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Effects of Paclobutrazol on yield and quality of Mao Laung (*Antidesma thwaitesianum* Müll. Arg.) Cultivar

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Abstract The effects of paclobutrazol at different rates on flowering, fruit and quality of Mao Luang were compared to investigate the residue of the chemical in soil and its products. Uniform plants of of Fah Prathan variety with 6 years old were selected in the farmer plantation. The treatments were significantly different for flowering development, inflorescence per branch, inflorescence length percentage of fruit maturity and yield per plant. Paclobutrazol at the concentration of 200 ppm had the highest flower induction, fruiting and quality of Mao Luang fruits, which showed good physical characteristics, and chemical compositions of fruits. This concentration had the highest total flavonoid content in fruits. Paclobutrazol residues in soil, leaf and fruits were lower than the maximum limited residues. The results indicated that application of paclobutrazol on Mao Luang is safe for consumers.

Keywords: Chemical compositions, Flowering induction, Fruit quality, Maximum limit, Soil chemical residue, Value-added products

Introduction

Mao Luang (*Antidesma thwaitesianum* Müll. Arg.) is a neglected and underutilized wild species of Phyanthaceae family. This genus is native to tropical Africa, South Asia, East Asia, Southeast Asia, Australia, and various oceanic islands, and there are 101 accepted species in the genus. Mao Luang distributes in Southeast Asia, and high diversity is found in Thailand especially in the Northeast.

Mao Luang berries contain several phytochemicals such as phenolic compounds, flavonoid and anthocyanin beneficial to human health (Butkhup

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and Samappito, 2008; Jorjong *et al.*, 2015). These phytochemicals have medicinal properties as antioxidants to prevent cancer, diabetes and other non contagious diseases (Belina-Aldemita *et al.,* 2013).

Mao Luang industry has been well established in Sakhon Nakhon province in the Northeast of Thailand, generating important income for farmers and its related industries. The products are currently available in supermarkets. The demand for processed products such as wine and ready to drink juice and concentrated juice increases constantly as Mao Luang is known among health lover consumers.

In 2017, the province has 15 processing plants, and the demand for ripe berries has been estimated at 550 tons per annum. However, the production capacity is only 200-300 tons per annum. The current climate changes also causes yield reduction and yield fluctuation in Mao Luang. These problems make the berry shortage more severe because some Mao Luang growers are reluctant to continue their plantations, and small numbers of new plantations are established. Moreover, the processing plants reduced their production capacity.

In 2021, the province has 15 processing plants, and the demand for ripe berries has been estimated at 200-300 tons per annum. However, the production capacity is only 100-150 tons per annum. The unsustainable situation of the industry required a timely and rapid solving of the problems. This is because Mao Luang is a wild plant which was domesticated for some decades, and the production of Mao Luang is difficult to obtain high yield and high quality fruits. There are two main problems of the industry including raw material shortage and short production season of Mao Luang, which cause low efficiency of the industry.

Bulk information is available for application of paclobutrazol in fruit crops to induce flowering especially for off-season production of fruit crops. Paclobutrazol inhibits gibberellins synthesis and increases cytokinins (Desta and Amare, 2021). As gibberellins is responsible for promotion of vegetative growth, reduction in gibberellins synthesis results in accumulation of more food for flowering, fruit setting and fruit yield (Srivastava, 2002). Furthermore, paclobutrazol increases leaf chlorophyll and photosynthesis and induces resistance to adverse environments (Xia *et al.,* 2018).

However, the information on the responses of Mao Luang to paclobutrazol is not well understood. Moreover, it residues in soil and fruits have not been clearly studied. The objectives were to find the suitable concentration of paclobutrazol in inducing flowering and fruit setting in Mao Luang and providing high quality fruits, to investigate residues of paclobutrazol in soil, leaves and ripe fruits of Mao Luang and to stuydy the effects of

paclobutrazol on total flavonoid content, total phenolic content and antioxidant capacity determined by DPPH method. The information gained from our study is important for improving production efficiency of Mao Luang.

Materials and methods

Plant materials and experimental design

Mao Luang, Fah Prathan variety, was used for the experiment. Luang is a Thai word of the North dialect and the Northeast dialect, which means “big”. This species is characterized by its big fruits compared to other species in the same genus suitable for processing. The experiment was conducted in a famer plantation, which was planted for six years.

Five paclobutrazol concentrations including 0 ppm (control), 100 ppm, 200 ppm, 400 ppm and 600 ppm were laid out in a completely randomized design with four replications, and there was one plant in each replication.

The plants were pruned, and after pruning, the crop was irrigated for a week to provide sufficient soil moisture. Cattle manure was applied to the crop at the rate of 20 kg per plant, and mixed chemical fertilizer formula 15-15-15 of N-P-K was applied at the rate of 1 kg per plant.

Paclobutrazol was applied on the soil surface under the canopy on 1 December 2017 at approximately three months after the crop was harvested. The application rates were according to the treatments, and blank water was also applied as a control treatment.

Paclobutazol residues in the soil, leaves and ripe fruit were determined. Antioxidant activity determined by DPPH, total phenolic content (TPC), and total flavonoid content (TFC) were also determined in ripe fruits.

Sample preparation

Sample preparation method of ripe fruits for total phenolic content total flavonoid contents and antioxidant capacity is described below. The berry samples were harvested from the plantation at physiological ripening stage. The samples were kept in sealable plastic bags, stored in ice box and transported immediately to the laboratory.

The samples were washed with distilled water and pitted to remove seeds. The pitted fruits were weighted, and 5 g of the samples were fine ground using mortar and pestle. The ground samples were added with 25 ml ethanol, and the mixed samples were sonicated for 20 minutes. Then, the sonicated samples were centrifuged at 19,000 rounds per minutes. After centrifugation, the

samples were set aside at room temperature for 10 minutes. This extraction method has been proved to be adequate for complete extraction. After 10-min of centrifugation, the supernatant was collected for the determination of antioxidant activities and polyphenolic compounds.

Sample preparation for paclobutrazol determination was described as follows. 5 g of soil samples, leaf samples and fruit samples were loaded in 50 ml centrifuge tubes. Water of 10 ml was added to each tube, and the samples were shaken for 1 minute. The samples were then sonicated for 15 minutes. After sonication, 5.5 g of QuEChERS Extract Pouch, EN Method (5982-0650) was added to the samples, and the samples were centrifuged at 5000 rpm for 5 minutes. 6 ml of the supernatants were loaded in centrifuge tube (volume 15 ml) containing Elut QuEChERS Dispersive Universal SPE and shaken for 1 minute. The samples were centrifuged at 5000 rpm for 5 minutes. The samples were filtered with filter paper (0.2 µm), and paclobutrazol concentration was determined in liquid chromatography mass spectrometry (LC-MS/MS).

Determination of total phenolic content

Total phenolic content (TPC) was determined spectrophotometrically by Folin-Ciocalteau assay (Singleton and Rossi, 1965). Standard solutions of gallic acid at different concentrations (0.5–100 mg/L) were used for the calibration curve. Briefly, 12.5 µL of the fruit extract or gallic acid standard was mixed with 12.5 µL of 10% Folin-Ciocalteu reagent and 125 µL of 20% Na_2CO_3 solution. The mixture was incubated in the dark for 90 min at room temperature and then measured at 760 nm. TPC was expressed as mg gallic acid equivalent per 100 g fresh weight (mg GAE/100 g FW).

Determination of total flavonoid content

Total flavonoid contents (TFC) were quantified using a colorimetric assay with slight modifications. Standard solutions of catechin at different concentrations (0.5–150 mg/L) were used for the calibration curve. In brief, 25 µL of the leaf extract or catechin standard was mixed with 75 µL of 5% $NaNO_3$ and the mixture was left at room temperature for 5 min. After the addition of 150 µL of 10% $AlCl_3$ and left at room temperature for 5 min, 50 µL of 1 M NaOH was added to the mixture. Then the mixture was determined at 510 nm. TFC was expressed as mg catechin equivalent per 100 g fresh weight (mg CE/100 g FW).

DPPH scavenging assay

The antioxidant capacity of the leaf extracts was evaluated by DPPH (2,2-diphenyl-1-picrylhydrazyl hydrate) free radical method as described earlier by Akowuah *et al.* (2005) with minor modifications. Standard solutions of vitamin C at different concentrations (0–200 µM) were used for the calibration curve. In brief, 0.2 mM DPPH methanolic solution and the leaf extract were mixed in equal amounts (100 µL). After thorough mixing and incubation under dark conditions for 1 h, the mixture was determined at 520 nm. The results were expressed in mg vitamin C equivalent antioxidative capacity per g fresh weight (mg VCEAC/g FW).

Determination of paclobutrazol residues

EN Method (5982-0650) was used for sample preparation of soil, leaves and ripe berries. The samples were analyzed for paclobutrazol residues using liquid chromatography–mass spectrometry (LC-MS/MS)**.** LC/MS/MS (Shimadzu LCMS-8030 triple quadrupole mass spectrometer) was carried out in electrospray ionization (ESI) mode, and a HPLC system (Shimadzu, Kyoto, Japan) was also used. Gradient elution of curcuminoids was carried out on an InertSustain® C18 (2.1 × 150 mm, 3 µm) column coupled with a guard column. Formic acid 0.1% (v/v) in deionized water and acetonitrile at a flow rate of 0.2 mL/min and 37 ℃ column temperature (solvent A were used as solvent B in mobile phase. Gradient elution times consisted of 0–3 min from 90% B to 50% B, 3–5 min from 50% B to 20% B, and 5–8 min from 20% B to 90%. The MS/MS condition was carried out according to the method suggested by El-Hawaz *et al.* (2016).

Data collection

Plant data were recorded for number of leaves per branch, percentage of defoliated leaves, percentage of detached leaves, SPAD chlorophyll meter reading (SCMR), percentage of flower shoots per branch at emergence and 30 days after emergence, flower cluster length, flower cluster diameter, flower cluster weight, number of fruit clusters per branch, fruit cluster length, fruit cluster weight, fruit cluster diameter, ripe fruit percentage and fruit weight per plant.

Chemical data were recorded for pH, total soluble solid, antioxidant activity determined by DPPH, total phenolic content (TPC), total flavonoid content (TFC) and paclobutazol residues in soil, leaves and ripe fruits.

Statistical analysis

Plant data and the data for phytochemicals and antioxidant capacity were analyzed statistically according to a completely randomized design using SPSS statistical software. Means were separated by Duncan's multiple range test at 0.05 and 0.01 probability levels. Data for paclobutazol residues were not analyzed statistically. However, standard deviations are provided.

Results

Effects on growth parameters

Concentrations of paclobutazol were not significantly different for number of leaves per branch, percentage of defoliated leaves per branch, percentage of detached leaves per branch, and SPAD chlorophyll meter reading (SCMR) (Table 1). Numbers of leaves per branch ranged from 203.25 leaves in concentration of 200 ppm to 259.00 leaves in concentration of 600 ppm.

Table 1. Means for number of leaves per branch, percentage of defoliated leaves per branch, percentage of detached leaves per branch, and SPAD chlorophyll meter reading (SCMR) of Mao Luang as affected by different concentrations of paclobutrazol

Concentration of paclobutrazol	Number of leaves/branch	Percentage of defoliated leaves per branch	Percentage of detached leaves per branch	SCMR
0 ppm	216.61	78.77	21.23	41.11
100 ppm	204.25	78.99	21.01	38.74
200 ppm	203.25	81.78	18.22	41.16
400 ppm	242.25	82.25	17.75	38.92
600 ppm	259.00	79.27	20.73	42.01
F-ratio	ns	ns	ns	ns
C.V.	23.48	14.67	58.80	9.895

Means in the same column with the same letter(s) are not significantly different by DMRT at 0.05 probability level.

Percentages of defoliated leaves per branch ranged between 78.77 leaves in concentration of 0 ppm and 82.25 leaves in concentration of 400 ppm. Percentages of detached leaves per branch ranged between 17.75 leaves in concentration of 400 ppm and 21.23 leaves in concentration of 100 ppm. Values of SCMR ranged between 38.74 in concentration of 100 ppm and 41.16 in concentration of 0 ppm.

Effects on flowering traits

Concentrations of paclobutrazol were significantly different ($P \leq 0.05$ and 0.01) for percentage of flower shoots at first day of emergence and 30 days of

emergence (Table 2). At the first day of emergence, the concentrations of 200 ppm and 600 ppm were significantly higher than the concentration of 0 ppm, whereas the concentrations of 100 ppm and 400 ppm were similar to control. At 30 days after emergence, percentages of flower shoots ranged between 13.58 % in the concentration of 0 ppm to 75.49% in the concentration of 600 ppm, and all paclobutrazol treatments (100-600 ppm) were significantly higher than control (0 ppm). The results indicated that application of paclobutrazol at all concentrations could effectively induce flowering.

However, application of paclobutrazol at all concentrations did not significantly affect length of flower cluster, diameter of flower cluster and flower cluster weight (Table 3). Lengths of flower cluster ranged between 10.27 and 11.55 cm. Flower cluster diameters ranged between 0.75 and 0.94 cm, and flower cluster weights ranged between 1.51 and 1.98 g.

Table 2. Means for percentage of flower shoots per branch at first day and percentage of flower shoots per branch at 30 days of Mao Luang as affected by different concentrations of paclobutrazol

Concentration of paclobutrazol	percentage of flower shoots per branch at first day	percentage of flower shoots per branch at 30 days
0 ppm	2.80^{c}	13.58^{c}
100 ppm	3.83^{bc}	43.13^{b}
200 ppm	5.82^{ab}	69.00^{ab}
400 ppm	3.62^{bc}	65.01^{ab}
600 ppm	7.83^{a}	75.49^{a}
F-ratio	*	**
C.V.	37.37	22.65

Means in the same column with the same letter(s) are not significantly different by DMRT at 0.05 probability level.

Table 3. Means for flower cluster length, flower cluster diameter and flower cluster weight of Mao Luang as affected by different concentrations of paclobutrazol

Concentration of paclobutrazol	Flower cluster length (cm)	Flower cluster diameter (cm)	Flower cluster weight (g)
0 ppm	10.52	0.89	1.51
100 ppm	11.55	0.94	1.68
200 ppm	10.99	0.87	1.55
400 ppm	10.27	0.85	1.56
600 ppm	10.78	0.94	1.98
F-ratio	ns	ns	ns
CV.	8.31	7.48	12.70

Means in the same column with the same letter(s) are not significantly different by DMRT at 0.05 probability level.

Effects on fruit traits

At ripening stage, significant differences among the concentrations of paclobutrazol were observed for number of fruit clusters per branch and fruit cluster length, but they were not significantly different for fruit cluster weight (Table 4). Number of fruit clusters per branch was lowest in the concentration of 0 ppm (19.35 clusters) and highest in the concentration of 200 ppm (56.13 clusters). Although application of paclobutrazol at all concentrations resulted in the increase in number of fruit clusters, only two concentrations consisting of 200 and 400 ppm were significantly higher than control (0 ppm) (Figure 1).

Figure 1. Fruit clusters per branch as affected by paclobutrazol at different concentrations: 0 ppm (A), 100 ppm (B), 200 ppm (C), 400 ppm (D) and 600 ppm (E)

Table 4. Means for number of fruit clusters per branch, fruit cluster length and fruit cluster weight of Mao Luang as affected by different concentrations of paclobutrazol

Concentration of paclobutrazol	Number of fruit clusters/branch	Fruit cluster length (cm)	Fruit cluster weight (g)
0 ppm	19.35[b]	16.40[a]	21.57
100 ppm	34.81[ab]	16.00[ab]	20.88
200 ppm	56.13[a]	15.36[ab]	20.54
400 ppm	51.13[a]	13.86[c]	13.86
600 ppm	35.25[ab]	12.56[bc]	14.99
F-ratio	**	**	ns
C.V.	28.47	11.81	22.13

Means in the same column with the same letter(s) are not significantly different by DMRT at 0.05 probability level.

Table 5. Means for fruit cluster diameter, ripe fruit percentage and Fruit weight per plant of Mao Luang as affected by different concentrations of paclobutrazol

Concentration of paclobutrazol	Fruit cluster diameter (cm)	Ripe fruit percentage	Fruit weight/plant (kg)
0 ppm	27.73	34.02[c]	8.73b[c]
100 ppm	28.01	41.62[c]	19.60[a]
200 ppm	26.67	54.38[b]	21.15[a]
400 ppm	27.81	67.56[a]	15.33[ab]
600 ppm	27.41	72.52[a]	5.25[c]
F-ratio	ns	**	**
C.V.	7.17	10.63	30.20

Means in the same column with the same letter(s) are not significantly different by DMRT at 0.05 probability level.

Fruit cluster lengths ranged between 16.40 cm in 0 ppm and 12.56 cm in 600 ppm. Application of paclobutrazol at all concentration resulted in the significant reduction in fruit cluster length. Application of paclobutazol at all concentrations did not significantly affect fruit cluster weight, ranging from 13.86 g in 400 ppm to 21.57 g in 0 ppm. It seemed likely that it had a tendency to reduce fruit cluster weight.

Concentrations of paclobutrazol were not significantly different for fruit cluster diameter, but they were significantly different ($P \leq 0.01$) for ripe fruit percentage and fruit weight per plant (Table 5). Fruit cluster diameters were similar among the treatments, ranging from 26.67 to 28.01cm. Application of paclobutazol increased ripe fruit percentage as all concentrations of paclobutrazol were higher than control (0 ppm). However, three concentrations

including 200, 400 and 600 ppm were significantly higher than control. Application of paclobutrazol significantly increased fruit weight per plant in the concentrations of 100, 200 and 400 ppm. However, it seemed to have detrimental effect on fruit weight per plant in the concentration of 600 ppm (Figure 2).

Figure 2. Fruit cluster size (cluster length and cluster diameter) and percentage of ripe fruits as affected by paclobutrazol at different concentrations: 0 ppm (A), 100 ppm (B), 200 ppm (C), 400 ppm (D) and 600 ppm (E)

Effects on pH, total soluble solid, phytochemicals and antioxidant activity

Application of paclobutrazol did not have significant effect on pH, total soluble solid and antioxidant capacity determined by DPPH method (Table 6). However, it had significant effects on total phenolic content, total flavonoid content. The values of pH were in a range between 3.13 and 3.35, whereas the values of total soluble solid were in a range between 14.38 and 15.63 °Brix. The values of antioxidant capacity ranged between 114.59 and 129.03 mg VCEA/g FW.

Total phenolic contents ranged between 223.06 and 272.40 mg GAE/ 100g FW, whereas total flavoloid contents ranged from 413.99 to 547.58 mg CE/ 100 g FW. Although the concentrations were different for total phenolic content, total flavonoid content, the patterns of the effects were rather confounding. For total phenolic content, the increases in phenolic content were found in 100 and 600 ppm, whereas paclobutrazol did not have significant effect in 200 and 400 ppm. For total flavonoid content, the increases in this

phytochemical were found in 200 and 400 ppm, and the reduction was observed in 100 ppm, whereas the effect was not significant in 600 ppm.

Table 6. Means for pH, total soluble solid (TSS), antioxidant activity determined by DPPH, total phenolic content (TPC) and total flavonoid content (TFC) of Mao Luang as affected by different concentrations of paclobutrazol

Concentration of paclobutrazol	**pH**	**TSS °Brix**	**DPPH (mg VCEA/g FW)**	**TPC (mg GAE/100 g FW)**	**TFC (mg CE/ 100g FW)**
0 ppm	3.20	15.00	114.59	223.63^{b}	459.86^{b}
100 ppm	3.13	14.38	129.02	272.40^{a}	413.99^{c}
200 ppm	3.35	15.25	129.02	223.06^{b}	547.58^{a}
400 ppm	3.13	14.75	129.03	248.96^{ab}	520.85^{a}
600 ppm	3.30	15.63	129.00	265.35^{a}	418.55^{bc}
F-ratio	ns	ns	ns	**	**
C.V.	3.94	5.08	5.11	4.32	3.42

Means in the same column with the same letter(s) are not significantly different by DMRT at 0.05 probability level.

Paclobutrazol residues

Residues of paclobutrazol in soil, leaves and ripe fruits of Mao Luang were determined at harvest compared to that before treatment. The residues in soil and leaves were not detected before treatment (Table 7). The residues in soil were lowest at 0 ppm (1.43 μg/kg) and highest at 600 ppm (58.96 μg/kg), and the range from 0 ppm to 400 ppm was between 1.43 and 15.27 μg/kg, indicating that the residue increased considerably at 600 ppm.

The residues in leaves were not detected at concentrations of 0, 100 and 200 ppm, but they were detected at 400 (2.33 μg/kg) and 600 ppm (11.20 μg/kg). The results also indicated that the residue in leaves increased considerably at 600 ppm.

The residues in ripe fruits were detected at trace amount at concentrations of 0 and 100 ppm. The residues in ripe fruits were 0.04 a μg/kg t 200 ppm, 2.02 μg/kg at 400 ppm and 1.81 μg/kg at 600 ppm. It is interesting to note here that although at high concentration (600 ppm) the residue in ripe fruits was still low similar to that at 400 ppm.

Table 7. Residue of paclobutrazol in soil, leaves and ripe fruits of Mao Luang as affected by different concentrations of paclobutrazol

Concentration of paclobutrazol	Residue of paclobutrazol (µg/kg sample)		
	Soil	Leaves	Ripe fruits
Before treatment	ND	ND	-
0 ppm	1.43 ± 0.07	ND	Trace
100 ppm	1.89 ± 0.07	ND	Trace
200 ppm	2.32 ± 1.34	ND	0.04 ± 0.01
400 ppm	15.27 ± 1.96	2.33 ± 0.10	2.02 ± 0.29
600 ppm	58.96 ± 18.67	11.20 ± 0.11	1.81 ± 0.43

ND= Not detected, Trace= paclobutrazol concentration lower than 0.00098 µg/ml
Remark: Means were averaged from two replications. As the data had zero values, the data were not analyzed statistically.

Discussion

Effects on growth parameters

Mao Luang is a wild plant and it is grown commercially for its ripe fruits to produce juice, concentrated juice and wine, and fresh fruits are also consumed by rural people. Harvest time is during September. Old leaves fall in the dry season during Jaunty to March, and new shoots germinate during leaf falling, and flower shoots also germinate after new shoots during early rainy season. Fruit setting is largely dependent on rainfall and air humidity in the early rainy season. Although irrigation is available, fruit setting is still low if air humidity is low.

Paclobutazol treatments were imposed to Mao luang on 1 December 2017. Number of leaves was evaluated at the same time of paclobutrazol treatment. Leaf falling, leaf persistence and SCMR were evaluated on 1 February 2018 at 60 days after application of paclobutrazol. This study did not find significant effects of paclobutrazol on leaf falling and SCMR.

The information on the effects of paclobutrazol in Mao Laung is rare. However, bulk information is available in other fruit crops. According to Xia *et al*. (2018), application of paclobutrazol significantly increased SPAD chlorophyll meter reading (SCMR) in herbaceous peony (*Paeonia lactiflora* Pall. Application of paclobutrazol increased SCMR of trees of Callery pear and honeylocust (Cregg and Ellison-Smith, 2020).

The differences in the results would be due to the fact that the leaf samples used in this study were old leaves that were mature prior to treatment

of paclobutrazol. From our observation, however, new leaves of the plants treated with paclobutrazol were smaller and darker than those of untreated control. Cregg and Ellison-Smith (2020) found that application of paclobutrazol reduced leaf size of Callery pear trees, but it did not significantly affect leaf size of honeylocust trees. According to Lolaei *et al.* (2013), application of paclobutrazol reduced shoot length and leaf area and increased total leaf chlorophyll content. Sheena and Sheela (2010) found that application of paclobutrazol reduced liaf size and, thus, reduced leaf area. Similar results were also repotede in cassava. Gomathinayagam *et al*. (2007) found that application of triazole also reduced the leaf area. The effects of paclobutrazol on morphological traits varied depending on crop species, growth stage, rate of application and method used (Yeshitela *et al.,* 2004; Bañón *et al.,* 2002). Plants of different species might respond differently to paclobutrazol application for leaf size.

Paclobutrazol increased leaf longavity, and the reduction in leaf size was compensated by the leaf durability (Tekalign and Hammes, 2005). In this study, leaf falling and leaf persistence were not different from untreated control.

Effects on flowering traits

Number of flower shoots was recorded on the first day, when flower shoots were found on 1 February 2018 at 60 days after application of paclobutrazol, and percentage of flower shoots was recorded on 1 March 2018 at 90 days after application of paclobutrazol. The effect of paclobutrazol in inducing flowering in Mao Luang was found at first day, and the effect was more pronounced at 30 days. The concentration of 200 ppm had the highest flower number.

As Mao Luang was domesticated for some decades, and the information on the responses to paclobutrazol is not available. However, paclobutrazol is known to ireduce shoot growth in many fruit crop species (Kishore *et al.,* 2015), and it can be applied by different methods such as, soil injection, drenching, trunk injection, foliar spray (Griffin *et al.,* 1993), application on soil surface and incorporation into the soil or potting medium (Jungklang and Saengnil, 2012). It has an effect on inhibiting biosynthesis of gibberellic acid, and, therefore, makes hormone balance and induces flowering (Zhang *et al.,* 2016).

However, paclobutazol did not have significant effects on flower cluster length, flower cluster diameter and flower cluster weight. The main contribution to yield increase if any would be higher number of flower clusters.

Effects on fruit traits

Ripe fruits were harvested on 1 September 2018 at about 300 days after application of paclobutazol. In this study, application of paclobutrazol to Mao Luang resulted in higher fruit cluster number, shorter fruit cluster length, and, therefore, it did not have significant effect on fruit weight. However, fruit cluster weight seemed to be reduced at 400 and 600 ppm. The results indicated that although paclobutrazol increased fruit cluster number it increased at low concentrations, and it also reduced fruit cluster length at all concentrations. However, at high concentrations (400 and 600 ppm), it also reduced fruit cluster weight.

Number of fruit clusters per branch is an important component contributing to fruit yield. It is interesting to note here that the highest number of fruit clusters, which was about three times higher than untreated control, was obtained at the concentration of 200 ppm. In young Mclntosh apple trees, application of paclobutrazol at the highest rate of 200 and 50 mg L-' increased flower clusters and fruit numbers in the second and third years fafter application (Estabrooks, 1993). In durian trees,application of paclobutrazol at any tines and ages of the trees increased the number of branches bearing flowers, but the numberof branches bearing fruits and the number of fruits per tree were not significantly affected (Subhadrabandhu and Kaiviparkbunyay, 1998). In rambutan, application of paclobutrazol at the range between 500 and 1000 ppm induceed early flowering and increased flower number, fruit weight, and fruit number. However, application of paclobutrazol seemed to reduce shoot length, panicle length and leaf area (Na Nakorn *et al.*, 2017).

Numbers of fruit clusters per branch as affected by paclobutrazol observed on the trees are presented in Figure 1, and fruit cluster characters are presented in Figure 2. This study also found fruit yield increase at 100, 200 and 400 ppm but not at 600 ppm. Yield increase was largely due to the increase in number of fruit clusters, and low fruit yield at 600 ppm was also caused by low fruit cluster number. The appropriate concentrations are dependent on plant age, plant size, crop species, crop varieties methods of application, times of application, frequency of application and years. Care must be taken for application of this growth regulator in order to obtain optimum benefit from this chemical.

Application of paclobutrazol did not have significant effect on fruit cluster diameter. However, it caused high percentage of ripe fruits at all concentrations. Early ripening of fruits is an advantage because it can expand harvest season for some extent.

Effects on phytochemicals and antioxidant activity

pH and total soluble solid are very important traits related to quality of fruits. In this study, the effects of paclobutrazol on phytochemicals and antioxidant activity were not significant. In rambutan (*Nephelium lappaceum*), application of paclobutrazol did not show significant effects on total soluble solids (TSS) of aril (Na Nakorn *et al.,* 2017). In West Indian cherry (*Malpighia emarginata* D.C.), the effects paclobutrazol on pH and fruit acidity were not significant (Sousa *et al.,* 2020). In mango, paclobutrazol did not have negative effects in the quality of fruit, and the treatments with higher doses of paclobutrazol had higher content of total soluble solid (Rebolledo-Mart ńez *et al.,* 2008). In pine apple, application of paclobutrazol did not have significant effects on fruit traits and it did not show physical chage in fruits (Antunes *et al.,* 2008).

However, contrasting results were also reported. In mango, application of paclobutrazol increased fruit yield and fruit firmness, but it reduced fruit length, fruit weight, pH and totyal soluble solids (Oliveira *et al.,* 2013). According to Kumar *et al.* (2019), the rate of paclobutrazol, time of application and variety of mango were the factors affecting total soluble solids and acidity. The authors found that the best rate for treating mango was 1.0 gram, and the best time was in October. Based on the results in this study and other studies, the effects of paclobutrazol on fruits quality of Mao Luang might be different under different environmental conditions and varieties, and further studies on different varieties, time of application and locations are required to verify the results.

Application of paclobutrazol did not have significant effect on total anthocyanin content, and its effect on antioxidant capacity (DPPH) was not significant. However, the treatments were significantly different for total phenolic content and total flavonoid content. Although they were different, this study did not find consistent pattern of changes in these phytochamicals.

In this study, Mao Luang has low anthocyanin, and, thus, anthocyanin is not the main source of antioxidant. In previous study, antioxidant in Mao Laung is mainly contributed by phenolic compounds and anthocyanins (Krongyut and Sutthanut, 2019). In our previous work, major components of phenolic compounds in Mao-Luang consisted of Gallic acid, (-)-epicatechin, (+)-catechin, and cyanidin-3-O-glucoside, and these compounds contributed to antioxcidant capacity ub Mao Luang (Jorjong *et al.,* 2015). The differences in the results of different studies would be due to different materials and ripening stages of fruits. In bignay fruit (*Antidesma bunius* Spreng), the related species of Mao Luang, total phenolic in juice was highest (1202.5 mg GAE/100 mL)

followed by ascorbic acid (48.931 mg/100 mL) and flavonoid (3.78 mg/100 mL), respectively. The scavenging activities determined by DPPH and ABTS methods were recorded at 0.110 mg/mL and 0.126 mg/mL, respectively (Hardinasinta *et al.*, 2020). This species had greater total phenolic than Mao Luang in this study, but it had lower flavoloid.

Paclobutrazol residues

When chemicals are used in agriculture, most people worry about chemical residues in soil and agricultural products, which might be harmful to environments and health. In this study, paclobutrazol was detected in the soil even in untreated control. This would be possibly due to water runoff because the treatments were in the same plantation. However, they were found at low amounts (1.43 to 2.32 μg/kg sample) in 0, 100 and 200 ppm. At these concentrations, paclobutrazol was not detected in leaves, but it was detected at trace amounts in fruits at 0 and 100 ppm and at small amounts (0.04 μg/kg sample) at 200 ppm.

The amounts of paclobutazol were rather high in soil, leaves and fruits at 400 and 600 ppm. These concentrations are not recommended as they may be harmful to environments and consumers. Therefore, the most appropriate concentration was at 200 ppm.

Paclobutrazol is most effective compared to other growth regulators used in the same purpose, and this is the reason why it is used widely in agriculture in most if not all types of crop species (Davis, 1991). The methods used and rates of application would be varied depending on crop types and species (Desta and Amare, 2021).

There are four methods popularly used for application of Paclobutrazol including soil drenching (applying below the soil surface near the roots or trunk), applying on the soil surface, spraying on the leaves and mixing in soil or potting medium. Paclobutrazol found in soil of untreated control and even in fruits at 0 ppm might indicate its possibility to contaminate soil if it is not properly used. According to Costa *et al*. (2009), application of paclobutrazol had residue effects on treated plants for one or more years, and application rates at latter years if it is necessary should be reduced. According to Liu *et al.* (2015), paclobutrazol had the half-life of 20.64 days after aftewr application to the soil, and the residue at 50 days after spraying was below 0.22 mg/kg. Unfortunately, the effect of two or more years was not studied and further investigations are still required for more effective use of paclobutrazol in Mao Luang.

High residue contamination in fruits is at the risk because it cannot be removed by washing or peeling. It presents in fruits largely by absorption and

cannot be metabolized in plant. The maximum residue levels (MRLs) of paclobutrazol in fruits and their processed products allowed by European union (EU) were in a range between 0.01 and 0.5 mg/kg depending on fruit commodities (Commission Regulation, 2019), and the maximum values have a trend to lower than the existing values in the future. In this study, ripe fruits at concentration of 200 ppm had the residue lower than the existing range of maximum residues, and ripe fruits at the concentrations of 400 and 600 ppm had the residues exceeded the maximum values. However, the information obtained in this study is limited to the residues in ripe fruits, and further studied on processed products of Mao Luang are still required.

In conclusion, this study evaluated the affects of paclobutrazol on growth parameters, flowering parameters, fruit parameters, chemical properties of fruits and antioxidant capacity. The study also evaluated paclobutrazol residues in soil, leaves and fruits of Mao Luang. The purposes were to select the most appropriate concentrations of paclobutazol for application in Mao Luang to increase production efficiency and to evaluate the possible risk in using paclobutrazol in Mao Luang production. The concentration of 200 ppm was identified as the most suitable rate for soil surface application. This concentration could produce the highest fruit yield, and it had the lowest risk to soil and fruit contamination of paclobutrazol. Application of paclobutrazol on Mao Luang did not affect food quality parameters including pH and total soluble solid.

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