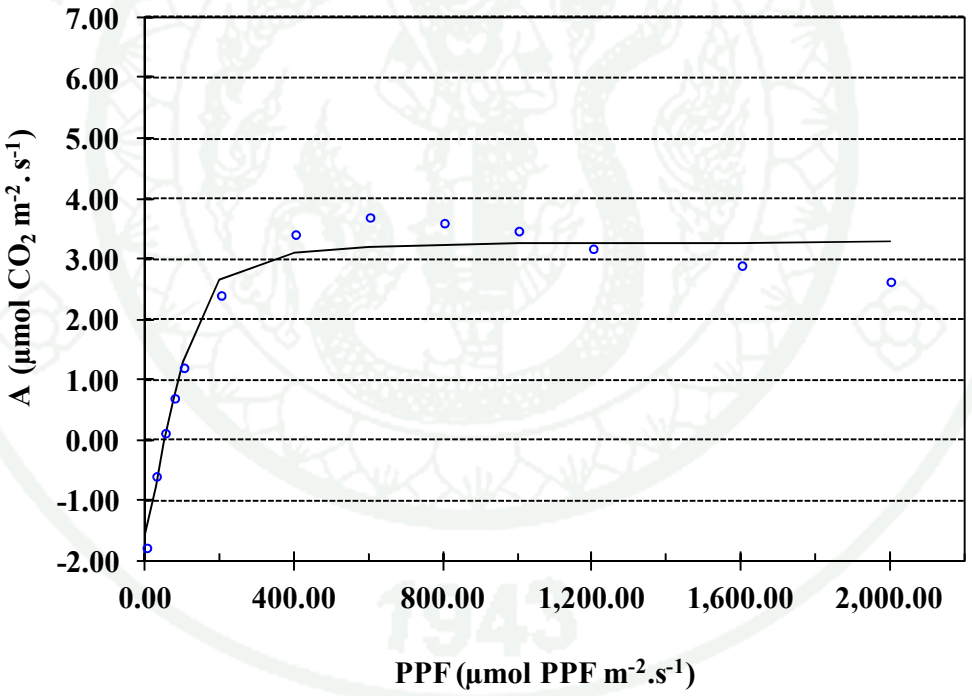

Figure 4 Net rate of photosynthesis (A) versus photosynthetic photon flux (PPF) light response curve of mango leaves measured in CO_2 (400 µmol.mol^{-1}) in an atmospheric chamber at leaf temperature of 37 °C.

Experiment 2 Effects of kaolin on photosynthesis, carbohydrate content, fruit yield and fruit quality.

Microclimates

Microclimates data were logged by quantum sensor with connected data-logger. The report was divided into two periods from April to July 2010 and November 2010 to January 2011.

The weather was generally warm and humid from April to July 2010. The value of PPF varied from 400-1,300 µmol PPF $m^{-2}.s^{-1}$. The average PPF was 1,052.70 µmol PPF $m^{-2}.s^{-1}$. Relative humidity (RH) ranged from 55-90% during the day and 70-100% at night. The average RH was 65.93%. The average of temperature (T_{air}) was 33.17°C, while the max and min mean of T_{air} were 41.72°C and 23.94°C, respectively. The maximum of VPD_{air} value was 4.89 kPa. However, the average VPD_{air} for this time was 1.92 kPa (Figure 5 and Table 5).

During November 2010 to January 2011, the weather was usually cooler and damper than in April to July 2010. The average PPF was 790.10 µmol PPF $m^{-2}.s^{-1}$. The values of RH were between 55-90% during the day and 60 -100% at night. The average RH was 68.49%. T_{air} increased along with the increase of PPF, while the average T_{air} for this time was 30.25°C. VPD_{air} in November 2010 to January 2011 were mostly lower than VPD_{air} in April to July 2010. The average value of VPD_{air} was 1.42 kPa (Figure 6 and Table 5).

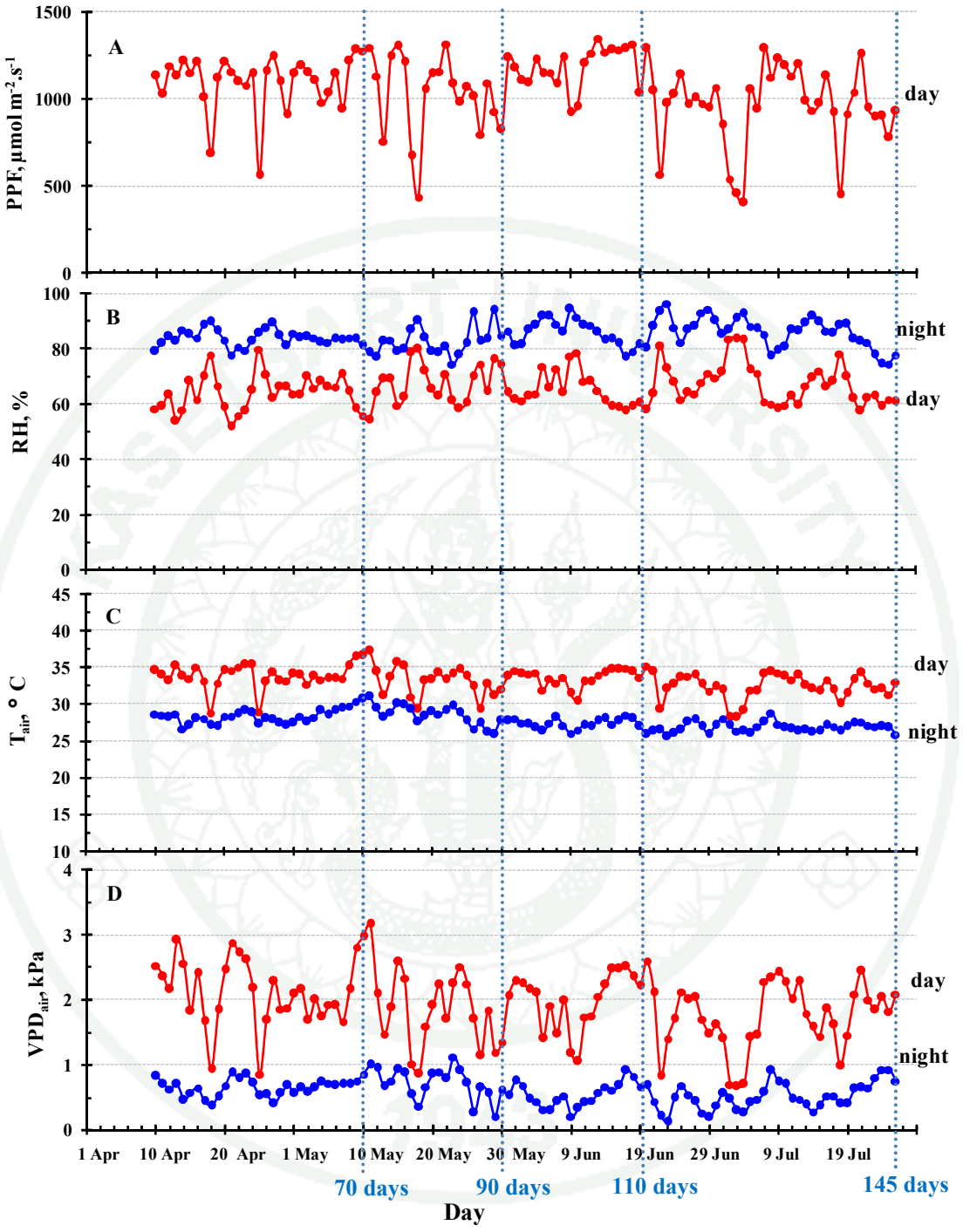

Figure 5 Changes in microclimate of average photosynthetic photon flux (PPF) (A), relative humidity (RH) (B), air temperature (T_{air}) (C) and air vapour pressure deficit (VPD_{air}) (D) measured from April to July 2010. Microclimatic data at the leaves age of 70, 90, 110 and 145 days highlighted in blue.

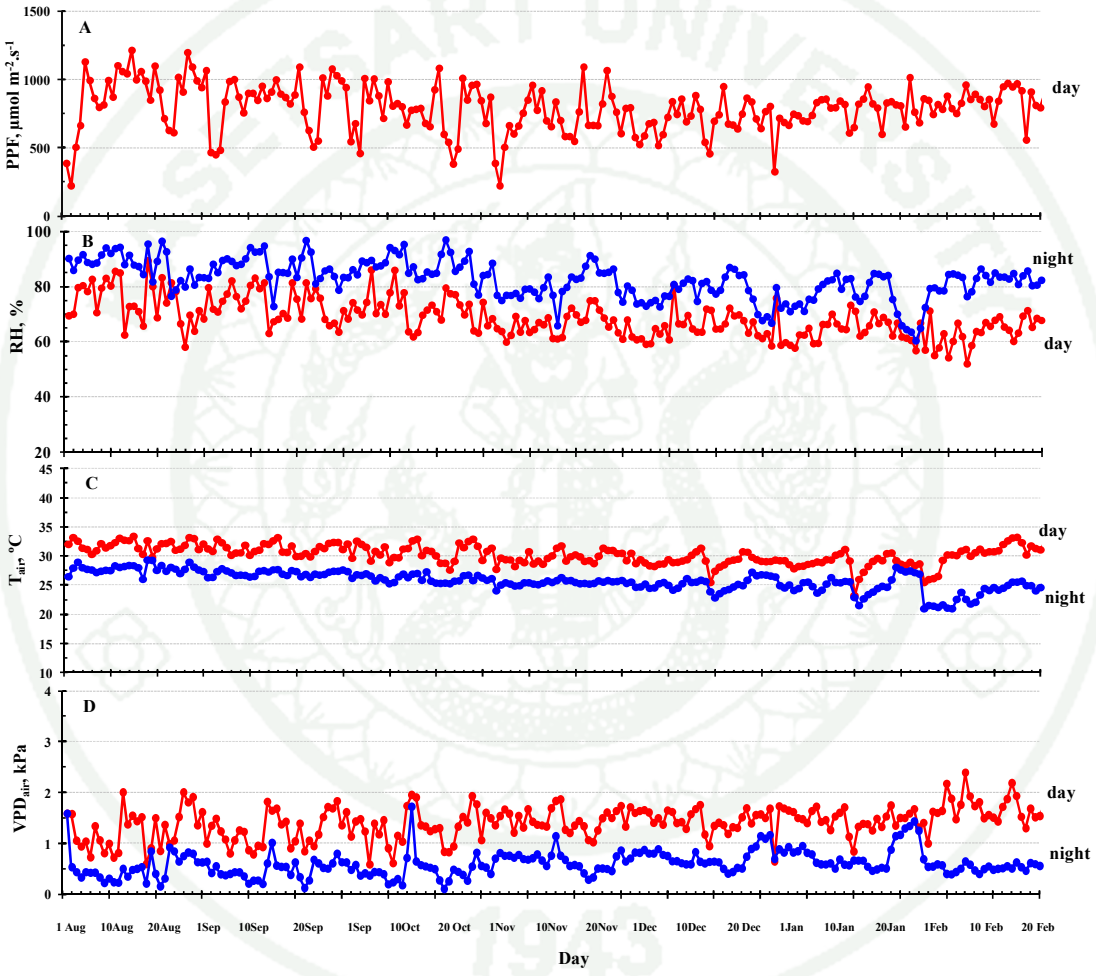

Figure 6 Changes in microclimate of average photosynthetic photon flux (PPF) (A), relative humidity (RH) (B), air temperature (T_{air}) (C) and air vapour pressure deficit (VPD_{air}) (D) measured from November 2010 to January 2011.

Table 5 Microclimatic parameters measured from April to July 2010 and November 2010 to January 2011.

Microclimate	April – July 2010 (Summer)	November 2010 – January 2011 (Winter)
Average PPF, µmolPPF$m^{-2}.s^{-1}$	1052.70	790.10
PPF max, µmolPPF$m^{-2}.s^{-1}$	2456.00	2446.00
PPF min, µmolPPF$m^{-2}.s^{-1}$	0.00	0.00
Average RH_{air}, %	65.93	68.49
RH_{air} max, %	100.00	99.40
RH_{air} min, %	34.60	30.50
Average T_{air}, ºC	33.17	30.25
T_{air} max, ºC	41.72	38.25
T_{air} min, ºC	23.94	19.94
Average VPD_{air}, kPa	1.92	1.42
VPD_{air} max, kPa	4.89	3.92
VPD_{air} min, kPa	0.00	0.02

PPF = Photosynthetic photon flux, RH = Relative humidity, T_{air} = Air temperature, VPD_{air} = The air vapor pressure deficit.

Microclimatic conditions in the day measured gas exchange

The microclimate during the day measured gas exchange was different depending on the weather at that time. At 70 days of leaf ages, the average value of PPF 2214.37 μmol PPF $m^{-2}s^{-1}$ in midday. While, the average value of PPF for 90, 110 and 145 days of leaf ages were 1732.00, 1829.44 and 1394.69 μmol PPF $m^{-2}s^{-1}$, respectively (Table 6). Furthermore, RH was high in the morning and afternoon. It showed a dramatical decrease after 07:00 hours and a rapid increase at 18:00 hours (Figure 7). At midday of 70 day leaf age, the average RH showed the lowest for 46.44% while T_{air} had the highest average value for 39.83°C (Figure 7). During morning time, VPD_{air} was 1.00 kPa at 08:00 hours then increased sharply at midday and dramatically decreased after 16:00 hours. However, at 70 and 110 days of leaf ages showed VPD_{air} was greater than 2.5 kPa. The VPD_{air} value was 3.93 kPa for 70 days of leaf ages as the highest average value. While, the average values of VPD_{air} for 110 days of leaf ages was 3.24 kPa.

Thus, the microclimate of this study showed that at 70 and 110 days of leaf ages indicated more severe weather than that of 90 and 145 days of leaf ages. Subsequently, based on these climatic conditions, these days can be described generally as a sunny day. For 90 and 145 days of leaf ages, the climatic at these days were considered to be consistent with a cloudy day.

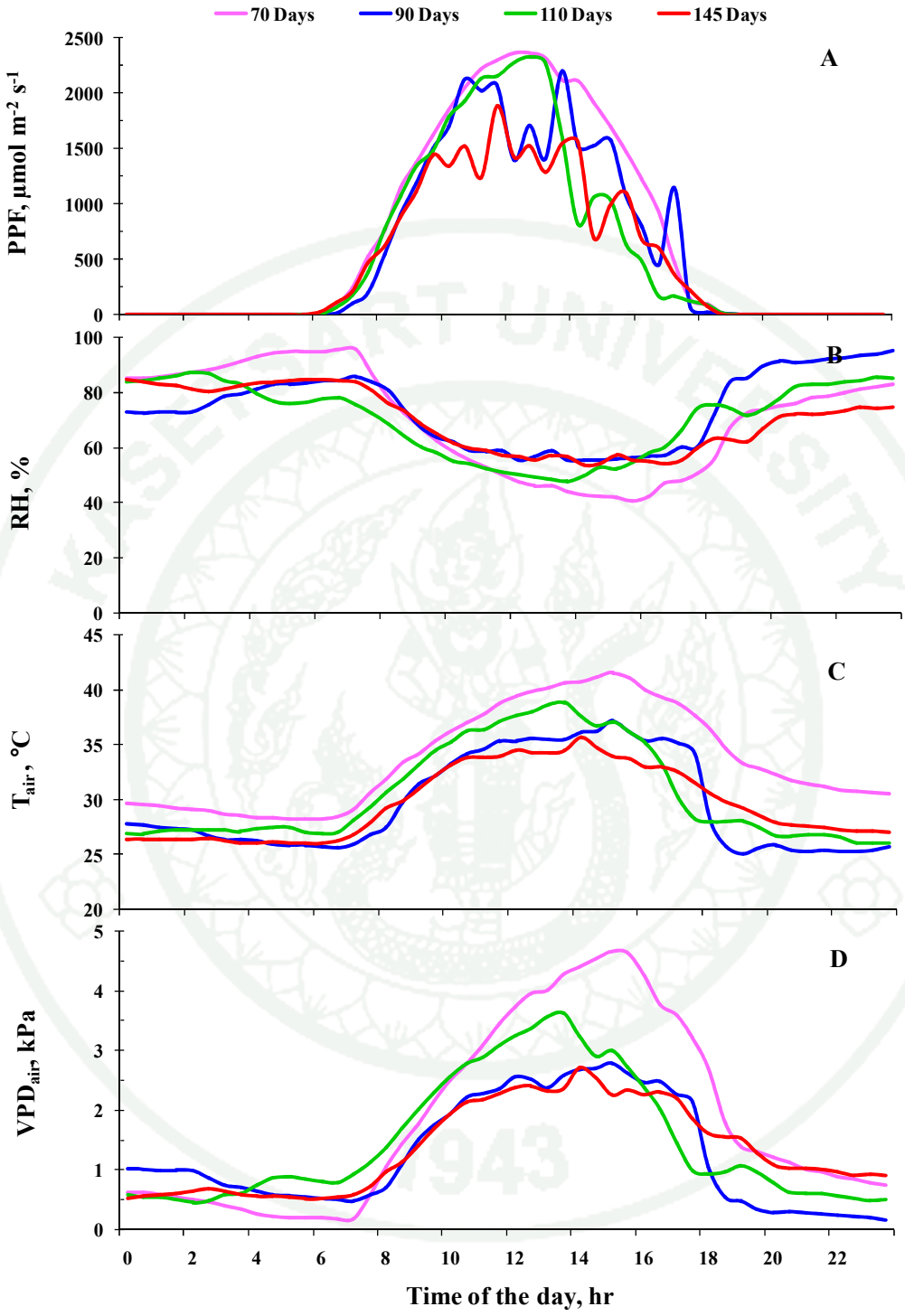

Figure 7 Diurnal changes of photosynthetic photon flux, PPF (A); relative humidity, RH (B); air temperature, T_{air} (C); and air vapor pressure deficit, VPD_{air} (D) were measured at 70, 90, 110 and 145 days of leaf ages.

Table 6 Environmental condition during the experimental period, considering the variation of microclimate in midday (11:00 to 14:00 hours).

Microclimate*	8 May 2010 (70 days of leaf ages)	29 May 2010 (90 days of leaf ages)	20 June 2010 (110 days of leaf ages)	26 July 2010 (145 days of leaf ages)
PPF, $\mu molPPFm^{-2}.s^{-1}$	2214.37±55.09	1732.00±113.65	1829.44±211.60	1394.68±122.01
RH_{air}, %	46.44±1.28	56.75±0.59	50.21±0.66	56.17±0.60
T_{air}, ºC	39.83±0.40	35.51±0.18	37.63±0.31	34.47±0.19
VPD_{air}, kPa	3.93±0.17	2.50±0.05	3.24±0.09	2.40±0.57

* Mean value of 8 replications (±S.E.)

PPF = Photosynthetic photon flux, RH = Relative humidity, T_{air} = Air temperature, VPD_{air} = The air vapor pressure deficit.

2.1 Effects of kaolin on photosynthesis

2.1.1 Diurnal changes of gas exchange

Gas exchange measurements in the first day (at 70 days of leaf ages) showed that PPF was 2214.38 μmol PPF $m^{-2}.s^{-1}$ while VPD_{air} was greater than 2.5 kPa (Figure 7, Table 6). Consequently, mango leaves were exposed to the weather as high PPF and temperature throughout the day. The A values in all treatments decreased continuously during 08:00 to 16:00 hours. However, the average A value in kaolin-sprayed leaves twice a week was not significantly higher than in other treatments especially during 11:00 to 14:00 hours (Figure 8E and Table 7). Similarly, mango leaves treated with kaolin had higher average g_s values than in untreated leaves at midday (11:00 to 14:00 hours) which had average g_s values that were not significantly different (Figure 8I and Table 7). Diurnal variation of T_{leaf} and $VPD_{leaf-air}$ in all treatments increased and reached the maximum value at midday. Then, these values were gradually decreased until 16:00 hours. Mango leaves treated with kaolin once and twice a week had lower average T_{leaf} values than in untreated leaves which had average T_{leaf} values that were not significantly different. The average of T_{leaf} during 11:00 to 14:00 hours for control leaves was 41.05 ºC, while those for kaolin-sprayed leaves once and twice a week were 40.68 and 41.61 ºC, respectively. Furthermore, the average of $VPD_{leaf-air}$ for control leaves, kaolin-sprayed leaves once and twice a week were 4.59, 4.41 and 4.80 kPa, respectively. These values were not significantly different (Figure 8M, 8Q and Table 7). The C_i values in all treatments generally decreased during morning hours but increased in the afternoon with lower values in kaolin sprayed leaves. In addition, the values of C_i in kaolin-sprayed leaves twice a week (255.06±8.83 μmol CO_2 mol^{-1}) was significantly lower than those in other treatments (295.88±13.83 μmol CO_2 mol^{-1} for control and 290.31±10.69 μmol CO_2 mol^{-1} for kaolin-sprayed leaves once a week) during 11:00 to 14:00 hours (Figure 8 U and Table 7). Diurnal variation of E in all treatments decreased in the morning and reached the maximum value during 12:00 to 13:00 hours, while E values in control leaves were throughout the day generally lower. The values of E for control leaves (1.32±0.16 mmol H_2O $m^{-2}.s^{-1}$) had lower average E values than in kaolin

treatment (1.64±0.13 and 1.66±0.22 mmol H_2O $m^{-2}.s^{-1}$, respectively) which had average E values that were not significantly different (Figure 8V and Table 7).

On the second day of gas exchange measurements (at 90 days of leaf ages), the PPF was 1732.00 µmol PPF m^{-2} $s^{-1}$and VPD_{air} was 2.5 kPa (Figure 7, Table 6). This indicated that mango leaves were not exposed to severe weather with a high PPF and VPD_{air} at midday. The A values dropped rapidly after 08:00 hours and gradually increased after 12:00 to 16:00 hours. The values of A in non sprayed trees were higher than that in kaolin sprayed trees during 09:00 to 11:00 hours. However, the averages of A in all treatments were not significantly different at midday (Figure 8F and Table 8). The g_s value in all treatments showed diurnal change in the same tends with A (Figure 8J and Table 8). T_{leaf} and $VPD_{leaf-air}$ in these three treatments increased along with the increase of PPF and reached the maximum value at 12:00 hours. After 13:00 hours, the values of T_{leaf} and $VPD_{leaf-air}$ dropped and fairly constant (T_{leaf} = 32-33 ºC, $VPD_{leaf-air}$= 1.75-2.22 kPa) until 16:00 hours. However, the averages of T_{leaf} and $VPD_{leaf-air}$ in all treatments were not significantly different at midday. In addition, the average values of T_{leaf} and $VPD_{leaf-air}$ in the second days of measurements were lower than those in first measurements (Figure 8N, 8R and Table 8). C_i values showed the similar pattern in all treatments with decrease in the morning hour during 08:00 to 10:00 hours. Then, these values increased slowly after 10:00 hours and fairly constant until 16:00 hours. The C_i values in all treatments were not significantly different (Figure 8R and Table 8). The values of E in all treatments decreased during 08:00 to10:00 hours, then fairly constant throughout the day (Figure 8V and Table 8).

The measurements of gas exchange on the third days (at 110 days of leaf ages) showed that the average of PPF on the leaf surface reached 1829.44 µmol PPF $m^{-2}s^{-1}$ and VPD_{air} was greater than 2.5 kPa during 11:00 to 14:00 hours (Figure 7, Table 6).Thus, mango leaves exposed to the weather as high PPF and temperature throughout the day. However, the weather was less severe than the first measurement. Higher value of A was observed in control leaves during 08:00 to 9:00 hours, however it was decreased after 10:00 hours. A values showed the similar pattern in

these three treatments with the values less than 1 μmol CO_2 m^{-2} s^{-1} during 10:00 to 16:00 hours (Figure 8G and Table 9). The g_s values showed diurnal change in the same tends with g_s in the first measurements. However, the average of g_s in both kaolin-sprayed leaves (31.28±4.20 and 30.73±2.82 mmol H_2O m^{-2} $.s^{-1}$, respectively) was not significantly higher than in unsprayed control (21.48±3.01 mmol H_2O m^{-2} $.s^{-1}$) especially during 11:00 to 14:00 hours (Figure 8K and Table 9). The average T_{leaf} and $VPD_{leaf-air}$ values in all treatments were not significantly different at midday (T_{leaf} = 35.73-35.81 ºC, $VPD_{leaf-air}$= 2.60-2.61 kPa) (Figure 8O, 8S and Table 9). The C_i in all treatments generally decreased during 08:00 to 09:00 hours, then slightly increased and fairly constant between 350-380 μmol CO_2 mol^{-1} throughout the day (Figure 8W and Table 9). Diurnal variation of E values in all treatments decreased during morning hours, then fairly constant throughout the day. In addition, the averages of E in all treatments were not significantly different at midday (Figure 8AA and Table 9).

In the fourth day of gas exchange measurements (at 145 days of leaf ages), the average of PPF was 1394.69 μmol PPF $m^{-2}s^{-1}$ and VPD_{air} was less than 2.5 kPa (Figure 7, Table 6). Thus, mango leaves were not exposed to the weather with a high PPF and VPD_{air} at midday. Mango leaves treated with kaolin twice a week had higher average A values than in other treatments throughout the day. At 11:00 hours, the values of A in untreated leaves dropped rapidly as A values decreased to 1 μmol CO_2 m^{-2} s^{-1}. Then, A values increased after 12:00 hours and fairly constant until 16:00 hours. At midday, the average A value in kaolin-sprayed leaves twice a week (3.97±0.48 μmol CO_2 m^{-2} s^{-1}) was not significantly higher than those in untreated leaves (2.65±0.54 μmol CO_2 m^{-2} s^{-1}); but was significantly higher than those in kaolin-sprayed leaves once a week (2.03±0.37 μmol CO_2 m^{-2} s^{-1}) (Figure 8H and Table 10). Similarly, mango leaves treated with kaolin twice a week had higher average g_s values than in other treatments throughout the day. Furthermore, g_s value in kaolin-sprayed leaves twice a week (51.60±4.11 mmol H_2O m^{-2} $.s^{-1}$) was significantly higher than those in the kaolin once a week (30.81± 2.40 mmol H_2O m^{-2} $.s^{-1}$) and untreated leaves (33.35± 4.62 mmol H_2O m^{-2} $.s^{-1}$) at midday. (Figure 8L and Table 10). The T_{leaf} values of three treatments slightly increased in the morning

from 08:00 to 12:00 hours and was 33–34 °C from around midday until 16:00 hours (Figure 8P and Table 10). The $VPD_{leaf-air}$ values in all treatments increased and reached the maximum value at 11:00 hours. Then, these values were gradually decreased and constant until 16:00 hours (Figure 8T and Table 10). The C_i value in kaolin-sprayed leaves once a week was higher than those in other treatments from 09:00 until 13:00 hours. At 11:00 hours, the C_i value of the control leaves increased and maximum which close to the other treatments. However, the C_i values in all treatments were not significantly different during midday (Figure 8X and Table 10). The values of E in mango leaves treated with kaolin twice a week had higher average E values than in other treatments throughout the day. Moreover, E value in kaolin-sprayed leaves twice a week (1.05±0.08 mmol H_2O $m^{-2}.s^{-1}$) was significantly higher than those in those in the kaolin once a week (0.67±0.06 mmol H_2O $m^{-2}.s^{-1}$) and untreated leaves (0.70±0.08 mmol H_2O $m^{-2}.s^{-1}$) (Figure 8BB and Table 10).

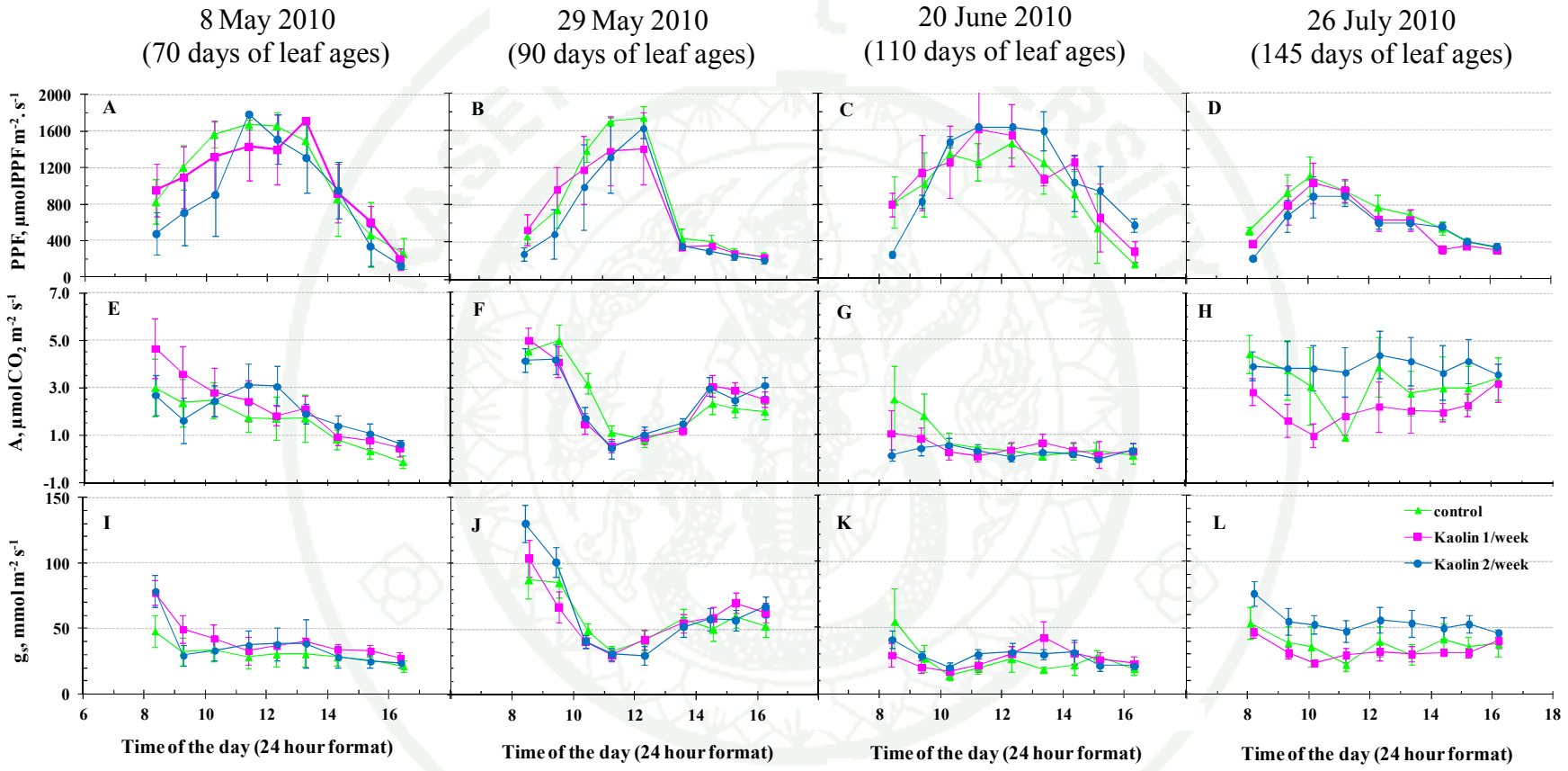

Figure 8 Diurnal changes of average photosynthetic photon flux (PPF) (µmol PPF $m^{-2}.s^{-1}$) (A-D), net photosynthesis rate (A) (E-H), stomatal conductance (g_s) (I-L), leaf temperature (T_{leaf}) (M-P) leaf-air vapour pressure difference ($VPD_{leaf-air}$) (Q-T), intercellular CO_2 concentration (C_i) (U-X), and transpiration rate (E) (Y-BB) measured of kaolin-treated and untreated trees at 70, 90, 110 and 145 days of leaf ages. Each symbol represents the mean value of the four replications (± SE).

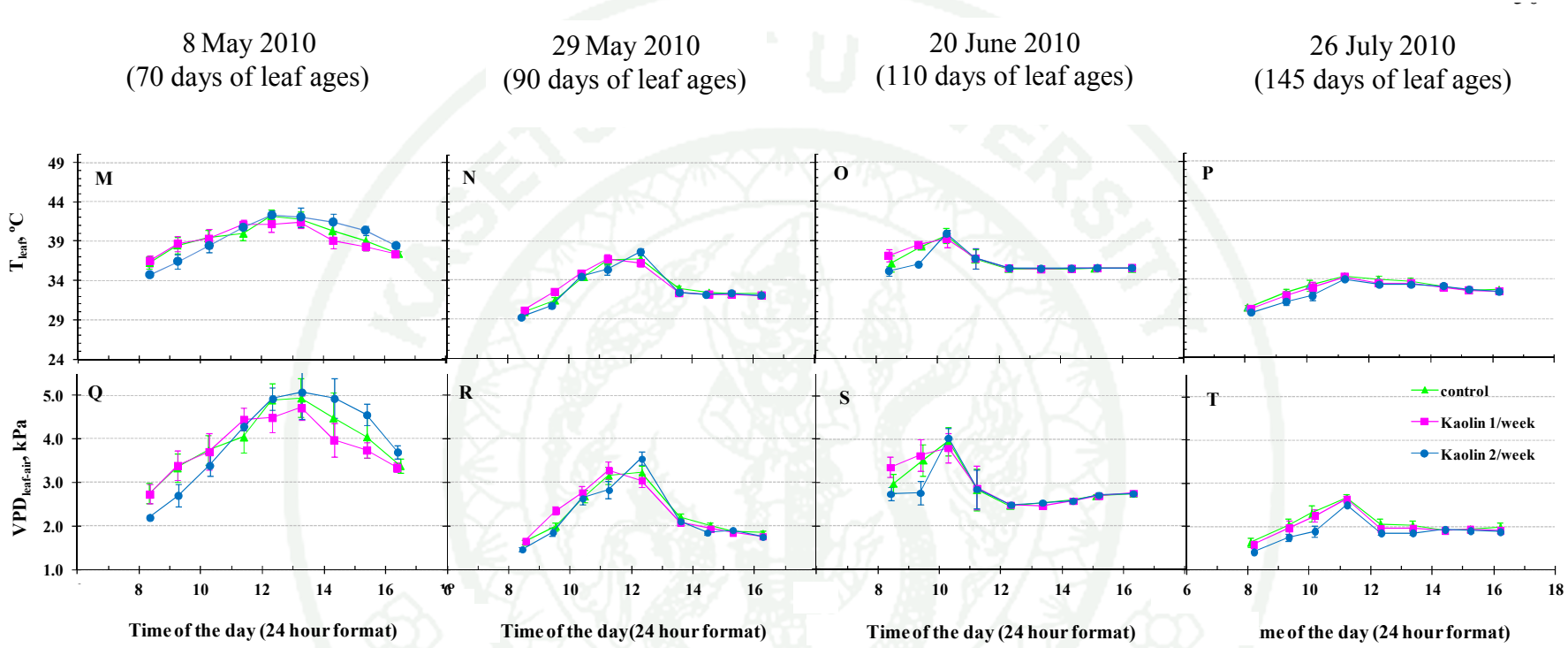

Figure 8 (Continued)

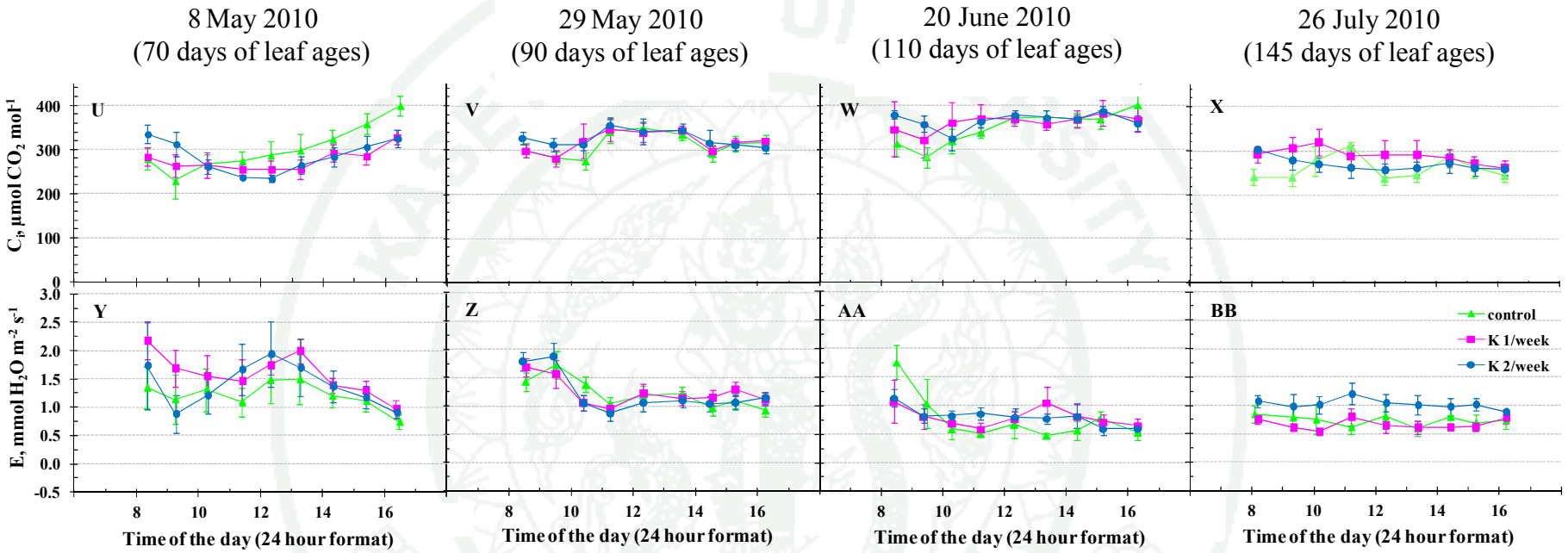

Figure 8 (Continued)

Table 7 Physiological variation related to the photosynthesis after spraying kaolin onto mango leaves at 70 days of leaf ages during midday (11:00 to 14:00 hours).

Treatment	Physiological variables*						
	PPF (μmolPPF $m^{-2}.s^{-1}$)	A (μmol CO_2 $m^{-2}.s^{-1}$)	g_s (mmol $H_2Om^{-2}.s^{-1}$)	T_{leaf} (°C)	$VPD_{leaf-air}$ (kPa)	C_i (μmolCO_2 mol^{-1})	E (mmol H_2O $m^{-2}.s^{-1}$)
Control	1419.31±135.98	1.49±0.36	29.33±3.92	41.05±0.53	4.59±0.22	295.88±13.83a[1/]	1.32±0.16
K 1/week	1364.75±157.36	1.82±0.30	36.04±2.75	40.68±0.45	4.41±0.16	290.31±10.69a	1.64±0.13
K 2/week	1388.94±148.05	2.36±0.36	35.66±5.94	41.61±0.42	4.80±0.19	255.06±8.83b	1.66±0.22

* Mean value of 16 replications (±S.E.)

[1/] Means followed by the same lowercase superscript letter within a column are not significantly different by Duncan's multiple range test at the $P \leq 0.05$ level.

PPF = photosynthetic photon flux, A = Net photosynthesis, g_s = Stomatal conductance, T_{leaf} = Leaf temperature, $VPD_{leaf-air}$ = Leaf to air vapor pressure deficit, C_i = Intercellular CO_2 concentration, E = Transpiration rate

Table 8 Physiological variation related to the photosynthesis after spraying kaolin onto mango leaves at 90 days of leaf ages during midday (11:00 to 14:00 hours).

Treatment	Physiological variables*						
	PPF (μmolPPF $m^{-2}.s^{-1}$)	A (μmol CO_2 $m^{-2}.s^{-1}$)	g_s (mmol $H_2Om^{-2}.s^{-1}$)	T_{leaf} (°C)	$VPD_{leaf-air}$ (kPa)	C_i (μmolCO_2 mol^{-1})	E (mmol H_2O $m^{-2}.s^{-1}$)
Control	1071.88±172.54	1.41±0.24	45.46±8.47	34.65±0.58	2.65±0.17	329.56±10.02	1.09±0.16
K 1/week	873.63±180.18	1.44±0.32	46.35±4.75	34.37±0.65	2.58±0.19	332.13±9.83	1.11±0.65
K 2/week	900.94±174.56	1.50±0.45	42.65±5.97	34.39±0.65	2.58±0.20	340.25±11.22	1.02±0.99

* Mean value of 16 replications (±S.E.)

1/ Means followed by the same lowercase superscript letter within a column are not significantly different by Duncan's multiple range test at the $P \leq 0.05$ level.

PPF = photosynthetic photon flux, A = Net photosynthesis, g_s = Stomatal conductance, T_{leaf} = Leaf temperature, $VPD_{leaf-air}$ = Leaf to air vapor pressure deficit, C_i = Intercellular CO_2 concentration, E = Transpiration rate

Table 9 Physiological variation related to the photosynthesis after spraying kaolin onto mango leaves at 110 days of leaf ages during midday (11:00 to 14:00 hours).

Treatment	Physiological variables*						
	PPF (μmolPPF $m^{-2}.s^{-1}$)	A (μmol CO_2 $m^{-2}.s^{-1}$)	g_s (mmol H_2O $m^{-2}.s^{-1}$)	T_{leaf} (°C)	$VPD_{leaf-air}$ (kPa)	C_i (μmolCO_2 mol^{-1})	E (mmol H_2O $m^{-2}.s^{-1}$)
Control	1193.81±142.34	0.29±0.12	21.48±3.01	35.73±0.31	2.61±0.11	363.19±7.23	0.56±0.07 b[1/]
K 1/week	1434.75±123.85	0.38±0.14	31.28±4.20	35.78±0.33	2.60±0.12	366.88±9.53	0.81±0.09 a
K 2/week	1484.19±119.46	0.22±0.11	30.73±2.82	35.81±0.32	2.61±0.11	371.00±6.05	0.82±0.07 a

* Mean value of 16 replications (±S.E.)

[1/] Means followed by the same lowercase superscript letter within a column are not significantly different by Duncan's multiple range test at the $P \leq 0.05$ level.

PPF = photosynthetic photon flux, A = Net photosynthesis, g_s = Stomatal conductance, T_{leaf} = Leaf temperature, $VPD_{leaf-air}$ = Leaf to air vapor pressure deficit, C_i = Intercellular CO_2 concentration, E = Transpiration rate

Table 10 Physiological variation related to the photosynthesis after spraying kaolin onto mango leaves at 145 days of leaf ages during midday (11:00 to 14:00 hours).

Treatment	Physiological variables*						
	PPF (μmolPPF $m^{-2}.s^{-1}$)	A (μmol CO_2 $m^{-2}.s^{-1}$)	g_s (mmol $H_2Om^{-2}.s^{-1}$)	T_{leaf} (°C)	$VPD_{leaf-air}$ (kPa)	C_i (μmolCO_2 mol^{-1})	E (mmol H_2O $m^{-2}.s^{-1}$)
Control	738.13±63.85	2.65±0.54 ab[1/]	33.35±4.62 b	33.76±0.21	2.16±0.09	269.38±10.69	0.70±0.08 b
K 1/week	660.00±71.56	2.03±0.37 b	30.81±2.40 b	33.55±0.16	2.11±0.08	288.44±12.76	0.67±0.06 b
K 2/week	586.06±56.62	3.97±0.48 a	51.60±4.11 a	33.42±0.11	2.03±0.07	262.88±8.84	1.05±0.08 a

* Mean value of 16 replications (±S.E.)

[1/] Means followed by the same lowercase superscript letter within a column are not significantly different by Duncan's multiple range test at the $P \leq 0.05$ level.

PPF = photosynthetic photon flux, A = Net photosynthesis, g_s = Stomatal conductance, T_{leaf} = Leaf temperature, $VPD_{leaf-air}$ = Leaf to air vapor pressure deficit, C_i = Intercellular CO_2 concentration, E = Transpiration rate

2.1.2 CO_2 response (A/C_i) curves

There was no significant difference in $V_{c\ max}$, J_{max}, $J_{max}/V_{c\ max}$ and TPU parameters estimated by Sharkey *et al* (2007) among the treatment group (Figure 9 and Table 11).

Changing of the maximum rate of ribulose-1,5-bisphosphate (RuBP) carboxylation ($V_{c\ max}$) in leaves treated with kaolin once and twice a week were high at 70 days of leaf ages and gradually decreased when the leaves of mango reached to 90 until 145 days. However, the $V_{c\ max}$ of control kaolin untreated leaves has decreased from 70 to 90 days of leaf but has increased to be higher than the other treatments at 110 days of leaf ages (Figure 9A).

The maximum rate of electron transport driving RuBP regeneration (J_{max}) in leaves treated with kaolin twice a week gradually decreased from leaf ages of 70 to 145 days. Furthermore, the J_{max} in leaves untreated with kaolin had a pattern of changing similar to $V_{c\ max}$ (Figure 9B).

The balance between RuBP regeneration and carboxylation was studied by the evaluation of the $J_{max}/V_{c\ max}$ ratio of every treatment. Results of the study found that mango leaves on every treatment at every leaf age had the ratio value closed to 1. Mango leaves that received foliar application of kaolin twice a week had the highest J_{max}/V_{cmax} ratio of higher than 2.0 at 110 days leaf ages (Figure 9C).

There were no significant different in rate of triose phosphate use (TPU) in mango leaves after the spraying of kaolin at 70, 90, 110 and 145 days of leaf ages among the treatments. However, mango leaves treated with kaolin spraying twice a week at 70 and 90 days of leaf ages tended to have higher value of TPU than those of the control by 25.61 and 14.28 %, respectively (Table 11).

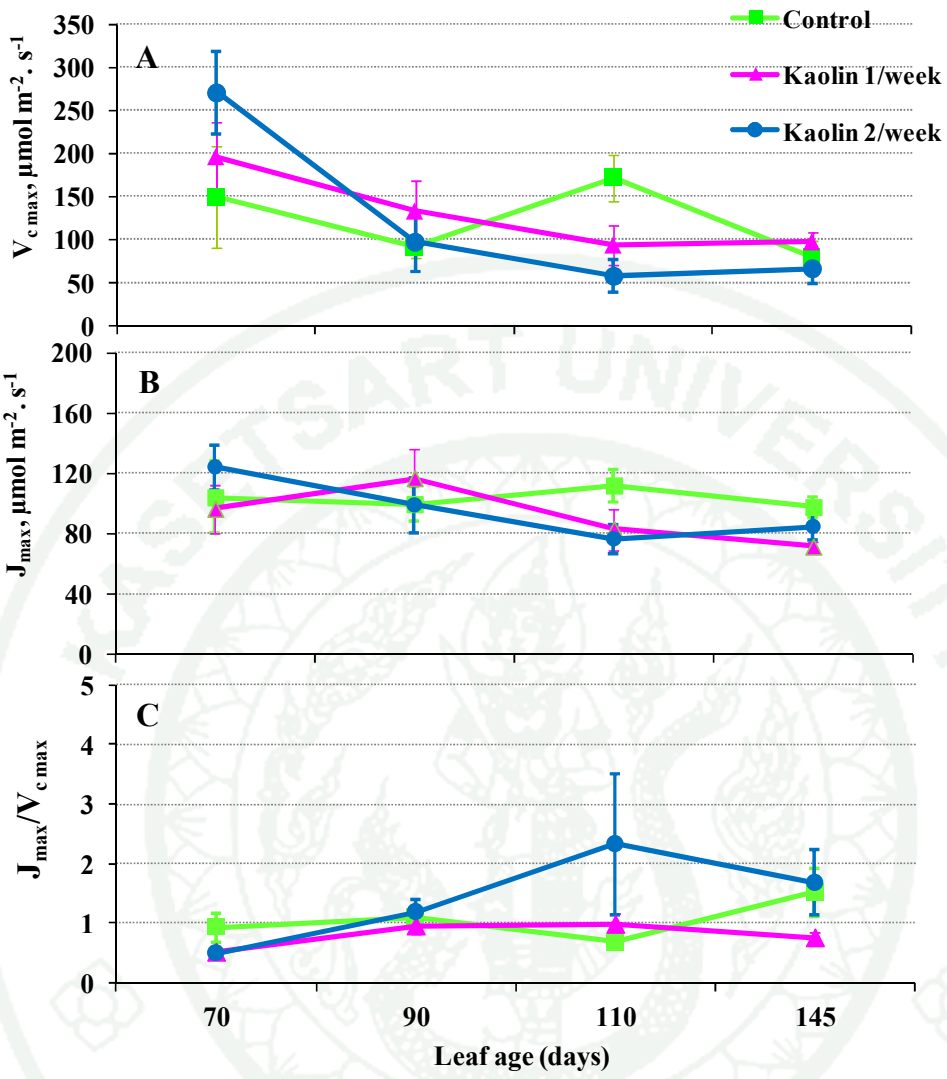

Figure 9 Changes in the maximum rate of ribulose-1,5-bisphosphate (RuBP) carboxylation ($V_{c\ max}$) in (A), the maximum rate of electron transport driving RuBP regeneration (J_{max}) in (B), and the relationship between J_{max} and $V_{c\ max}$ in (C) in mango leaves after spraying kaolin at 70, 90, 110 and 145 days of leaf ages. Each symbol represents the mean value of the four replications (± SE).

Table 11 Effect of kaolin on triose phosphate use (TPU) in mango leaves after spraying kaolin at 70, 90, 110 and 145 days of leaf ages

Treatment	Leaf age (Days)			
	70	90	110	145
Control	4.88±0.59	5.25±0.63	4.13±0.52	5.00±0.57
K1/week	5.13±4.12	5.88±0.83	3.00±0.71	3.25±0.14
K2/week	6.13±0.89	6.00±0.71	3.50±0.28	4.00±0.57

Mean value of 4 replications (±S.E.)

2.1.3 Measurement of Chlorophyll content

Change in chlorophyll a, b and total chlorophyll of three treatments in mango leaves after spraying kaolin at 70, 90, 110 and 145 days of leaf ages was shown in Figure 9. Chlorophyll a content was not significantly different among all treatments in all sampling dates (Figure 10A and Appendix Table B1).

Chlorophyll b in leaves treated with kaolin was not significantly different among untreated leaves at 70, 90 and 110 days of leaf ages. In addition, mango leaves without spraying kaolin tended to give less chlorophyll b values than in leaves treated with kaolin. At 145 days of leaf ages, untreated leaves had the lowest chlorophyll b values and was significantly different those with kaolin application (Figure 10B and Appendix Table B2).

Total chlorophyll content showed that there was no difference among three treatments of each sampling date. Nevertheless, non-kaolin-treated mango leaves tended to give less total chlorophyll content values than in leaves treated with kaolin at 90, 110 and 145 days of leaf ages (Figure 10C and Appendix Table B3).

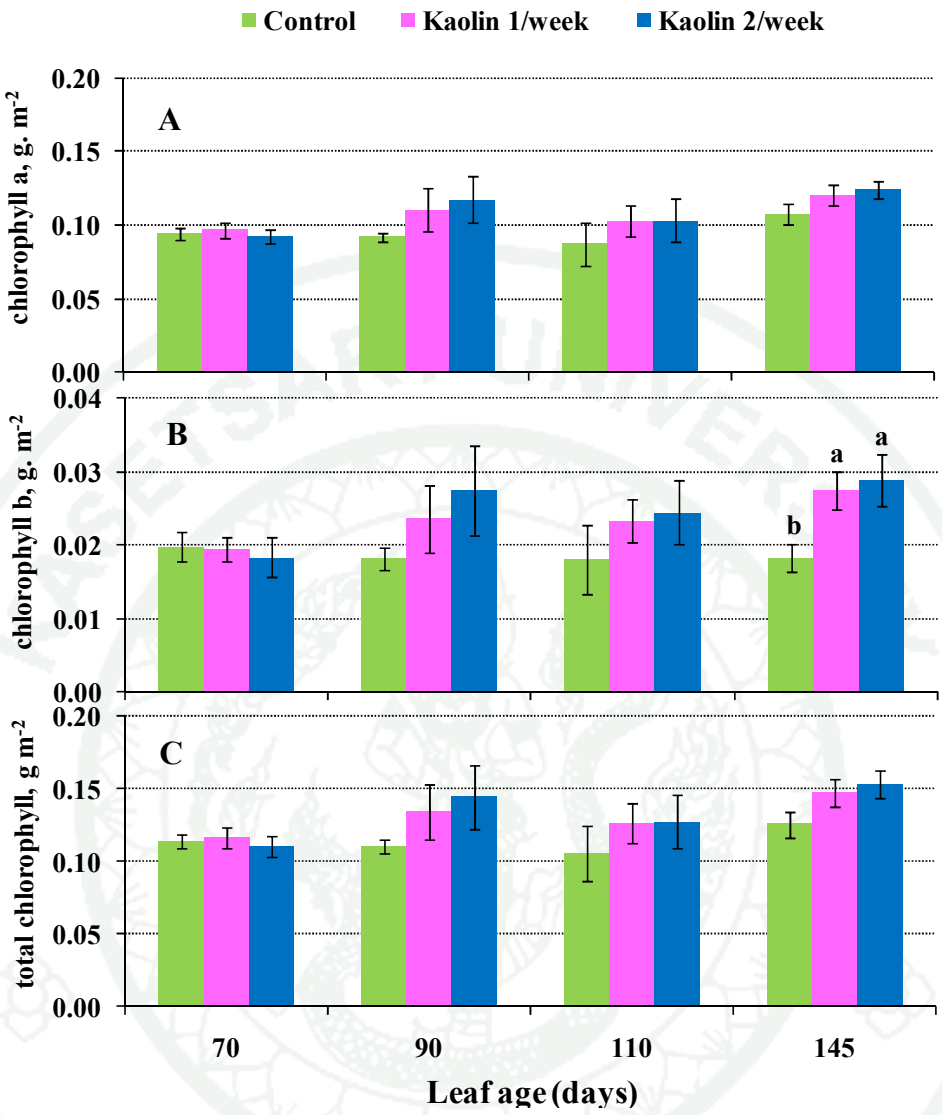

Figure 10 Changes in chlorophyll a (A), chlorophyll b (B) and total chlorophyll (C) in mango leaves after spraying kaolin at 70, 90, 110 and 145 days of leaf ages. Each symbol represents the mean value of the four replications (± SE).

2.2 Effects of kaolin on carbohydrate content

2.2.1 Non- structural carbohydrate levels in leaves

The variation of sucrose accumulation was found in kaolin treatments twice a week were significantly higher than those in untreated leaves. At 2nd and 5th week, once and twice treated leaves showed similar trend which were higher than the control. At 8th week, leaves treated with kaolin twice a week contained the highest sucrose content while the control leaves contained the lowest content. At 13th week, sucrose contents in both kaolin treatments were not significantly different. Twice a week treatment, however, was equal to the control treatment (Figure 11A and Appendix Table B4). When comparing sucrose accumulation in each sampling time, the results showed that the significantly lowest value (2.43±0.38) of sucrose accumulation was found at 13th week (Appendix Table B65). Throughout sampling time, the average value of sucrose accumulation in mango leaves treated with kaolin twice and once a week (6.59±0.73 and 5.66±0.53, respectively) were significantly higher than those in untreated leaves (2.26±0.27) (Appendix Table B65).

The amount of glucose in leaves of spraying kaolin once and twice a week were significantly lower than those in untreated leaves at the 2nd week. However at 5th week, glucose in leaves of spraying kaolin once and twice a week were significantly higher than those in untreated leaves. At the 8th and 13th week, there was no significant difference in glucose accumulation between kaolin-application and control group (Figure 11B and Appendix Table B5). The average value of glucose accumulation at 8th week was the highest which was 8.66±0.44 when compared other sampling time (5.86±0.50 for 2nd week, 5.40±0.57 for 5th week and 5.84±0.32 for 13th week) (Appendix Table B65). There was no significant difference in any kaolin treatment throughout sampling time (Appendix Table B65). In addition, for the comparison between sampling time and treatments, the highest value of glucose was 9.39 ±0.96 found in untreated leave at 8th week. Furthermore at the same week, glucose accumulations in both of kaolin treatment were not significantly

different with untreated leaves. The lowest content of glucose was at 5th week of control treatment (Appendix Table B5).

The significantly variation of fructose was found at only 2th and 8th week. At 2nd week, the content of fructose in leaves treated with kaolin twice a week was low but not significantly difference from the control. At the 8th week, the control leaves had high fructose content but not significantly different from leaves treated with kaolin once a week. Between kaolin treatments, however, the content of fructose was not significantly difference from each other (Figure 11C and Appendix Table B65). Fructose accumulation showed the highest average value at 8th week (11.68±0.91). While the other sampling times show no difference values. (6.50±0.61, 5.68±0.60 and 5.85±0.62 for 2nd, 5th, 13th week, respectively) (Appendix Table B65). The average value of fructose accumulation in mango leaves treated with kaolin twice a week (6.77±0.67) was not significantly difference from that of once a week treated leaves (7.18±0.89). The latter, however, was equivalent to that of untreated leaves (8.34±0.98) (Appendix Table B65). Moreover, there was a significant interaction between sampling time and all treatments. The highest values of fructose were found in 8th week for control (13.70 ±1.05) and leaves treated with kaolin once a week (12.61±0.85) (Figure 11C and Appendix Table B6).

For total carbohydrate contents, there were no significant differences in all treatments in leaves at 2, 5, and 13 weeks. However at 8th week, in leaves with spraying kaolin twice a week had the lowest total carbohydrate contents and significantly difference from the other two groups (Figure 11D and Appendix Table B7). The average total carbohydrate content was the highest (468.49±11.36) at 13th week while at 2nd weeks it was the lowest (206.13±4.87) (Appendix Table B65). Among treatment, the content of average total carbohydrate in mango leaves treated with kaolin once a week (309.08±28.24) was significantly higher than those in untreated leaves (286.11±24.09) and leaves treated with kaolin twice a week (280.43±30.42) (Appendix Table B65).

2.2.2 Non- structural carbohydrate levels in shoot tips

The content of sucrose in shoot tips showed that there was no difference between three treatments of each sampling time (Figure 12A and Appendix B Table 4). However, average sucrose content showed highest accumulation at the 5th week (2.00±0.27). The content of sucrose at fruit's age the 8th week was 0.68±0.12 which was higher than the content that of the 2nd and 13th week (0.11±0.01 and 0.10±0.01, respectively) (Appendix B Table 66). There was no significant difference of average sucrose in any kaolin treatments throughout sampling time (Appendix B Table 4). The comparison between sampling time and kaolin treatments showed significantly difference. The highest value of sucrose found in spraying kaolin once a week at 5th week, which had the values for 2.73±0.48 (Appendix B Table 4).

Glucose accumulation in shoot tips was no significant difference in any sampling time (Figure 12B and Appendix B Table 5). Nevertheless, accumulation of glucose in shoots of all treatments expressed the highest average values at the 8th week (18.30±0.98). While the average of glucose for the 13th week was 11.48±1.49 which was higher than that of 2nd and 5th showing the low values for 5.21±0.51 and 7.13±1.07, respectively (Appendix B Table 66). Throughout sampling time, there was no significant difference in all kaolin treatments (Appendix B Table 66).

The amount of fructose in shoot tips of all treatments showed no difference in any sampling time (Figure 12C and Appendix B Table 6). The accumulation of fructose in all treatments showed the highest average value at 8th week (28.30±0.85) when compared to the other sampling times. In addition, the average fructose of 13th week was 19.43±2.24 which was higher than that of 2nd and 5th which was 9.05±0.58 and 10.05±1.14, respectively. However, there was no significant difference of fructose content in all kaolin treatments (Appendix B Table 66).

The total carbohydrate contents in shoot tips showed no significant differences in all treatments (Figure 12D and Appendix B Table 7). However, the comparison between the averages of total carbohydrate in any sampling time showed

that the highest value was found at the 13th week (476.23±41.05) while the lowest value was found at 2th week (107.66±4.36) (Appendix B Table 66). In addition, there was no significant difference of average total carbohydrate content in all treatments (Appendix B Table 66).

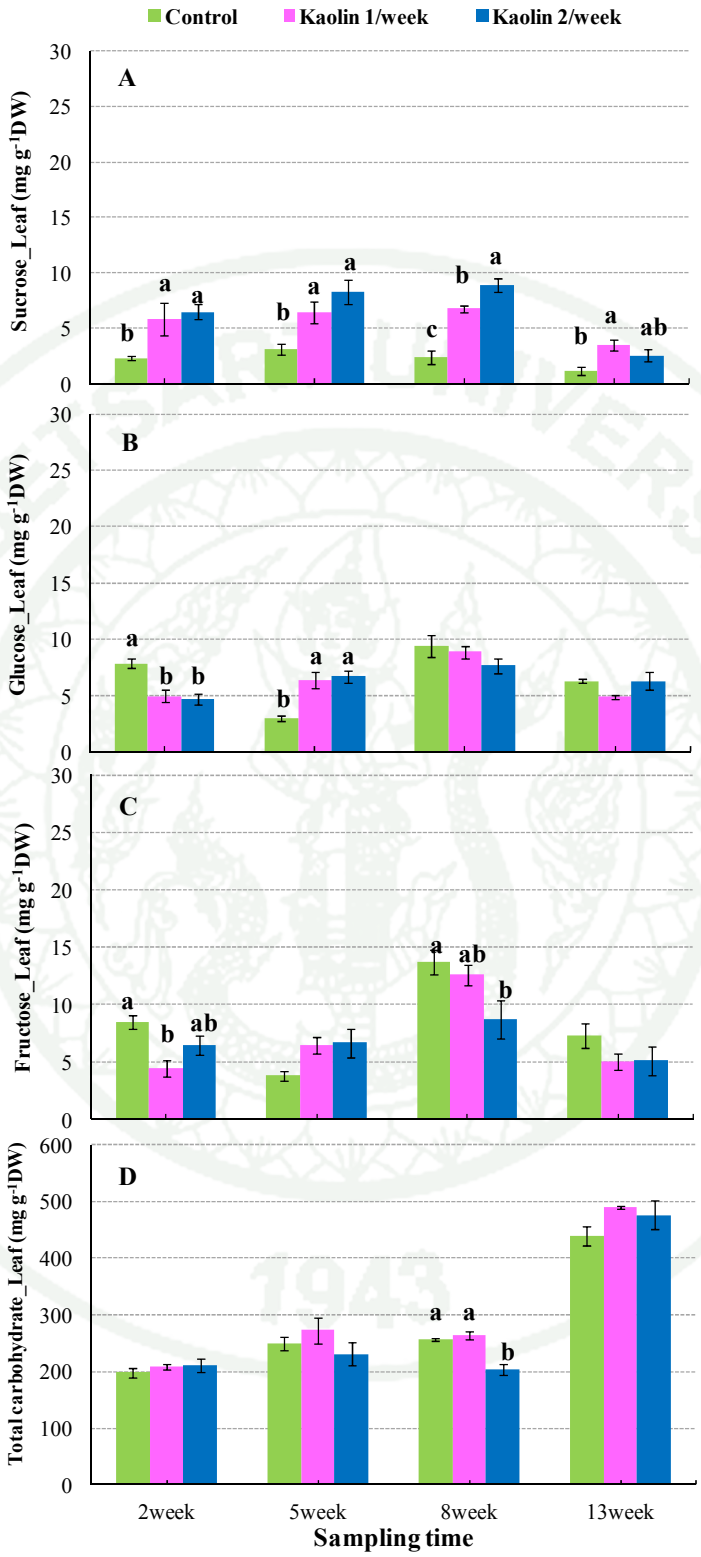

Figure 11 Changes in sucrose (A), glucose (B), fructose (C) and total carbohydrate (D) accumulation in mango leaves at fruit's age 2, 5, 8, and 13 weeks. Each symbol represents the mean value of the four replications (± SE).

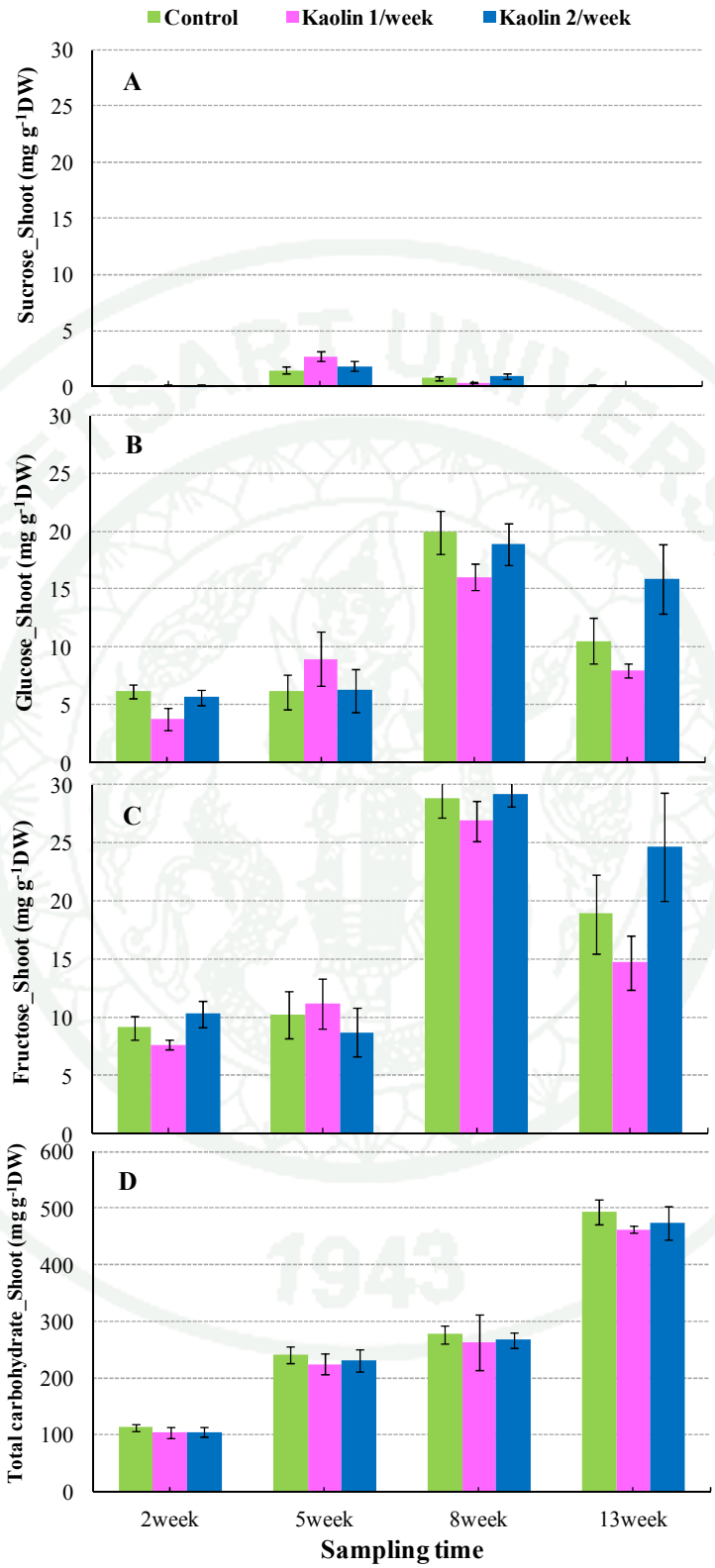

Figure 12 Changes in sucrose (A), glucose (B), fructose (C) and total carbohydrate (D) accumulation in mango shoots at fruit's age 2, 5, 8, and 13 weeks. Each symbol represents the mean value of the four replications (± SE).

2.3 Effects of kaolin on fruit yield and fruit quality

2.3.1 Fruit yield

The total number of fruit and the total fruit weight per mango trees were not significantly different in the all treatments. However, the kaolin-sprayed trees twice a week tended to increase the total number of fruit (152.75±16.46, 153.50±14.14 and 188.50±25.17 for control, kaolin-sprayed trees once a week and kaolin-sprayed trees twice a week, respectively) and the total fruit weight (45.50±3.57, 41.75±4.80 and 53.00±7.29 for control, kaolin-sprayed trees once a week and kaolin-sprayed trees twice a week, respectively (Table 12).

Fruit size (fruit width and thickness) of kaolin-sprayed tree was significantly smaller than in the unsprayed control. In addition, the average fruit width of control**,** kaolin-sprayed trees once and twice a week were 7.16±0.04, 6.95±0.03 and 6.96±0.04, respectively. While, the average fruit thickness of both kaolin application was significantly less than control. That was 6.08±0.03min for kaolin-sprayed trees once a week and 6.13±0.04 min for kaolin-sprayed trees twice a week while it was 6.35±0.03 for control. Fruit length of the kaolin-sprayed trees once a week showed the lowest value for 13.48±0.07. Moreover, there was no significant difference between fruit length from the kaolin-sprayed trees twice a week (13.86±0.07) compared with those in the unsprayed control (14.02±0.08) (Table 12).

2.3.2 Fruit quality

The values of peel brightness (*L*) of the kaolin-treated fruits were not significantly different in the all treatments, while the yellowness (*b*) values of the kaolin-sprayed fruit peels with once and twice a week (33.94±0.62 and 32.58±0.46, respectively) were significantly lower than those of the control (36.70±0.62) (Table 12).

There was no significant difference in TSS of fruits derived from the kaolin-sprayed trees compared with those in the unsprayed control. Nevertheless, the TA content was significantly lowered by the application of kaolin (0.12±0.002 for control, 0.11±0.003 for kaolin-sprayed trees once a week and 0.11±0.002 for kaolin-sprayed trees twice a week) (Table 12).

The kaolin film was able to reduce the severity of anthracnose and fruit rot during the post-harvest period. The results showed that the percentage of healthy fruit among fruits coated was significantly higher than that of the control for before and after ripening induction. Before ripening induction, the percentage of healthy fruit for control**,** kaolin-sprayed trees once and twice a week were 72.5±4.33, 88.75±5.54 and 87.5±2.50, respectively. The values of healthy fruit after ripening induction in kaolin-sprayed trees twice a week showed the highest values (12.5±4.78) (Figure 13 Appendix Table B8). The comparison between the levels of average severity of anthracnose and fruit rot showed that the percentage of disease severity of fruit during before ripening induction appeared only at the first level. Moreover, the severity level of the diseases in kaolin-coated fruits was significantly lower at the first level for before ripening induction. After ripening induction, the severity level of the diseases in all treatments was shown in all levels except 4th level. In addition, the percentage of disease severity of fruit in kaolin-coated fruits with twice a week was significantly lower than those of uncoated fruits at the 2nd level (Figure 14, 15, 16 and Appendix Table B8).

Table 12 Effect of kaolin on total number of fruit, total weight, peel colors and juice quality of mango

Treatment	Total number of fruit[A]	Total weight (kg/tree) [A]	Fruit size[B]			Peel color characteristics[C]		Juice quality[D]	
			Width (cm.)	Length (cm.)	Thickness (cm.)	*L*	*b*	TSS (°Brix)	TA
Control	152.75±16.46	45.50±3.57	7.16±0.04a[1/]	14.02±0.08a	6.35±0.03a	60.81±0.45	36.70±0.62a	14.70±0.13	0.12±0.002a
K 1/week	153.50±14.14	41.75±4.80	6.95±0.03b	13.48±0.07b	6.08±0.03b	59.53±0.83	33.94±0.62b	14.86±0.09	0.11±0.003b
K 2/week	188.50±25.17	53.00±7.29	6.96±0.04b	13.86±0.07a	6.13±0.04b	59.67±0.36	32.58±0.46b	14.59±0.12	0.11±0.002b

Mean value of 4 replications (±S.E.)

[1/] Means followed by the same lowercase superscript letter within a column are not significantly different by Duncan's multiple range test at the $P \leq 0.05$ level.

Total numbers and total weight per tree were counted and recorded at harvesting time; [A](n=4)

Fruit size were measured from twenty representative fruits per tree at harvesting time; [B] (n=80)

Peel colors: *L* = brightness, *b* = yellowness; [C] (n=80)

Juice quality: TSS=Total soluble solids, TA= titratable acidity; [D] (n=80)

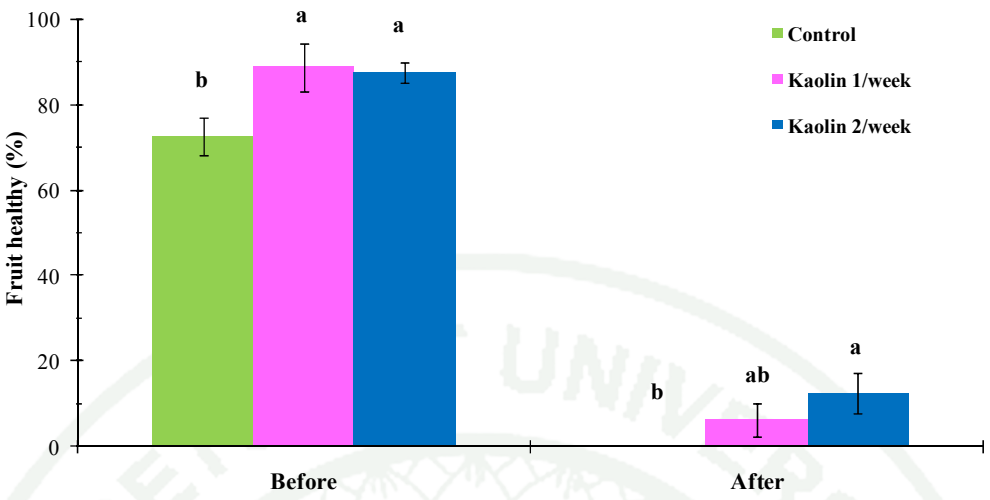

Figure 13 Effect of kaolin on healthy fruit (level 0) in mango before and after ripening induction. Each symbol represents the mean value ± SE of four replications (n=4).

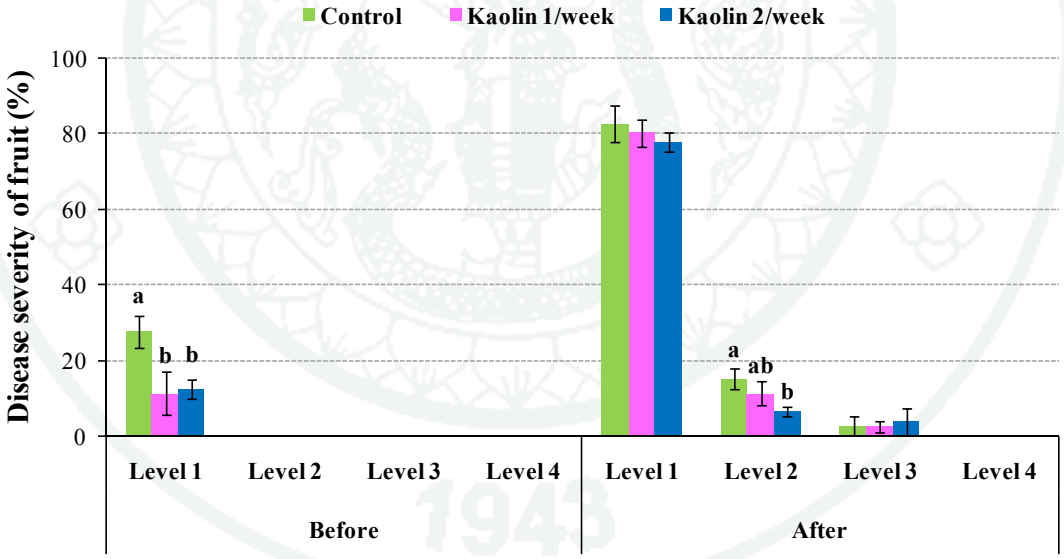

Figure 14 Effect of kaolin on fruit damage by anthracnose and fruit rot disease during before and after ripening induction. Each symbol represents the mean value ± SE of four replications (n=4). Disease severity of fruit: Level 1 = 1–25%, Level 2 = 26–50%, Level 3 = 51–75% and Level 4 = 76–100% disease area.

(A) (B) (C)

Figure 15 Effect of kaolin on fruit damage by anthracnose and fruit rot disease during before ripening induction. (A) Uncoated fruits, (B) Kaolin-coated fruits with once a week, and (C) Kaolin-coated fruits with twice a week.

(A) (B) (C)

Figure 16 Effect of kaolin on fruit damage by anthracnose and fruit rot disease during after ripening induction (A) Uncoated fruits, (B) Kaolin-coated fruits with once a week, and (C) Kaolin-coated fruits with twice a week.

Discussion

Experiment 1 Selection of the most appropriate particle film for coating mango leaves

Kaolin suspended well in water and precipitated slowly. The precipitation time of a coating substance depends on its particle size—the larger the particle size, the shorter the precipitation time. In this research, the precipitation of kaolin was slowest; this was due to its smallest particle size.

The results showed that among the four coating materials, kaolin was the best for reducing irradiation as measured by transmission of photons through the glass plate. Kaolin could increase the reflective irradiation, therefore, its measurement of PPF by the transmission of photons through the glass plate showed the lowest PPF percentage. An explanation of the mechanism of kaolin application on a treated plastic Petri plate was reported by Jifon and Syvertsen (2003), who demonstrated that kaolin particle film reduced PAR transmittance by approximately 28%. They also found that the loss of PAR absorption was entirely due to increased PAR reflection. Glenn and Puterka (2005) reported the kaolin sprayed onto tree foliage as a liquid suspension, leaving kaolin as a white, porous, protective, powdery film on the surface of leaves and fruits after water evaporation. The physical properties of the film were a reduction of damage from insects and plant pathogens as well as enhancing photosynthesis, yield and fruit quality, particularly in hot and dry climates. Kaolin particle film has been reported to increase foliage reflectivity and reduce the heat load on plants with some increases in plant productivity (Glenn *et al.*, 2001). The leaf is able to utilize PAR through the particle film, but the film reflects ultraviolet and infrared radiation from the leaf or fruit surface (Glenn and Puterka, 2005). However, the variability in light transmittance probably approximates nonuniform particle deposition on leaves as might occur in the field (Jifon and Syvertsen, 2003; Glenn and Puterka, 2005).

The natural microclimatic conditions indicated that mango leaves were exposed to high levels of PPF, T_{air} and VPD_{air} at midday. Consequently, the T_{leaf} and

$VPD_{leaf-air}$ values in mango leaves increased, leading to the closing of stomata and a midday depression of photosynthesis due to limited CO_2 uptake and decreased CO_2 availability. Furthermore, mango leaves received excess radiation of 1,462.67 µmol PPF $m^{-2}.s^{-1}$ which was higher than the light saturation point measurement in this study of around 600 µmol PPF $m^{-2}.s^{-1}$ or 232.93 µmol PPF $m^{-2}.s^{-1}$ from the calculation. Moreover, the saturated A values which were measured from the first mature leaves in exterior canopy positions showed a P_{max} value of 3.5 µmol CO_2 $m^{-2}.s^{-1}$ for the data. However, the A value of untreated leaves was 2.34 µmol CO_2 $m^{-2}.s^{-1}$. The decrease in photosynthesis in uncoated leaves due to the high VPD_{air} induced stomatal closure and limited the CO_2 uptake. The carbon metabolism may limit the consumption of photosynthetic energy under high light radiation resulting in excess photon absorption. As a consequence, non-utilized excitation energy can accumulate, promoting reductions in photosynthetic efficiency, which is termed photoinhibition. Thus, this condition could exacerbate photoinhibition in non-kaolin-treated mango leaves due to the excess of photosynthesis (Xu and Shen, 2005). Goldschmidt (1999) found that the excess radiation and high temperatures of leaves caused water deficits and reduced light-use efficiency, leading to photoinhibition, reduced photosynthesis, lower growth, less fruit yield and poorer quality.

The study on physiological variables relating to photosynthesis after applying coating materials to mango leaves showed that kaolin induced the highest A, g_s and E values compared with the bentonite and control treatments. In addition, the C_i and C_i:C_a values for the kaolin-coated leaves increased, compared with untreated leaves. It may be assumed that kaolin could help the reflection of excess radiation from the mango leaf. The uncoated leaves accumulated heat load inside the leaves which affected stomatal closure. Bentonite had a higher particle size than kaolin which caused low attachment on the leaves resulting in its poor suitability as a coating material. Its low reflection result led to the lowest A value and the highest C_i value since carbon dioxide use was inhibited. The study indicated that kaolin effectively increased A since it was able to decrease excess irradiation. Likewise, Glenn and Puterka (2005) suggested that kaolin was reflective to UV radiation but the formulation and particle distribution significantly influenced the degree of its UV

reflection. In addition, the physical presence of the clay particles apparently did not inhibit leaf gas exchange, perhaps due to the porous nature of kaolin clay. This desirable quality led to an increase of net carbon assimilation in the kaolin-treated plant. It suggested that an increase in yield and fruit quality on kaolin-treated apple trees was caused by an increase in whole-tree carbon assimilation under heat stress (Glenn *et al*., 2003).

Experiment 2 Effects of kaolin on photosynthesis, carbohydrate content, fruit yield and fruit quality

Diurnal changes of gas exchange after kaolin treatment

Microclimate data measured during the diurnal leaf gas exchange study indicated that there was a variation of PPF at the midday (11:00-12:00 hr.) among the study at each leaf ages. PPF of the study at leave age 70 and 110 days were exceeded 1000 μmol PPF $m^{-2}.s^{-1}$ which is ranging from 1364.75 - 1419.31 μmol PPF $m^{-2}.s^{-1}$ for leave age 70 days and 1193.81 - 1484.19 μmol PPF $m^{-2}.s^{-1}$ for leave age 110 days. Meanwhile PPF of the study at leave age 90 and 145 days were generally lower than 1000 μmol PPF $m^{-2}.s^{-1}$ which is ranging from 873.63- 1071.88 μmol PPF $m^{-2}.s^{-1}$ for leave age 90 days and 586.06- 738.13μmol PPF $m^{-2}.s^{-1}$ for leave age 145 days. (Figure 8 and Table 7-10)

Mango leaves at 70, 90, 110 and 145 days of age were subjected for midday gas exchange measurement by using portable photosynthesis system (LI-6400) in a closed chamber. Results of the study showed that the mango leaves treated with kaolin spraying had a higher photosynthetic efficiency than those of the untreated control group. At 70 days of age, mango leaves treated with kaolin spraying twice a week had A and g_s values of 2.36 μmol CO_2 $m^{-2}.s^{-1}$ and 35.66 mmol H_2O $m^{-2}.s^{-1}$, respectively which is 58.39 % and 21.58 % higher than those of the mango leaves in untreated control group, respectively (Figure 8 and Table 7). It may be assumed that kaolin could increase light reflection of the mango leaves and promote stomata opening on the day of high light intensity which led to an increasing of g_s values. In

addition, the mango leaves had C_i values of 255.06 µmol CO_2 mol^{-1} which is lower than 295.88 µmol CO_2 mol^{-1} of those the unsprayed control leaves. It is probably due to the cells in mesophyll layer of leaf have used carbon dioxide in photosynthetic activity and consequently decreases carbon dioxide in intercellular space. Moreover, the increased in g_s value of the twice a week kaolin sprayed leaves resulted in the increased of E value and is 25.75% higher than those of the control unsprayed leaves (Table 7). Results of the study indicated that twice a week foliar spray of kaolin affects the opening and closing of the mango leaves stomata which have limited the water loss and the conductivity carbon dioxide into intercellular space of leaves.

At 90 days of leave age, mango leaves of the control unsprayed group had higher A value in the midday than those on twice a week kaolin sprayed leaves. But the light intensity was dramatically reduced in the afternoon (16:00 hours.) due to raining. However, there was no significant different in g_s value of mango leaves treated or untreated with kaolin spraying. The data indicated that, under low light intensity condition, kaolin sprayed mango leaves tended to have more adverse effects on photosynthetic rate than the untreated leaves. In additional, the A and g_s value of mango leaves of the two treatments are almost similar and did not showed any statistically different (Table 8). Results of the Experiment 1 showed that mango leaves have a light saturation point of 600 µmol PPF $m^{-2}s^{-1}$ (Figure 4). A low light intensity of 500 PPF $m^{-2}s^{-1}$ in the afternoon and the light reflection of kaolin coated leaves which have reported to be 28% (Jifon and Syvertsen, 2003) may be responsible for the low A value of the kaolin treated leaves in the study.

At 110 days of leave age, the mango leaves were exposed to high PPF similar to those of the 70 days of leave age. However, there were no significantly different in A and g_s values among the mango leaves in all treatment. The A value of mango leaves of the untreated control, once and twice a week kaolin leave-sprayed group were 0.29, 0.38 and 0.22 µmol CO_2 $m^{-2}.s^{-1}$, respectively (Table 9). Mango leaves on once and twice a week kaolin leave sprayed group tended to have a higher g_s value (31.28±4.20 and 30.73±2.82 mmol H_2O m^{-2} $.s^{-1}$, respectively) than those on control unsprayed group (21.48±3.01 mmol H_2O m^{-2} $.s^{-1}$), however, the differences were not

statistically significant. The higher of g_s value resulted in the higher E value of mango leaves in once and twice a week kaolin leave sprayed group by 44.64 and 46.42% than those of the untreated control group, respectively. Data of the study indicated that kaolin did not result in the increasing of A value in the older leaves. Phattarlerphong (1997) suggested that A value of mango leaves was attained the highest level at 45 days of age and then dramatically reduced at 100 days old age. Results of the study indicated that leaf age was a major factor to limit A value in mango leaf with kaolin spraying treatment.

At 145 days of leaf age, although the mango leaves were exposed to midday low PPF similar to those of the 90 days of leave age, the mango leaf treated with twice a week kaolin leave spraying showed a significantly higher ($P<0.05$) A value than those of once a week kaolin leave spraying, but no significantly differences from the untreated control group. However, g_s value of mango leaves on twice a week kaolin leave spraying group was significantly higher than those of the other treatment groups. Data of the study suggested that the continuously leave spraying of kaolin for 26 days was able to reduced midday depression, photoinhibition and increase A value of the mango leaf. The results are in agreement with Ratichote (2014) who reported that twice a week foliar spraying of white clay kaolin for mango leaves was capable to increase Fv/Fm value at 145 days of age since kaolin can reduce the losing of heat energy and prevented photoinhibition in a photosynthetic activity.

A/Ci (CO_2 response) curves measurement

The response of A to CO_2 in Rubisco-limited and RuBP regeneration-limited conditions can be utilized for the estimation of photosynthesis parameters in dark reaction by the model of Sharkey *et al*. (2007). A/C_i curves were created by the measuring of three reactions. 1) Rubisco enzyme activity by measurement of maximum velocity of Rubisco in carboxylation ($V_{c\ max}$). 2) The turnover of 3-PGA into RuBP in Calvin Cycle which depended on an electron transport rate and the ability to use NADPH and ATP in light reaction. The second reaction is namely RuBP regeneration which was expressed as the maximum rate of electron transport

for driving RuBP regeneration; J_{max}. 3) The rate of triose phosphate use (TPU) which was the conversion of triose into sucrose.

Results of the study showed that at 70 days of leaf age, foliar application of kaolin twice a week had the highest $V_{c\ max}$. This indicated that kaolin application could promote the opening of stomata, thus increase CO_2 fixation which lead to promotion of RuBP carboxylation efficiency and raise the photosynthesis capacity in mango leaves. However, at 110 days of leaf age, the control untreated mango showed the highest $V_{c\ max}$ and had a higher rate of electron transport driving RuBP regeneration (J_{max}) than those kaolin treated plants. Results of the study indicated that foliar spraying of kaolin to old age mango leaves could not increase the photosynthetic capacity of the leaves.

There was no significant difference in triose phosphate use (TPU) among the treatment groups. However, foliar spraying of kaolin twice weekly had a higher TPU than those of the control group by 25.61 and 14.28% at 70 and 90 days leaf age, respectively. The data suggested that the twice weekly kaolin foliar spraying have increased the conversion of triose phosphate to sucrose in mango leaves.

Moreover, $J_{max}/V_{c\ max}$ ratio is a parameter to determine the balance between RuBP carboxylation and RUBP regeneration. Generally, $J_{max}/V_{c\ max}$ ratio ranging from 1.0 to 3.0 was classified as a well balance between RuBP carboxylation and RuBP regeneration (Ribeiro *et al*., 2009). Results of the present study showed that twice weekly kaolin foliar application of mango leaves provided the highest $J_{max}/V_{c\ max}$ ratio which was ranging from 1.0 to 3.0 at 110 days of the leaf age, and indicated that the balance between RuBP carboxylation and RuBP regeneration was maintained. However, the $J_{max}/V_{c\ max}$ ratio is depended on the climate temperature (von Caemmerer and Farquhar, 1981; Hikosaka, 2005; Onoda *et al*., 2005). These results are inconsistent with Ribeiro *et al*., 2009 who reported that the $J_{max}/V_{c\ max}$ ratio of orange leaves in winter season was higher than 3.0 due to the closure of their stomata during the night.

Chlorophyll content

No differences were observed on chlorophyll a, b and total chlorophyll in all treatments. Similar results were observed by Sotelo-Cuitiva *et al*. (2011), who observed that foliar applications of kaolin did not affect on chlorophyll content in rose cut plants (*Rose spp*. L.). However, only chlorophyll b was affected by kaolin treatments at 145 days of leaf ages. Chlorophyll b is most abundant in the antennae of the light harvesting complex, whereas chlorophyll a is concentrated around PSII. Griffin *et al*. (2004) found that shade-grown plants typically have more light harvesting complexes per unit area than do sun-grown plants that typically receive more light than needed to capture as much light as possible. An explanation of the mechanism of kaolin application on treated plants was reported by Glenn and Puterka (2005), who demonstrated that the physical properties of the kaolin film reduce damage from light reflection on the leaf surface. Therefore, it may be assumed that spraying kaolin could promote light harvesting complexes on the mango leaf. In a similar study, Tworkoski *et al*. (2002) indicated that the particle-film-type antitranspirant enhanced chlorophyll biosynthesis and increased the chlorophyll content of bean leaves. Thus, it could enhance chlorophyll formation and increased chlorophyll concentration in plant leaves due to reducing heat stress.

Carbohydrate content in leaf and shoot tips

Mango leaves and shoot tips at the fruit age 2, 5, 8 and 13 weeks were analyzed for total sugar (sucrose, glucose and fructose) and total carbohydrate content. Result of the study indicated that generally there were rather a similar content of each type of sugar in the mango leaves and shoot tips among the treatment groups at each age of the fruit studied. However, mango leaves treated with once or twice a week kaolin spraying had higher sucrose content than those without kaolin treatment at all fruit ages. The data indicated that long-term leaves treatment with kaolin spraying increased net photosynthetic rate which is related to the increased of A value of the mango leaves at 145 days of age. The sucrose content in mango shoot tips was rather low and is lower than those in the leaves indicating the major

synthesis and translocation of glucose in the leaves and a very limited amount of sucrose is transferred to the mango shoot tips. Glucose and fructose contents in both mango leaves and shoot tips tended to increase from the fruit age 2 weeks, attained the maximum at the fruit age 8 weeks and then decrease until the fruit age 13 weeks (Figure 11 and 12). Both glucose and fructose were carbon reactant derived from photosynthesis and subsequently changed to sucrose for deposition in sink (fruit and other parts). Wangwattana (1999) has shown that growth of mango fruit was a simple sigmoid curve. There was a slow growth of the fruit cells during 1-2 week after flowering, the highest growth rate at 3-8 week after flowering, and the maintain of stable growth rate at 9-15 week after flowering. However, glucose and fructose contents in mango shoot tips were higher than those in the leaves. The data indicated that during the high growth rate of mango fruit, sucrose was transferred from the leaves to accumulate in bark at the mango shoot tips before transferring into the fruits. Chauhun and Pondey (1984) found that during mangos flowering and fruiting, 25-48% of carbohydrate synthesized from photosynthetic activity was transferred from flowered shoots to flower spikes, fruits and other parts of a branch.

At the fruit aged 13 weeks, the content of glucose, fructose and sucrose in both mango leaves and shoot tips were dramatically reduced while the total carbohydrate contents were maintained at a high level. Mango fruits aged 13 week may reach a maturity and the sucrose produced in mango leaves and shoot tips may be transferred for accumulation in the fruits (Lalonde *et al*., 1999). Therefore, after the fruit maturity, mango leaves began to accumulate sugars without the transferring of sugar to sink which is led to an increasing of total carbohydrate content in the mango leaves and shoot tips. However, there were no difference in total carbohydrate content in leaves and shoot tips of mango in all treatments.

Fruit yield and fruit quality

It was hypothesized that an increase in photosynthesis after the kaolin application would increase the fruit yield and quality by increasing the carbohydrate supply. Nevertheless, a number of harvested fruits including the total fruit weight

showed no statistical difference among treatments even though the kaolin-sprayed mango twice a week produced a greater total number of fruit over the control.

Peel colors were affected by the kaolin treatment, especially yellowness (*b*). It is well known that mango cv. Nam Dok Mai peel has greenish to bright yellow coloration when ripe. However, kaolin treated plants was found to be associated with reduced yellowness on the fruit skin. Decreasing in yellowness in study was possibly due to reduction of chlorophyll concentrations by declination and cultivar specific where light intensity was optimal. The present results are consistent with those reported by Onnom (2007), who reported that using plastic roof could reduce photosynthetic photon flux by 29% led to the reduction of yellowness on the fruit skin in mango. In addition, previously published evidence demonstrate that kaolin coating in mango cv. Mahajanaka affected the ripe fruit peel colors especially redness (Chamchaiyaporn *et al*., 2013). While Glenn *et al*. (2001) reported that the fruit color of apple in Santiago trials could be enhanced by kaolin application. Kaolin-treated fruits show improved anthocyanin color, if the layer applied was not too heavy, indicating a possible promotional effect due to reduced day temperature (Erez and Glenn, 2004). However, the effects of light and temperature depend on the cultivar and the stage of fruit development (Ritenour and Khemira, 2007; Saengnil *et al.*, 2011).

With respect to the juice quality, the TA content in the control group was significantly higher than in the kaolin treatment, while the TSS in the control was not significantly different compared to the kaolin treatment. A probable explanation is that mango is a climacteric fruit. The taste development was marked by an increase in gluconeogenesis, hydrolysis of polysaccharides, especially starch, and a decrease in the acidity (Prasanna *et al.*, 2007, Brecht and Yahia, 2009). Thus, the juice quality in the control may have been subjected to less hydrolysis of the polysaccharides than in the kaolin treatment which led to the higher TA content. This may have been a result from the kaolin application increasing the efficiency of photosynthesis leading to the higher production of sugar which can accumulate in a starch form. However, maturity at harvest plays an important role for postharvest life and eating quality, in particular

for climacteric fruits where ripening is regulated by ethylene (Lelièvre *et al.*, 1997). In the immature fruit, sucrose is almost absent but it increases during ripening and becomes the major carbohydrate constituent in the ripe fruit (Wang *et al.*, 1996). During fruit maturation, starch that accumulates in chloroplasts is hydrolyzed to sucrose, glucose and fructose (Kumar and Dhawan, 1995).

In this study the kaolin coating effectively reduced the severity of anthracnose damage caused by *Collectotrichum gloeosporioides* and fruit rot caused by *Lasiodiplodia theobromae* during ripening at an ambient temperature of 30 ± 2 °C. Results of decreasing the anthracnose and fruit rot damage in mango field was similar to that reported previously, that kaolin coating reduced the severity of anthracnose and fruit rot during the post-harvest ripening period in mango cv. Mahajanaka (Chamchaiyaporn *et al*., 2013). The current study suggested that the kaolin coating prevented direct contact between pathogenic microbial inocula and plant surfaces by coating the leaves and fruit with barrier films and interfered with spore adhesion, thereby reducing infection and disease. Glenn *et al*. (1999) reported that kaolin contributed to the control of fungal and bacterial plant pathogens by preventing the formation of a liquid film on the surface of pear leaves. Similarily, kaolin controlled the fungal disease, Fabraea leaf spot, caused by *Fabraea maculata* Atk. in grape (Puterka *et al*., 2000).

CONCLUSIONS

1. Kaolin had good solubility and suspension in water which showed the slowest rate of precipitation when compared to bentonite, calcium carbonate and dolomite. In addition, kaolin was the most effective leaf coating material for reducing light transmission as it had the lowest photon transmittance through a glass plate.

2. At 70 days of leave age, mango leaves treated with kaolin spraying twice a week had A and g_s values of 2.36 μmol CO_2 $m^{-2}.s^{-1}$ and 35.66 mmol H_2O $m^{-2}.s^{-1}$, respectively which is 58.39 % and 21.58 % higher than those of the mango leaves in untreated control group, respectively. The mango leaves treated with kaolin spraying twice a week had C_i values of 255.06 μmol CO_2 mol^{-1} which is lower than 295.88 μmol CO_2 mol^{-1} of those the unsprayed control leaves. Moreover, the increased in g_s value of the twice a week kaolin sprayed leaves resulted in the increased of E value and is 25.75% higher than those of the control unsprayed leaves

3. At 90 days of leave age, mango leaves of the control unsprayed group had higher A value in the midday than those on twice a week kaolin sprayed leaves. But the light intensity was dramatically reduced in the afternoon (16.00 hr.) due to raining. The data indicated that, under low light intensity condition, kaolin sprayed mango leaves tended to have more adverse effects on photosynthetic rate than the untreated leaves.

4. At 110 days of leave age, the mango leaves were exposed to high PPF similar to those of the 70 days of leave age. However, there were no significantly different in A and g_s values among the mango leaves in all treatment. Mango leaves on once and twice a week kaolin leave sprayed group tended to have a higher g_s value (31.28±4.20 and 30.73±2.82 mmol H_2O m^{-2} $.s^{-1}$, respectively) than those on control unsprayed group (21.48±3.01 mmol H_2O m^{-2} $.s^{-1}$), however, the differences were not statistically significant. Data of the study indicated that kaolin did not result in the increasing of A value in the older leaves.

5. At 145 days of leaf age, although the mango leaves were exposed to midday low PPF similar to those of the 90 days of leave age, the mango leaf treated with twice a week kaolin leave spraying showed a significantly higher ($P<0.05$) A value than those of once a week kaolin leave spraying, but no significantly differences from the untreated control group. However, g_s value of mango leaves on twice a week kaolin leave spraying group was significantly higher than those of the other treatment groups. Thus, the continuously leave spraying of kaolin was able to reduced midday depression, photoinhibition and increase A value of the mango leaf.

6. At 70 days of leaf age, foliar application of kaolin twice a week tended to provide a higher leave $V_{c\ max}$ than those of the control by 80%, whereas, at 110 days of leaf age, kaolin application twice a week tended to provide a higher leave $J_{max}/ V_{c\ max}$ than those of the control by 180%. However, there was no significantly difference in TPU among treatments throughout the study period.

7. Foliar application of kaolin once or twice a week on mango leaves had higher sucrose content than those without kaolin treatment at all fruit ages. The sucrose content in mango shoot tips was rather low and is lower than those in the leaves indicating the major synthesis and translocation of glucose in the leaves and a very limited amount of sucrose is transferred to the mango shoot tips. Glucose and fructose contents in both mango leaves and shoot tips tended to increase from the fruit age 2 weeks, attained the maximum at the fruit age 8 weeks and then decrease until the fruit age 13 weeks. However, glucose and fructose contents in mango shoot tips were higher than those in the leaves. At the fruit aged 13 weeks, the content of glucose, fructose and sucrose in both mango leaves and shoot tips were dramatically reduced while the total carbohydrate contents were maintained at a high level. Therefore, after the fruit maturity, mango leaves began to accumulate sugars without the transferring of sugar to sink which is led to an increasing of total carbohydrate content in the mango leaves and shoot tips.

8. There was no significance in chlorophyll content of mango leaves among the treatments. However, foliar application of kaolin twice a week significantly increased chlorophyll b content in leaf at 145 days of leaf age.

9. Foliar application of kaolin twice a week tended to increase the total number of fruit and the total fruit weight when compared to the control. Spraying of kaolin on the leaves and fruits commencing from fruit set up until the mature stage reduced the severity of anthracnose and fruit rot diseases during the post-harvest period.

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APPENDICES

Appendix A

Appendix A

1. Reagents in extraction and quantification of starch

1.1 MOPS buffer (50 mM, pH 7.0), calcium chloride (5 mM) and sodium azide (0.02% w/v).The 11.55g of MOPS (sodium salt, Sigma cat. no.M-9381) was dissolved in 900 mL of distilled water, and pH was adjusted to 7.0 by the addition of 1 M (10% v/v) hydrochloric acid (HCl; approximately 17 mL is required). After that, 0.74 g of calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$) and 0.2 g of sodium azide (NaN_3) were added and dissolved. The volume was adjusted to 1 L.

1.2 Sodium acetate buffer (200 mM, pH 4.5), sodium azide (0.02% w/v).

The 11.8 mL of glacial acetic acid (1.05 g mL; CH_3COOH) was added to 900 mL of distilled water. The pH was adjusted to 4.5 with 1 M (4g 100mL) sodium hydroxide (NaOH) solution (approximately 60 mL was required). After that, 0.2 g of sodium azide (0.2 g) was added and dissolved. The volume was adjusted to 1 L and the buffer was stored at room temperature.

2. Calculations for quantification of starch

$$\text{Starch} = \Delta_A \times F \times 1000 \times \frac{1}{1000} \times \frac{100}{W} \times \frac{162}{180}$$

$$= \Delta_A \times \frac{F \times 90}{W}$$

where : Δ_A = Absorbance (reaction) read against the reagent blank.

$$F = \frac{100\ (\mu g \text{ of glucose})}{\text{absorbance of } 100\ \mu g \text{ of glucose}} \quad \text{(conversion from absorbance to } \mu g)$$

1000 = Volume correction (0.1 mL taken from 100 mL)

$\frac{1}{1000}$ = Conversion from µg to mg.

$\frac{100}{W}$ = Factor to express "starch" as a percentage of flour weight

W = The weight in milligrams ("as is" basis) of the flour analysed.

$\frac{162}{180}$ = Adjustment from free glucose to anhydro glucose (as occurs in starch).

Starch % (dry wt. basis):

$$= \text{Starch \% (as is)} \times \frac{100}{100 - \text{moisture content (\% w/w)}}$$

3. Titratable acidity (TA)

5 ml of pulp extract is determined by titration with 0.1 N sodium hydroxide (NaOH), using 1% phenolphthalein as indicator. Acidity is expressed as percent citric acid.

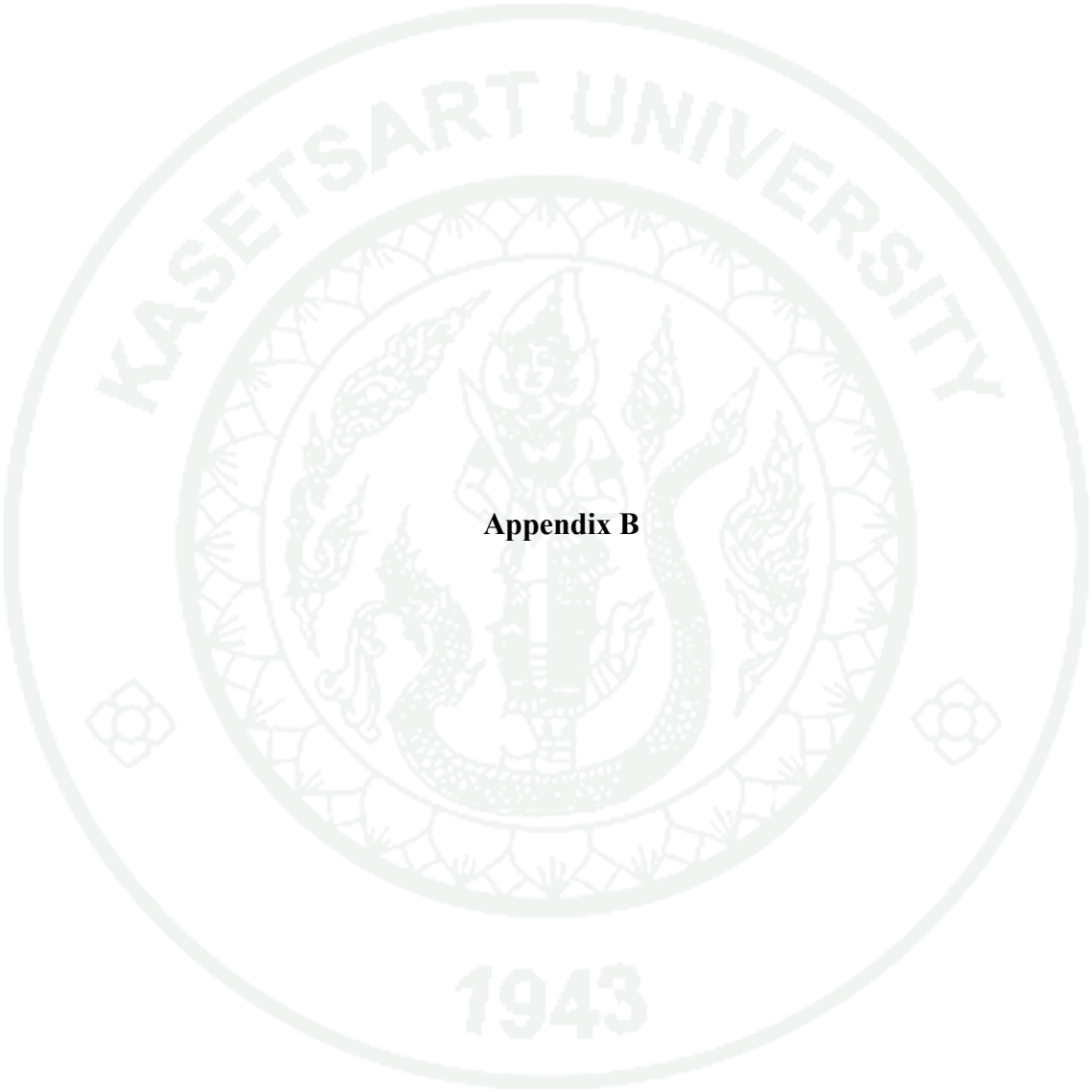
$$\%TA = \frac{(\text{ml NaOH})\,(\text{N NaOH})\,(\text{meq}) \times 100}{\text{ml of sample}}$$

Where; ml NaOH = Volume of 0.1 N NaOH used in ml

N NaOH = Concentration of NaOH in N; 0.1 N

Meq = Milliequivalent weight of citric acid in g/ milliequivalent; 0.064

ml of sample = Volume of sample in ml; 5 ml



Appendix B

Appendix B

Appendix Table B1 Effects of kaolin on chlorophyll a in mango leaves at 70, 90, 110 and 145 days of leaf ages.

Treatment	Leaf age (Days)			
	70	90	110	145
Control	0.094±0.0042	0.092±0.0034	0.087±0.0145	0.107±0.0071
K 1/week	0.096±0.0055	0.110±0.0149	0.102±0.0107	0.120±0.0072
K 2/week	0.092±0.0047	0.117±0.0158	0.103±0.0146	0.124±0.0118

Mean value of 4 replications (±S.E.)

[1/] Means followed by the same lowercase superscript letter within a column are not significantly different by Duncan's multiple range test at the $P \leq 0.05$ level.

Appendix Table B2 Effects of kaolin on chlorophyll b in mango leaves at 70, 90, 110 and 145 days of leaf ages.

Treatment	Leaf age (Days)			
	70	90	110	145
Control	0.019±0.0020	0.018±0.0016	0.018±0.0047	0.018±0.0018b[1/]
K 1/week	0.019±0.0016	0.023±0.0145	0.023±0.0029	0.027±0.0026a
K 2/week	0.018±0.0027	0.027±0.0061	0.024±0.0043	0.028±0.0036a

Mean value of 4 replications (±S.E.)

[1/] Means followed by the same lowercase superscript letter within a column are not significantly different by Duncan's multiple range test at the $P \leq 0.05$ level.

Appendix Table B3 Effects of kaolin on total chlorophyll in mango leaves at 70, 90, 110 and 145 days of leaf ages.

Treatment	Leaf age (Days)			
	70	90	110	145
Control	0.114±0.0048	0.110±0.0051	0.105±0.0191	0.125±0.0088
K 1/week	0.116±0.0071	0.133±0.0194	0.126±0.0136	0.147±0.0098
K 2/week	0.110±0.0075	0.144±0.0218	0.127±0.0188	0.153±0.0095

Mean value of 4 replications (±S.E.)

[1/] Means followed by the same lowercase superscript letter within a column are not significantly different by Duncan's multiple range test at the $P \leq 0.05$ level.

Appendix Table B4 Effect of kaolin on sucrose accumulation in leaves and shoots of mango at fruit's age 2, 5, 8, and 13 weeks.

Treatment	Leaf				Shoot			
	2 week	5 week	8 week	13week	2 week	5 week	8 week	13week
Control	2.33±0.15b[1/]	3.18±0.46b	2.39±0.59c	1.14±0.36b	0.08±0.00	1.47±0.30	0.76±0.13	0.12±0.03
K 1/week	5.85±1.46a	6.43±0.97a	6.82±0.30b	3.55±0.50a	0.12±0.01	2.73±0.47	0.37±0.07	0.10±0.02
K 2/week	6.53±0.68a	8.27±1.13a	8.95±0.61a	2.59±0.51ab	0.12±0.02	1.81±0.43	0.93±0.27	0.07±0.01

Mean value of 4 replications (±S.E.)

[1/] Means followed by the same lowercase superscript letter within a column are not significantly different by Duncan's multiple range test at the $P \leq 0.05$ level.

Appendix Table B5 Effect of kaolin on glucose accumulation in leaves and shoots of mango at fruit's age 2, 5, 8, and 13 weeks.

Treatment	Leaf				Shoot			
	2 week	5 week	8 week	13week	2 week	5 week	8 week	13week
Control	7.87±0.42a[1/]	3.03±0.25b	9.39±0.96	6.31±0.19	6.18±0.62	6.16±1.49	19.94±1.86	10.55±1.97
K 1/week	4.98±0.57b	6.44±0.71a	8.91±0.53	4.90±0.18	3.76±0.97	8.98±2.31	16.06±1.10	7.95±0.63
K 2/week	4.72±0.43b	6.72±0.51a	7.68±0.61	6.30±0.79	5.68±0.66	6.26±1.83	18.86±1.78	15.92±3.02

Mean value of 4 replications (±S.E.)

[1/] Means followed by the same lowercase superscript letter within a column are not significantly different by Duncan's multiple range test at the $P \leq 0.05$ level.

Appendix Table B6 Effect of kaolin on fructose accumulation in leaves and shoots of mango at fruit's age 2, 5, 8, and 13 weeks.

Treatment	Leaf				Shoot			
	2 week	5 week	8 week	13week	2 week	5 week	8 week	13week
Control	8.51±0.55a[1/]	3.86±0.44	13.70±1.05a	7.31±1.03	9.15±1.02	10.25±2.04	28.84±1.68	18.89±3.44
K 1/week	4.52±0.72b	6.50±0.71	12.61±0.85ab	5.09±0.75	7.66±0.38	11.18±2.12	26.87±1.70	14.74±2.33
K 2/week	6.48±0.81ab	6.68±1.29	8.74±1.69b	5.15±1.22	10.33±1.13	8.72±2.10	29.15±1.06	24.65±4.65

Mean value of 4 replications (±S.E.)

[1/] Means followed by the same lowercase superscript letter within a column are not significantly different by Duncan's multiple range test at the $P \leq 0.05$ level.

Appendix Table B7 Effect of kaolin on total carbohydrate in leaves and shoots of mango at fruit's age 2, 5, 8, and 13 weeks.

Treatment	Leaf				Stem			
	2 week	5 week	8 week	13week	2 week	5 week	8 week	13week
Control	198.62±8.29	249.34±11.95	257.30±2.04a[1/]	439.14±17.51	113.02±5.31	241.71±13.80	277.50±15.31	493.46±22.34
K 1/week	209.15±5.27	272.82±23.39	264.51±7.10a	489.84±2.36	104.60±10.44	225.41±17.87	262.24±48.98	461.92±5.94
K 2/week	210.60±11.66	231.34±20.35	203.31±9.06b	476.48±25.46	105.37±7.57	232.16±19.46	267.25±14.10	473.31±28.97

Mean value of 4 replications (±S.E.)

[1/] Means followed by the same lowercase superscript letter within a column are not significantly different by Duncan's multiple range test at the $P \leq 0.05$ level.

Appendix Table B8 Effect of kaolin on fruit damage by anthracnose and fruit rot disease of ripenning.

Treatment	Disease severity of fruit									
	Before					After				
	Level 0	Level 1	Level 2	Level 3	Level 4	Level 0	Level 1	Level 2	Level 3	Level 4
Control	72.5±4.33b[1/]	27.5±4.33a	0±0.00	0±0.00	0±0.00	0±0.00b	82.5±4.78	15±2.88a	2.5±2.50	0±0.00
Kaolin 1/week	88.75±5.54a	11.25±5.54b	0±0.00	0±0.00	0±0.00	6.25±3.75ab	80±3.53	11.25±3.14ab	2.5±1.44	0±0.00
Kaolin 2/week	87.5±2.50a	12.5±2.50b	0±0.00	0±0.00	0±0.00	12.5±4.78a	77.5±2.50	6.25±1.25b	3.75±3.75	0±0.00

Mean value of 4 replications (±S.E.)

[1/] Means followed by the same lowercase superscript letter within a column are not significantly different by Duncan's multiple range test at the $P \leq 0.05$ level.

Disease severity of fruit: Level 0 = no disease spots (healthy fruit), Level 1 = 1–25%, Level 2 = 26–50%, Level 3 = 51–75% and Level 4 = 76–100% disease area.

Appendix Table B9 Statistical analysis of average net photosynthesis (A) after spraying kaolin onto mango leaves at 70 days of leaf ages during midday (11:00 to 14:00 hours).

Source	Sum of squares	df	Mean square	F	Sig.
treatment	6.29	2	3.14	1.67	0.19
error	84.46	45	1.87		
total	90.75	47			

Appendix Table B10 Statistical analysis of stomatal conductance (g_s) after spraying kaolin onto mango leaves at 70 days of leaf ages during midday (11:00 to 14:00 hours).

Source	Sum of squares	df	Mean square	F	Sig.
treatment	454.22	2	227.11	0.73	0.48
error	14003.33	45	311.18		
total	14457.55	47			

Appendix Table B11 Statistical analysis of leaf temperature (T_{leaf}) after spraying kaolin onto mango leaves at 70 days of leaf ages during midday (11:00 to 14:00 hours).

Source	Sum of squares	df	Mean square	F	Sig.
treatment	6.91	2	3.45	0.97	0.38
error	159.86	45	3.55		
total	166.77	47			

Appendix Table B12 Statistical analysis of leaf to air vapor pressure deficit ($VPD_{leaf-air}$) after spraying kaolin onto mango leaves at 70 days of leaf ages during midday (11:00 to 14:00 hours).

Source	Sum of squares	df	Mean square	F	Sig.
treatment	1.25	2	0.63	1.02	0.36
error	27.60	45	0.61		
total	28.85	47			

Appendix Table B13 Statistical analysis of intercellular CO_2 concentration (C_i) after spraying kaolin onto mango leaves at 70 days of leaf ages during midday (11:00 to 14:00 hours).

Source	Sum of squares	df	Mean square	F	Sig.
treatment	15675.54	2	7837.77	3.83	0.29
error	92098.12	45	2046.62		
total	107773.67	47			

Appendix Table B14 Statistical analysis of transpiration rate (E) after spraying kaolin onto mango leaves at 70 days of leaf ages during midday (11:00 to 14:00 hours).

Source	Sum of squares	df	Mean square	F	Sig.
treatment	1.35	2	0.62	1.27	0.28
error	21.76	45	0.48		
total	22.99	47			

Appendix Table B15 Statistical analysis of average net photosynthesis (A) after spraying kaolin onto mango leaves at 90 days of leaf ages during midday (11:00 to 14:00 hours).

Source	Sum of squares	df	Mean square	F	Sig.
treatment	0.06	2	0.03	0.02	0.98
error	88.30	45	1.96		
total	88.36	47			

Appendix Table B16 Statistical analysis of stomatal conductance (g_s) after spraying kaolin onto mango leaves at 90 days of leaf ages during midday (11:00 to 14:00 hours).

Source	Sum of squares	df	Mean square	F	Sig.
treatment	119.02	2	59.51	0.08	0.92
error	31211.62	45	693.59		
total	31330.64	47			

Appendix Table B17 Statistical analysis of leaf temperature (T_{leaf}) after spraying kaolin onto mango leaves at 90 days of leaf ages during midday (11:00 to 14:00 hours).

Source	Sum of squares	df	Mean square	F	Sig.
treatment	0.79	2	0.39	0.06	0.94
error	287.71	45	6.39		
total	288.50	47			

Appendix Table B18 Statistical analysis of leaf to air vapor pressure deficit ($VPD_{leaf-air}$) after spraying kaolin onto mango leaves at 90 days of leaf ages during midday (11:00 to 14:00 hours).

Source	Sum of squares	df	Mean square	F	Sig.
treatment	0.05	2	0.03	0.05	0.95
error	25.33	45	0.56		
total	25.38	47			

Appendix Table B19 Statistical analysis of intercellular CO_2 concentration (C_i) after spraying kaolin onto mango leaves at 90 days of leaf ages during midday (11:00 to 14:00 hours).

Source	Sum of squares	df	Mean square	F	Sig.
treatment	996.29	2	498.14	0.28	0.75
error	77566.68	45	1723.70		
total	78562.97	47			

Appendix Table B20 Statistical analysis of transpiration rate (E) after spraying kaolin onto mango leaves at 90 days of leaf ages during midday (11:00 to 14:00 hours).

Source	Sum of squares	df	Mean square	F	Sig.
treatment	0.08	2	0.04	0.19	0.82
error	10.03	45	0.22		
total	10.12	47			

Appendix Table B21 Statistical analysis of average net photosynthesis (A) after spraying kaolin onto mango leaves at 110 days of leaf ages during midday (11:00 to 14:00 hours).

Source	Sum of squares	df	Mean square	F	Sig.
treatment	0.21	2	0.10	0.40	0.67
error	11.49	45	0.25		
total	11.70	47			

Appendix Table B22 Statistical analysis of stomatal conductance (g_s) after spraying kaolin onto mango leaves at 110 days of leaf ages during midday (11:00 to 14:00 hours).

Source	Sum of squares	df	Mean square	F	Sig.
treatment	971.05	2	485.52	2.62	0.08
error	8331.06	45	185.13		
total	9302.11	47			

Appendix Table B23 Statistical analysis of leaf temperature (T_{leaf}) after spraying kaolin onto mango leaves at 110 days of leaf ages during midday (11:00 to 14:00 hours).

Source	Sum of squares	df	Mean square	F	Sig.
treatment	0.04	2	0.02	0.01	0.98
error	74.12	45	1.65		
total	74.16	47			

Appendix Table B24 Statistical analysis of leaf to air vapor pressure deficit ($VPD_{leaf-air}$) after spraying kaolin onto mango leaves at 110 days of leaf ages during midday (11:00 to 14:00 hours).

Source	Sum of squares	df	Mean square	F	Sig.
treatment	0.002	2	0.00	0.004	0.99
error	9.44	45	0.21		
total	9.44	47			

Appendix Table B25 Statistical analysis of intercellular CO_2 concentration (C_i) after spraying kaolin onto mango leaves at 110 days of leaf ages during midday (11:00 to 14:00 hours).

Source	Sum of squares	df	Mean square	F	Sig.
treatment	488.79	2	244.39	0.25	0.77
error	43166.18	45	959.25		
total	43654.97	47			

Appendix Table B26 Statistical analysis of transpiration rate (E) after spraying kaolin onto mango leaves at 110 days of leaf ages during midday (11:00 to 14:00 hours).

Source	Sum of squares	df	Mean square	F	Sig.
treatment	0.68	2	0.34	3.34	0.04
error	4.63	45	0.10		
total	5.32	47			

Appendix Table B27 Statistical analysis of average net photosynthesis (A) after spraying kaolin onto mango leaves at 145 days of leaf ages during midday (11:00 to 14:00 hours).

Source	Sum of squares	df	Mean square	F	Sig.
treatment	31.20	2	15.60	4.36	0.02
error	161.00	45	3.58		
total	192.20	47			

Appendix Table B28 Statistical analysis of stomatal conductance (g_s) after spraying kaolin onto mango leaves at 145 days of leaf ages during midday (11:00 to 14:00 hours).

Source	Sum of squares	df	Mean square	F	Sig.
treatment	4119.47	2	2059.73	8.75	0.001
error	10587.38	45	235.27		
total	14706.85	47			

Appendix Table B29 Statistical analysis of leaf temperature (T_{leaf}) after spraying kaolin onto mango leaves at 145 days of leaf ages during midday (11:00 to 14:00 hours).

Source	Sum of squares	df	Mean square	F	Sig.
treatment	0.97	2	0.48	1.09	0.34
error	20.02	45	0.44		
total	20.99	47			

Appendix Table B30 Statistical analysis of leaf to air vapor pressure deficit ($VPD_{leaf-air}$) after spraying kaolin onto mango leaves at 145 days of leaf ages during midday (11:00 to 14:00 h).

Source	Sum of squares	df	Mean square	F	Sig.
treatment	0.14	2	0.07	0.66	0.52
error	4.80	45	0.11		
total	4.94	47			

Appendix Table B31 Statistical analysis of intercellular CO_2 concentration (C_i) after spraying kaolin onto mango leaves at 145 days of leaf ages during midday (11:00 to 14:00 h).

Source	Sum of squares	df	Mean square	F	Sig.
treatment	5648.37	2	2824.18	1.49	0.23
error	85317.44	45	1895.94		
total	90965.81	47			

Appendix Table B32 Statistical analysis of transpiration rate (E) after spraying kaolin onto mango leaves at 145 days of leaf ages during midday (11:00 to 14:00 h).

Source	Sum of squares	df	Mean square	F	Sig.
treatment	1.47	2	0.74	7.96	0.001
error	4.16	45	0.09		
total	5.64	47			

Appendix Table B33 Statistical analysis of sucrose accumulation in mango leaves at fruit's age 2 weeks.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	40.53	2	20.26	5.77	0.02
error	31.58	9	3.51		
total	72.12	11			

Appendix Table B34 Statistical analysis of sucrose accumulation in mango leaves at fruit's age 5 weeks.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	53.17	2	26.58	8.10	0.01
error	29.52	9	3.28		
total	82.69	11			

Appendix Table B35 Statistical analysis of sucrose accumulation in mango leaves at fruit's age 8 weeks.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	89.77	2	44.88	40.77	0.00
error	9.91	9	1.10		
total	99.68	11			

Appendix Table B36 Statistical analysis of sucrose accumulation in mango leaves at fruit's age 13 weeks.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	11.78	2	5.89	6.82	0.01
error	7.78	9	0.86		
total	19.57	11			

Appendix Table B37 Statistical analysis of glucose accumulation in mango leaves at fruit's age 2 weeks.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	24.41	2	12.21	13.06	0.002
error	8.41	9	0.93		
total	32.82	11			

Appendix Table B38 Statistical analysis of glucose accumulation in mango leaves at fruit's age 5 weeks.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	33.79	2	16.90	15.45	0.001
error	9.84	9	1.09		
total	43.64	11			

Appendix Table B39 Statistical analysis of glucose accumulation in mango leaves at fruit's age 8 weeks.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	6.25	2	3.12	1.47	0.28
error	19.14	9	2.12		
total	25.38	11			

Appendix Table B40 Statistical analysis of glucose accumulation in mango leaves at fruit's age 13 weeks.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	5.24	2	2.62	2.77	0.11
error	8.49	9	0.94		
total	13.74	11			

Appendix Table B41 Statistical analysis of fructose accumulation in mango leaves at fruit's age 2 weeks.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	31.82	2	15.91	8.02	0.01
error	17.85	9	1.98		
total	49.67	11			

Appendix Table B42 Statistical analysis of fructose accumulation in mango leaves at fruit's age 5 weeks.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	19.89	2	9.94	3.13	0.09
error	28.53	9	3.17		
total	48.42	11			

Appendix Table B43 Statistical analysis of fructose accumulation in mango leaves at fruit's age 8 weeks.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	54.10	2	27.05	4.32	0.04
error	56.28	9	6.25		
total	110.38	11			

Appendix Table B44 Statistical analysis of fructose accumulation in mango leaves at fruit's age 13 weeks.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	12.73	2	6.36	1.53	0.26
error	37.47	9	4.16		
total	50.21	11			

Appendix Table B45 Statistical analysis of total carbohydrate accumulation in mango leaves at fruit's age 2 weeks.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	340.80	2	170.40	0.55	0.59
error	2793.12	9	310.35		
total	3133.92	11			

Appendix Table B46 Statistical analysis of total carbohydrate accumulation in mango leaves at fruit's age 5 weeks.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	3461.60	2	1730.80	1.17	0.35
error	13252.77	9	1472.53		
total	16714.37	11			

Appendix Table B47 Statistical analysis of total carbohydrate accumulation in mango leaves at fruit's age 8 weeks.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	8948.50	2	4474.25	24.54	0.00
error	1641.07	9	182.34		
total	10589.58	11			

Appendix Table B48 Statistical analysis of total carbohydrate accumulation in mango leaves at fruit's age 13 weeks.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	5524.40	2	2762.20	2.16	0.17
error	11527.43	9	1280.83		
total	17051.83	11			

Appendix Table B49 Statistical analysis of sucrose accumulation in mango shoots at fruit's age 2 weeks.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	0.003	2	0.002	1.27	0.327
error	0.012	9	0.001		
total	0.015	11			

Appendix Table B50 Statistical analysis of sucrose accumulation in mango shoots at fruit's age 5 weeks.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	3.38	2	1.69	2.49	0.14
error	6.09	9	0.67		
total	9.47	11			

Appendix Table B51 Statistical analysis of sucrose accumulation in mango shoots at fruit's age 8 weeks.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	0.65	2	0.33	2.52	0.13
error	1.16	9	0.13		
total	1.82	11			

Appendix Table B52 Statistical analysis of sucrose accumulation in mango shoots at fruit's age 13 weeks.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	0.004	2	0.002	0.89	0.44
error	0.022	9	0.002		
total	0.027	11			

Appendix Table B53 Statistical analysis of glucose accumulation in mango shoots at fruit's age 2 weeks.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	13.01	2	6.51	2.74	0.12
error	21.36	9	2.37		
total	34.37	11			

Appendix Table B54 Statistical analysis of glucose accumulation in mango shoots at fruit's age 5 weeks.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	20.40	2	10.20	0.69	0.52
error	131.62	9	14.62		
total	152.02	11			

Appendix Table B55 Statistical analysis of glucose accumulation in mango shoots at fruit's age 8 weeks.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	32.00	2	16.00	1.53	0.27
error	94.25	9	10.47		
total	126.25	11			

Appendix Table B56 Statistical analysis of glucose accumulation in mango shoots at fruit's age 13 weeks.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	131.92	2	65.95	3.67	0.06
error	161.60	9	17.95		
total	293.52	11			

Appendix Table B57 Statistical analysis of fructose accumulation in mango shoots at fruit's age 2 weeks.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	14.27	2	7.14	2.15	0.17
error	29.78	9	3.31		
total	44.06	11			

Appendix Table B58 Statistical analysis of fructose accumulation in mango shoots at fruit's age 5 weeks.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	12.27	2	6.14	0.35	0.71
error	157.91	9	17.54		
total	170.18	11			

Appendix Table B59 Statistical analysis of fructose accumulation in mango shoots at fruit's age 8 weeks.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	12.20	2	6.10	0.67	0.54
error	82.24	9	9.14		
total	94.44	11			

Appendix Table B60 Statistical analysis of fructose accumulation in mango shoots at fruit's age 13 weeks.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	197.95	2	98.97	1.91	0.20
error	467.30	9	51.92		
total	665.26	11			

Appendix Table B61 Statistical analysis of total carbohydrate accumulation in mango shoots at fruit's age 2 weeks.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	173.55	2	86.77	0.33	0.72
error	2338.37	9	259.82		
total	2511.92	11			

Appendix Table B62 Statistical analysis of total carbohydrate accumulation in mango shoots at fruit's age 5 weeks.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	536.37	2	268.19	0.22	0.80
error	10664.76	9	1184.97		
total	11201.14	11			

Appendix Table B63 Statistical analysis of total carbohydrate accumulation in mango shoots at fruit's age 8 weeks.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	483.69	2	241.85	0.06	0.94
error	34001.07	9	3777.89		
total	34484.77	11			

Appendix Table B64 Statistical analysis of total carbohydrate accumulation in mango shoots at fruit's age 13 weeks.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	2041.83	2	1020.92	0.55	0.59
error	16491.17	9	1832.35		
total	18533.00	11			

Appendix Table B65 Mean of sucrose, glucose, fructose and total carbohydrate accumulation after spraying kaolin in mango leaves at fruit's age 2, 5, 8, and 13 weeks. Each symbol represents the mean value of the four replications (± SE).

	sucrose	glucose	fructose	total carbohydrate
Treatments				
Control	2.26±0.27Y	6.65±0.65	8.34±0.98X	286.11±24.09Y
K 1/week	5.66±0.53X	6.31±0.48	7.18±0.89XY	309.08±28.24X
K 2/week	6.59±0.73X	6.35±0.39	6.77±0.67Y	280.43±30.42Y
Sampling times				
2 week	4.91±0.74A	5.86±0.50B	6.50±0.61B	206.13±4.87C
5 week	5.96±0.79A	5.40±0.57B	5.68±0.60B	251.17±38.98B
8 week	6.05±0.87A	8.66±0.44A	11.68±0.91A	241.71±8.96B
13 week	2.43±0.38B	5.84±0.32B	5.85±0.62B	468.49±11.36A

Treatments mean followed by the same letter (X, Y) are not significantly different ($P \leq 0.05$) using Duncan's multiple range tests.

Sampling times mean followed by the same letter (A, B. C) are not significantly different ($P \leq 0.05$) using Duncan's multiple range tests.

Appendix Table B66 Mean of sucrose, glucose, fructose and total carbohydrate accumulation after spraying kaolin in mango shoots at fruit's age 2, 5, 8, and 13 weeks. Each symbol represents the mean value of the four replications (± SE).

	sucrose	glucose	fructose	total carbohydrate
Treatments				
Control	0.61±0.16	10.71±1.61	16.79±2.27	281.43±36.00
K 1/week	0.83±0.30	9.19±1.30	15.12±2.03	263.55±35.27
K 2/week	0.73±0.22	11.68±1.75	18.22±2.58	269.52±35.19
Sampling times				
2 week	0.11±0.01C	5.21±0.51C	9.05±0.58C	107.66±4.36D
5 week	2.00±0.27A	7.13±1.07C	10.05±1.14C	233.10±9.21C
8 week	0.68±0.12B	18.30±0.98A	28.30±0.85A	269.00±16.16B
13 week	0.10±0.01C	11.48±1.49B	19.43±2.24B	476.23±41.05A

Sampling times means followed by the same letter (A, B, C) are not significantly different ($P \leq 0.05$) using Duncan's multiple range tests.

Appendix Table B67 Statistical analysis of effect of kaolin on sucrose accumulation in mango leaf comparisons between sampling time (at fruit's age 2, 5, 8, and 13 weeks) and all treatments.

Source	Sum of squares	df	Mean square	F	Sig.
Corrected Model	297.887(a)	11	27.081	12.373	.000
Intercept	1123.576	1	1123.576	513.337	.000
TIME	102.611	3	34.204	15.627	.000
TREAT	166.037	2	83.018	37.929	.000
TIME * TREAT	29.238	6	4.873	2.226	.063
Error	78.796	36	2.189		
Total	1500.258	48			
Corrected Total	376.683	47			

Appendix Table B68 Statistical analysis of effect of kaolin on glucose accumulation in mango leaf comparisons between sampling time (at fruit's age 2, 5, 8, and 13 weeks) and all treatments.

Source	Sum of squares	df	Mean square	F	Sig.
Corrected Model	150.356(a)	11	13.669	10.723	.000
Intercept	1989.670	1	1989.670	1560.817	.000
TIME	80.650	3	26.883	21.089	.000
TREAT	1.093	2	.547	.429	.655
TIME * TREAT	68.613	6	11.436	8.971	.000
Error	45.891	36	1.275		
Total	2185.918	48			
Corrected Total	196.248	47			

Appendix Table B69 Statistical analysis of effect of kaolin on fructose accumulation in mango leaf comparisons between sampling time (at fruit's age 2, 5, 8, and 13 weeks) and all treatments.

Source	Sum of squares	df	Mean square	F	Sig.
Corrected Model	411.980(a)	11	37.453	9.620	.000
Intercept	2649.739	1	2649.739	680.620	.000
TIME	293.439	3	97.813	25.125	.000
TREAT	21.367	2	10.683	2.744	.078
TIME * TREAT	97.174	6	16.196	4.160	.003
Error	140.152	36	3.893		
Total	3201.871	48			
Corrected Total	552.132	47			

Appendix Table B70 Statistical analysis of effect of kaolin on total carbohydrate accumulation in mango leaf comparisons between sampling time (at fruit's age 2, 5, 8, and 13 weeks) and all treatments.

Source	Sum of squares	df	Mean square	F	Sig.
Corrected Model	530893.607(a)	11	48263.055	59.473	.000
Intercept	4089121.231	1	4089121.231	5038.897	.000
TIME	512618.294	3	170872.765	210.561	.000
TREAT	7363.684	2	3681.842	4.537	.017
TIME * TREAT	10911.629	6	1818.605	2.241	.061
Error	29214.401	36	811.511		
Total	4649229.238	48			
Corrected Total	560108.007	47			

Appendix Table B71 Statistical analysis of effect of kaolin on sucrose accumulation in mango shoot comparisons between sampling time (at fruit's age 2, 5, 8, and 13 weeks) and all treatments.

Source	Sum of squares	df	Mean square	F	Sig.
Corrected Model	32.984(a)	11	2.999	14.796	.000
Intercept	25.306	1	25.306	124.872	.000
TIME	28.941	3	9.647	47.603	.000
TREAT	.400	2	.200	.986	.383
TIME * TREAT	3.644	6	.607	2.997	.018
Error	7.296	36	.203		
Total	65.586	48			
Corrected Total	40.280	47			

Appendix Table B72 Statistical analysis of effect of kaolin on glucose accumulation in mango shoot comparisons between sampling time (at fruit's age 2, 5, 8, and 13 weeks) and all treatments.

Source	Sum of squares	df	Mean square	F	Sig.
Corrected Model	1408.667(a)	11	128.061	11.276	.000
Intercept	5320.081	1	5320.081	468.464	.000
TIME	1211.333	3	403.778	35.555	.000
TREAT	50.438	2	25.219	2.221	.123
TIME * TREAT	146.896	6	24.483	2.156	.071
Error	408.831	36	11.356		
Total	7137.579	48			
Corrected Total	1817.498	47			

Appendix Table B73 Statistical analysis of effect of kaolin on fructose accumulation in mango shoot comparisons between sampling time (at fruit's age 2, 5, 8, and 13 weeks) and all treatments.

Source	Sum of squares	df	Mean square	F	Sig.
Corrected Model	3170.032(a)	11	288.185	14.072	.000
Intercept	13395.813	1	13395.813	654.131	.000
TIME	2933.315	3	977.772	47.746	.000
TREAT	77.004	2	38.502	1.880	.167
TIME * TREAT	159.713	6	26.619	1.300	.282
Error	737.236	36	20.479		
Total	17303.081	48			
Corrected Total	3907.268	47			

Appendix Table B74 Statistical analysis of effect of kaolin on total carbohydrate accumulation in mango shoot comparisons between sampling time (at fruit's age 2, 5, 8, and 13 weeks) and all treatments.

Source	Sum of squares	df	Mean square	F	Sig.
Corrected Model	846098.853(a)	11	76918.078	43.610	.000
Intercept	3538152.695	1	3538152.695	2006.028	.000
TIME	842863.393	3	280954.464	159.293	.000
TREAT	2651.354	2	1325.677	.752	.479
TIME * TREAT	584.107	6	97.351	.055	.999
Error	63495.376	36	1763.760		
Total	4447746.924	48			
Corrected Total	909594.229	47			

Appendix Table B75 Statistical analysis of effect of kaolin on total number of fruit in mango.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	3338.167	2	1669.083	1.133	0.364
error	13254.750	9	1472.750		
total	16592.917	11			

Appendix Table B76 Statistical analysis of effect of kaolin on total weight in mango.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	262.500	2	131.250	1.106	0.372
error	1067.750	9	118.639		
total	1330.250	11			

Appendix Table B77 Statistical analysis of effect of kaolin on fruit size (Width) in mango.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	2.280	2	1.140	10.074	0.000
error	26.824	237	0.113		
total	29.104	239			

Appendix Table B78 Statistical analysis of effect of kaolin on fruit size (Length) in mango.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	12.244	2	6.122	12.619	0.000
error	114.982	237	0.485		
total	127.226	239			

Appendix Table B79 Statistical analysis of effect of kaolin on fruit size (Thickness) in mango.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	3.306	2	1.653	17.673	0.000
error	22.171	237	0.094		
total	25.477	239			

Appendix Table B80 Statistical analysis of effect of kaolin on peel color characteristics (*L* = brightness) in mango.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	77.562	2	38.781	1.400	0.249
error	6565.136	237	27.701		
total	6642.698	239			

Appendix Table B81 Statistical analysis of effect of kaolin on peel color characteristics (*b* = yellowness) in mango.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	704.172	2	352.086	13.339	0.000
error	6255.535	237	26.395		
total	6959.708	239			

Appendix Table B82 Statistical analysis of effect of kaolin on juice quality (TSS=Total soluble solids) in mango.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	2.886	2	1.443	1.312	0.271
error	260.667	237	1.100		
total	263.553	239			

Appendix Table B83 Statistical analysis of effect of kaolin on juice quality (TA= titratable acidity) in mango.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	0.013	2	0.007	12.058	0.000
error	0.129	237	0.001		
total	0.142	239			

Appendix Table B84 Statistical analysis of effect of kaolin on healthy fruit (level 0) in mango before ripening induction.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	654.167	2	327.083	4.402	0.046
error	668.750	9	74.306		
total	1322.917	11			

Appendix Table B85 Statistical analysis of effect of kaolin on healthy fruit (level 0) in mango after ripening induction.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	312.500	2	156.250	3.169	0.091
error	443.750	9	49.306		
total	756.250	11			

Appendix Table B86 Statistical analysis of effect of kaolin on fruit damage (level 1) by anthracnose and fruit rot disease during before ripening induction.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	654.167	2	327.083	4.402	0.046
error	668.750	9	74.306		
total	1322.917	11			

Appendix Table B87 Statistical analysis of effect of kaolin on fruit damage (level 1) by anthracnose and fruit rot disease during after ripening induction.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	50.000	2	25.000	0.450	0.651
error	500.000	9	55.556		
total	550.000	11			

Appendix Table B88 Statistical analysis of effect of kaolin on fruit damage (level 2) by anthracnose and fruit rot disease during before ripening induction.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	0.000	2	0.000	-	-
error	0.000	9	0.000		
total	0.000	11			

Appendix Table B89 Statistical analysis of effect of kaolin on fruit damage (level 2) by anthracnose and fruit rot disease during after ripening induction.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	154.167	2	77.083	2.921	0.105
error	237.500	9	26.389		
total	391.667	11			

Appendix Table B90 Statistical analysis of effect of kaolin on fruit damage (level 3) by anthracnose and fruit rot disease during before ripening induction.

Source	Sum of squares	df	Mean square	F	Sig.
treatment	0.000	2	0.000	-	-
error	0.000	9	0.000		
total	0.000	11			

Appendix Table B91 Statistical analysis of effect of kaolin on fruit damage (level 3) by anthracnose and fruit rot disease during after ripening induction.

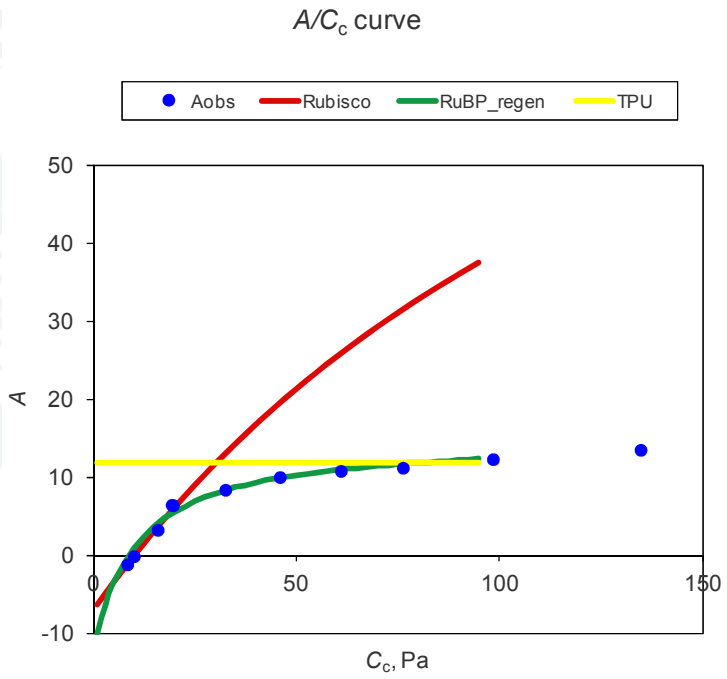
Source	Sum of squares	df	Mean square	F	Sig.
treatment	4.167	2	2.083	0.070	0.933
error	268.750	9	29.861		
total	272.917	11			

Appendix Table B92 Statistical analysis of effect of kaolin on fruit damage (level 4) by anthracnose and fruit rot disease during before ripening induction.

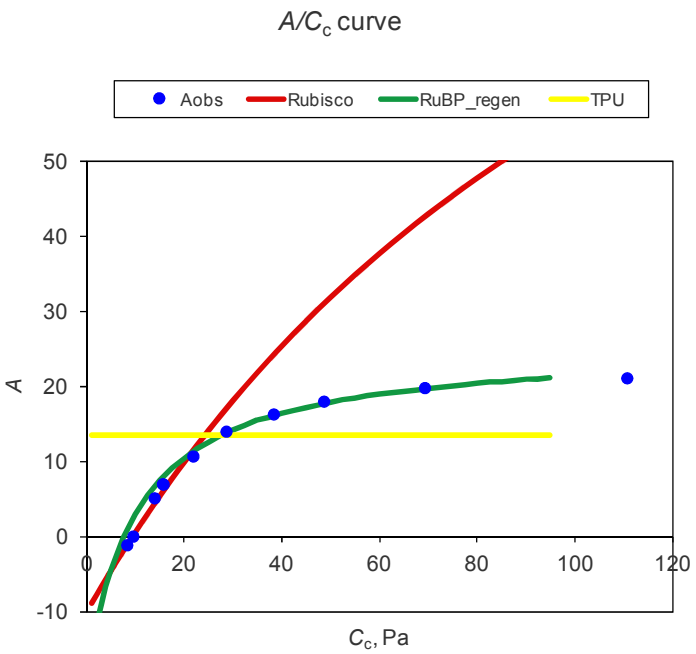
Source	Sum of squares	df	Mean square	F	Sig.
treatment	0.000	2	0.000	-	-
error	0.000	9	0.000		
total	0.000	11			

Appendix Table B93 Statistical analysis of effect of kaolin on fruit damage (level 4) by anthracnose and fruit rot disease during after ripening induction.

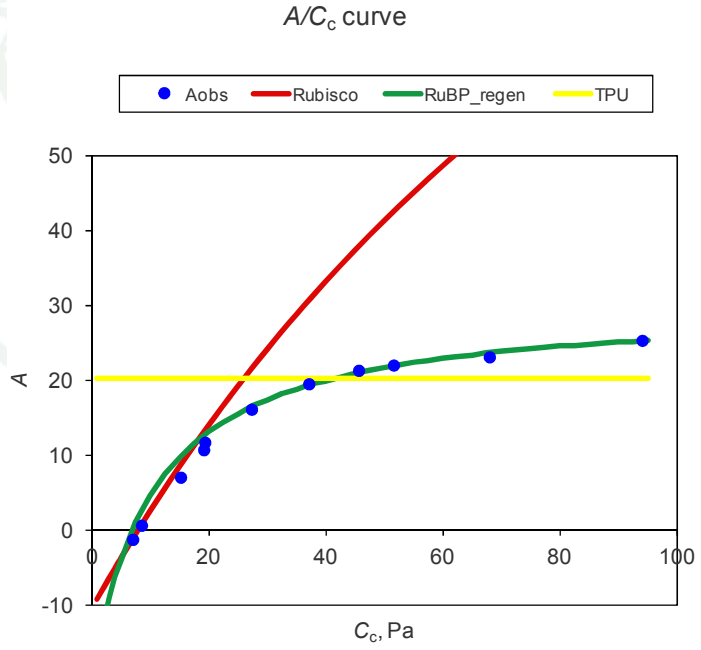
Source	Sum of squares	df	Mean square	F	Sig.
treatment	0.000	2	0.000	-	-
error	0.000	9	0.000		
total	0.000	11			



Appendix Figure B1 Example of A-C_i curve fitting in leaves untreated with kaolin at 70 days of leaf ages.



Appendix Figure B2 Example of A-C_i curve fitting in leaves treated with kaolin once a week at 70 days of leaf ages.



Appendix Figure B3 Example of A-C_i curve fitting in leaves treated with kaolin twice a week at 70 days of leaf ages.

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