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Research Article
Gas Exchange, Growth and Flowering of *Lagerstroemia indica* Treated with Different Concentration and Application Techniques of Paclobutrazol

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Abstract

Background and Objectives: *Lagerstroemia indica* is a fast growing landscape shrub that require frequent pruning. The pruning process is a costly operation, temporary and partially successful in controlling tree growth. This study reports the effect of different concentration and application techniques of paclobutrazol, a growth and development of the plant in view of its management in height control and flowering enhancement. **Materials and Methods:** The study involved four different concentration (0 (control), 1500, 3000 and 4500 mg L^{-1}) and two application techniques (foliar spray and soil drenching) of paclobutrazol (PBZ) on 2 month-old plants which were raised from semi hardwood cuttings. Changes in leaf photosynthesis, vegetative growth and flowering were measured. **Results:** Increasing PBZ rate to 3000 and 4500 mg L^{-1} reduced leaf photosynthesis and differ the rates markedly from plants of other treatments. The PBZ application, given as foliar spray and soil drenching reduced plants height. Among the two application techniques, the respective reduction in plant height for foliar sprayed and soil drenched plants were almost 75 and 90% compared to the control plants. The PBZ at 1500 mg L^{-1}, given as soil drenching increased the number of flowers by 25% and at 3000 mg L^{-1} in foliar spray increased the number of flowers by 21% over the non-treated plants. Foliar PBZ sprayed plants produced significantly more leaves compared to those produced by the soil drenched plants. Paclobutrazol applications inhibited extension growth of stem thus reduced the overall height of plants which is linked to shorted internode but at the same time increased branch, leaf and flower number. **Conclusion:** These effects of PBZ observed here could be regarded as positive effects as the treatments would produce shorted statured plants which are normal desirable to landscape enthusiasts.

Key words: Paclobutrazol, plant growth regulator, landscape shrubs, crape myrtle

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Lagerstroemia indica is an attractive, blossoming shrub. It is usually known as crape myrtle and a member of the Lythraceae[1,2] that would be a fantastic addition to any landscape[3]. Under favorable growing condition, the species has a high rate of growth and this could impose a problem in its management as the plants require frequent pruning to maintain its form and height[4]. The pruning process is a costly operation and time consuming and it is temporary and partially successful in controlling tree growth.

Besides mechanical pruning, chemical pruning by using Plant Growth Regulator (PGR) such as paclobutrazol, chlormequat and daminozide were found effective in controlling the rate of plant growth[5] and the techniques are now being widely practiced in production of ornamental pot plants and cut flowers. However, the use of retardant is hardly practiced in the management of landscape shrubs and trees despite of positive findings recorded[6-8]. Based on the concentration used, paclobutrazol (PBZ) is the most competent growth retardant available[5] and perhaps the most suitable to be used for woody species or trees. This assumption was based on our reviewed as we noticed that research involving woody species generally require high dosage of PBZ[7-9]. Applied at appropriate dosage and application techniques, PBZ being an effective anti-gibberellins was reported to reduce the rate of vegetative growth while maintaining leaf photosynthesis[9,10] and works without interfering biosynthesis of secondary metabolites that involves in the production of phytochemicals such as tannins, phenolics and terpenoids[11]. However, with a given situation, the impact of PBZ on production metabolites is species and time dependent[9]. Chorbadjian *et al.*[9] in their study on *Pinus nigra* and *Betula papyrifera* reported that application of PBZ could only enhance generation of tannin in PBZ applied *Betula papyrifera* and not *Pinus nigra* and the effect was only observed in 2004 which could contribute to the plant's resistance to gypsy moth and white-marked tussock moth. Wieland and Wample[12] showed that paclobutrazol did not affect chlorophyll content, transpiration and photosynthesis of 'Topred delicious' apples treated with paclobutrazol at 25, 50 and 150 mg. In contrast, Abod and Jeng[13] reported that PBZ reduced the leaf photosynthesis, transpiration and stomatal conductance of *Acacia mangium* seedlings. In potatoes, Tekalign and Hammes[14] found that leaf chlorophyll content of PBZ treated plants possessed higher chlorophyll content.

Following its impact of cell division and growth which reduces terminal growth, PBZ treated plants could induce lateral growth and side branching, thus produces a more compact, well balanced plants while encouraging plants' flowering capacity. Arnold and McDonald[15] reported that PBZ created a more compact growth form of bush morning glory without adverse residual impacts in the landscape. Working with *Nerium oleander* seedlings, Ochoa *et al.*[6] found that PBZ has successfully reduced the plants height. Nazarudin *et al.*[8] found that paclobutrazol was effective in reducing the growth of *Syzygium myrtifolium* when the plants were treated with PBZ at the rates of 0, 1.25, 2.50 and 3.75 g L^{-1}. Paclobutrazol was effective in increasing the number of okra leaves, whereby the leaf number in the PBZ treated plant was 38.8 while the leaf number of control plants[16] was only 30.6. Smiley *et al.*[7] examined the effects of paclobutrazol at 1000 and 4000 ppm, given as foliar sprays on the growth of four shrub species, *Abelia*×*grandiflora*, *Ligustrum japonicum*, *Ligustrum sinense* and *Loropetalum chinensis* revealed that paclobutrazol was effective in reducing the growth of the four shrub species tested without any distortion in growth. Matysiak[17] reported that paclobutrazol control the shoot elongation and flower initiation of magnolia when the plants were treated with PBZ at 100 mg L^{-1} resulted in 152% increase in the number of flower buds in cv Alexandrina whereas 100% more flower buds were observed in cv Susan treated with 400 mg L^{-1} PBZ. The objective of this study was to assess the effect of different concentration and application techniques of paclobutrazol on leaf photosynthesis, vegetative growth and flowering of *Lagerstroemia indica* in view of PBZ application to inhibit the growth of the species and at the same time to promote its flowering.

MATERIALS AND METHODS

Plant culture and treatments: The effect of different concentration and application techniques of PBZ on vegetative and reproductive growth of *Lagerstroemia indica*, in view of improving the plant architecture and flowering of the species was studied under a naturally ventilated greenhouse condition at Faculty of Agriculture, Universiti Putra Malaysia during a 5 month period. The study utilized 2 month-old plants which were raised from semi hardwood cuttings. Upon receiving the plants from a local supplier, the plants were transferred to black perforated poly bags containing 1.5 kg clay loam top soil. After 2 weeks of establishment, the plants were pruned to an approximate height of 20-25 cm. Fifteen days later, PBZ treatments composed of four concentrations of PBZ (0, 1500, 3000 and 4500 ppm, 50 mL $plant^{-1}$) delivered in two different application techniques (foliar spray and soil drenching) were given. The treatments were factorially arranged in a randomized complete block design. For the foliar treatment, plants were sprayed with a hand sprayer and for the soil drench treatment, the solutions were applied to the soil around the base of the stem. A compound fertilizer (8.0 N:8.0 P_2O_5:8.0 K_2O:3 MgO), 18 g plant^{-1} was given in three split application throughout the 5 months study period. The plants were watered manually and pest control given as necessary. The average temperature recorded during the study were between 25 (night) and 33°C (day) with relative humidity of 70 (±10%).

Data collection: Beside growth and flowering data, information on leaf photosynthesis, stomatal conductance and transpiration were measured at 2 months after the PBZ treatment by using Li-6400XT Portable Photosynthesis System (Li-Cor, Lincoln, Nebraska, USA) at 9.00-11.00 am. The measurements were carried out under 1000 µmol m^{-2} sec^{-1} PAR and 400 ppm CO_2. Relative leaf chlorophyll index was measured randomly on selected plants at 84 days after treatments by using a minolta-chlorophyll meter (SPAD-502 plus, Konika Minolta Optic, Inc, Japan).

Changes in the growth of the plants were monitored by measuring several growth parameters. Extension in plant height was measured as differences between the length of stem of plant at the start of the treatment and at the harvest date. The number of branches, healthy leaves and deformed leaves were counted and the lengths of branches were measured on 42 days after treatment. The length of internode of a branch was determined by dividing length of branch with their respective number of leaves. The number of flower buds and flowers emerged was counted on 26 July, 2014. At the end of the study (6 November, 2014), the leaves were excised from the plants and total leaf area was measured using an area meter (Model Li-3100, LiCOR, Inc., Lincoln, Nebraska, USA).

Experimental design and data analysis: The treatments were arranged in a randomized complete block (RCBD). Data generated were subjected to analysis of variance (ANOVA) utilizing SAS version 9.3 (SAS Institute, Cary, NC, USA). Differences between treatment means were compared by using Duncan's Multiple Range Test (DMRT) at 5% level of probability.

RESULTS AND DISCUSSION

Effects of paclobutrazol on gas exchange: Increasing concentration of PBZ reduced rate of leaf photosynthesis and transpiration but the treatment did not affect stomata conductance (Table 1). Control plants had a photosynthetic rate of 11.16 µmol CO_2 m^{-2} sec^{-1} and photosynthesis rate of plants treated with 4500 mg L^{-1} was the lowest (8.85 µmol CO_2 m^{-2} sec^{-1}). There was no marked different in leaf photosynthesis of control plants and photosynthesis of plants treated with 1500 mg L^{-1} for both application techniques. Results recorded here on the effects of PBZ on photosynthesis is contrasting to some of the previously reported study. This is not surprising as the rate of PBZ used in the present study were much higher than the results reported earlier[18-20] and the effect of PBZ on photosynthesis is species dependent[9]. Rate of photosynthesis in plants is controlled by many external and internal photosynthetic barriers. Although, stomatal conductance and hence CO_2 intake was not affected by PBZ and PBZ was found to elevate chlorophyll concentration (Table 1) but the biochemical pathways that occur along the photosynthetic process could have been damaged under high concentration of PBZ. The PBZ was also found to affect the rate of transpiration but relative to the transpiration of the control plants, the significant effects of PBZ treatment was only detected in plants sprayed with 4500 mg L^{-1} PBZ which has reduced the rate from 2.84-1.81 mmol m^{-2} sec^{-1}. Results of

Table 1: Effects of paclobutrazol concentration and application technique on photosynthesis, transpiration, stomatal conductance and relative chlorophyll content

Application technique	PBZ concentration (mg L^{-1})	Photosynthesis (mmol CO_2 m^{-2} sec^{-1})	Stomatal conductance (mol m^{-2} sec^{-1})	Transpiration (mmol m^{-2} sec^{-1})	Relative chlorophyll content
Control	-	11.16^{ab}	0.30^{a}	2.84^{a}	51.68^{c}
Foliar spray	1500	10.83^{ab}	0.27^{a}	2.96^{a}	52.51^{b}
	3000	10.06^{b}	0.25^{a}	2.42^{ab}	51.58^{c}
	4500	8.82^{c}	0.19^{a}	1.81^{b}	51.57^{c}
Soil drenching	1500	10.77^{ab}	0.20^{a}	2.84^{a}	54.76^{a}
	3000	8.54^{c}	0.23^{a}	2.37^{ab}	54.13^{a}
	4500	8.89^{c}	0.25^{a}	2.13^{ab}	54.06^{a}
F-test					
Application technique (AT)	-	ns	ns	ns	***
PBZ concentration (PC)	-	***	ns	*	***
AT×PC	-	ns	ns	ns	***

Ns: Not significant, *,***Significant level at p≤0.05 and p≤0.001, respectively, means in each column with the same letter(s) for each variable are not significantly different according to DMRT at p≤0.05

earlier study indicated that the leaves of PBZ treated plants had thicker epicuticular wax and epidermal layer compared to control plant[20-22] which may directly reduce transpiration. This is an interesting to note that the effect of PBZ on leaf morphology, structure and function could have been more obvious when the leaves are directly exposed to high concentration of PBZ. Beside affecting cuticle and epidermal layers, PBZ was also found to reduce diameter of xylem, the main passage way for water transport in plants[20]. This phenomenon would reduce overall plant's water use, leading to a higher drought and heat tolerance[23] which is a clear beneficial effect of PBZ in management of landscape plants under urban settings.

The PBZ treated plants have higher chlorophyll content than those in the control plants (Fig. 1). Among the two application techniques, plants of soil drenched PBZ contained higher chlorophyll than those received PBZ via foliar spray. Overall, there was no different in chlorophyll contents among plants treated with soil drenched PBZ which was significantly higher than those of foliar sprayed plants. Among the foliar sprayed plants, PBZ applied with 1500 mg L^{-1} contained higher chlorophyll that those treated with 3000 and 4500 mg L^{-1}, strengthening the fact that direct exposure of leaves to high concentration of PBZ would produce a higher detrimental effect on leaf structure and function as in the case of transpiration. The increase in chlorophyll in PBZ treated plants is a general phenomenon which is associated with the size and number of chloroplast[21].

Effect of paclobutrazol on vegetative growth: Extension of plant height was significantly affected by different concentration, application technique and their interaction. Both application techniques, foliar spray and soil drench reduced plant height extension (Fig. 2).

At final harvest (130 days after PBZ application) the height extension of plants received PBZ foliar spray treatment were 34.19, 20.72, 10.28 and 8.66 cm for 0, 1500, 3000 and 4500 mg L^{-1} PBZ, respectively and the corresponding values for the soil drenched plants were 34.19, 8.97, 3.94 and 3.53 cm (Table 2), indicating that the respective reduction in plant height extension of PBZ foliar spray and soil drenching plants

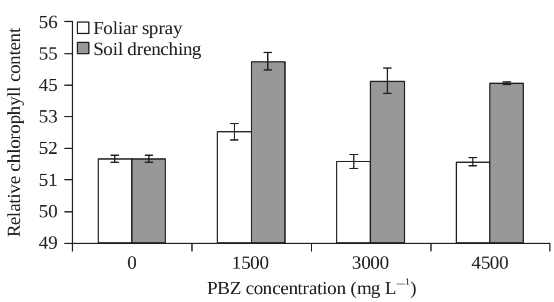

Fig. 1: Effects of paclobutrazol application technique and concentration on relative chlorophyll content of *Lagerstroemia indica*. Vertical bars represent standard error of means

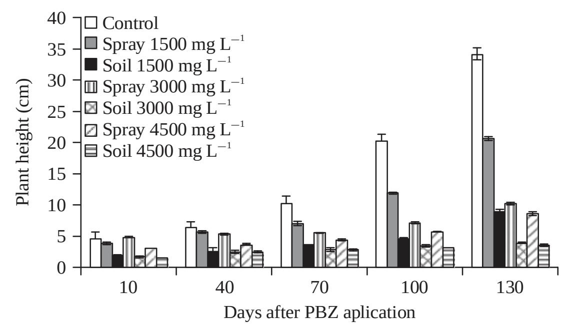

Fig. 2: Effects of paclobutrazol application technique and concentration on plant height of *Lagerstroemia indica* during 130 days. Vertical bars represent standard error of means

Table 2: Effects of paclobutrazol application technique and concentration on plant height, internode length, branch number, leaf number, No. of deformed leaves, leaf area, No. of flower buds and No. of flowers

Application technique	PBZ concentration	Plant height (cm)	Internode length	Branch number per plant	Leaf number per plant	No. of deformed leaves per plant	Leaf area (cm)^2	No. of flower buds	No. of flowers
Control		34.19^{a}	2.63^{a}	8.63^{f}	109.52^{f}	2.61^{e}	699.21^{a}	74.0^{ab}	71.87^{b}
Foliar spray	1500	20.72^{b}	1.76^{b}	14.81^{c}	153.54^{c}	3.25^{e}	575.07^{b}	81.0^{a}	79.75^{ab}
	3000	10.28^{c}	1.60^{b}	15.75^{b}	163.16^{a}	5.50^{e}	474.80^{d}	92.25^{a}	86.75^{a}
	4500	8.66^{d}	1.56^{b}	17.06^{a}	156.87^{b}	23.74^{c}	466.59^{d}	58.0^{b}	50.25^{c}
Soil drenching	1500	8.97^{d}	1.61^{b}	15.25^{bc}	155.83^{bc}	9.91^{d}	527.84^{c}	92.25^{a}	89.5^{a}
	3000	3.94^{e}	1.24^{c}	14.06^{d}	138.41^{d}	71.46^{b}	343.90^{e}	60.0^{b}	46.25^{c}
	4500	3.53^{e}	1.11^{c}	13.06^{e}	120.08^{e}	89.50^{a}	326.88^{f}	23.5^{c}	5.5^{c}
F-test									
Application technique (AT)		***	***	***	***	***	***	*	***
PBZ concentration (PC)		***	***	***	***	***	***	***	***
AT×PC		***	ns	***	***	***	***	*	***

Ns: Not significant, *,***Significant level at $p \leq 0.05$ and $p \leq 0.001$, respectively, means in each column with the same letter(s) for each variable are not significantly different according to DMRT at $p \leq 0.05$

were 75 and 90% as the concentration of PBZ has increased from 0-4500 mg L^{-1} PBZ. Higher impact of soil drench PBZ application provides more evident to the fact that paclobutrazol could have been absorbed and translocated better with soil application to both the shoots and roots by the xylem[13,24].

Decrease in overall height was positively correlated with internode length. As for plant height extension, the magnitude of PBZ effect on internode length was more apparent with soil application, whereby soil application caused a reduction of 58% in the internode length compared to the control plants while the reduction in foliar sprayed plants was 41% (Fig. 3).

PBZ is known to inhibit growth of shoot apex by reducing apical dominance for terminal growth and consequently promotes lateral growth or side branching. In the present study, this event was manifested by the increase in number of branches in the PBZ treated plants. Beneficial effect of PBZ in enhancing side branching was more apparent in foliar sprayed plants than the soil drenched plants with their respective increases of 95 and 62% respectively as the PBZ concentration had increased from 0-4500 mg L^{-1} (Fig. 4). Similar effect of PBZ was reported earlier by El-Quesni *et al.*[25]. Promotional effect of PBZ was also detected in leaf number. Plants with higher number of leaves, coupled with short interval resulted in short-statured plants and such shrubs are preferred to be used for landscaping.

PBZ applied as a soil PBZ drenching at 3000 and 4500 mg L^{-1} produced fewer leaves compared to those treated with 1500 mg L^{-1}, which may be due to toxicity levels to the plants, killing the new shoots and leaves (Fig. 5). Number of leaves of PBZ treatments increased over the control plants is linked to the increasing in the number of branches. A positive relationship between number of leaves and number of branches was reported by Benjawan *et al.*[16]. Despite of having more leaves, the leaf area had significantly reduced with both PBZ application techniques (foliar spray and soil drenching) as compared to the control plants (Table 2). Wanderley *et al.*[26] showed similar effect of PBZ on *Arundina graminifolia* orchids when treated with PBZ at 10 and 20 mg L^{-1}. The PBZ in soil drenching was more effective than the foliar spray whereby foliar spray and soil drenching decreased the leaf area by 33 and 53%, respectively when PBZ concentration had increased from 0-4500 mg L^{-1} (Fig. 6).

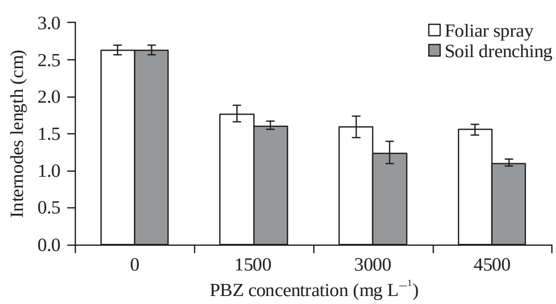

Fig. 3: Effects of paclobutrazol application technique and concentration on the internode length of *Lagerstroemia indica*. Vertical bars indicate standard error of means

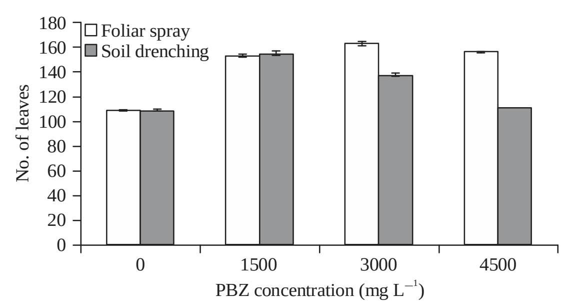

Fig. 5: Effects of paclobutrazol application technique and concentration on the number of leaves of *Lagerstroemia indica.* Vertical bars indicate standard error of means

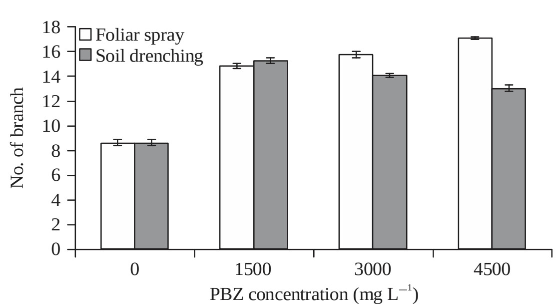

Fig. 4: Effects of paclobutrazol application technique and concentration on the number of a branch of *Lagerstroemia indica*. Vertical bars indicate standard error of means

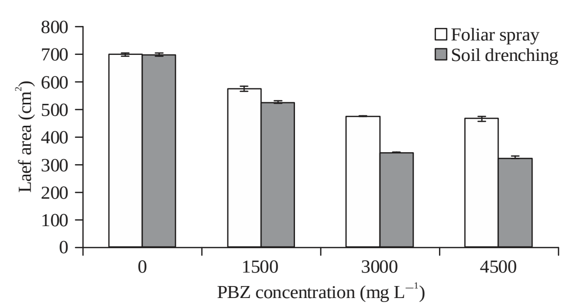

Fig. 6: Effects of paclobutrazol application technique and concentration on the leaf area of *Lagerstroemia indica*. Vertical bars indicate standard error of means

The results obtained on height extension, leaf number, leaf size and internode length are inline with the hypotheses that claim PBZ, as a plant growth retardant which blocks three steps in the terpenoid pathway for the production of gibberellins with one of the main roles is the stimulation of cell elongation[27]. Blockage of the pathway inhibits gibberellin production and this does not stop cell division to retard the elongation of new cells, resulting the generation of similar number of leaves and branches but with shorter internodes[28,29]. In this case, the number of leaves and branches were promoted at 'Intermediate' PBZ concentration. However, higher concentrations, due to severe leaf deformation, especially when given as soil drench, PBZ reduced the number of leaves and branches. Wanderley *et al.*[26] reported that higher PBZ concentration reduced number of shoots and flowers and killing new growth as high concentration of PBZ would be toxic to plants.

Effects of paclobutrazol on flowering: The PBZ at different concentration with different application techniques affected the flowering behaviour of the plants and the effects of the two factors are interdependent as shown by significant interaction between PBZ concentration and application technique (Table 2). The highest number of flowers was observed at 3000 mg L^{-1} in foliar sprayed plants and at 1500 mg L^{-1} in soil drenched plants with their respective increase of almost 21 and 25% compared with control plants. Overall, PBZ application to the plants at higher concentration (3000 and 4500 mg L^{-1} for soil drench and 4500 mg L^{-1} for foliar spray) depressed the generation of number of flowers (Fig. 7). Results generated here are similar to those reported by Matysiak[17] on magnolia, Karaguzel *et al.*[30] on *Lupinus varius* and Kumar *et al.*[31] on *Camelia sativa*. In addition, Wilkinson and Richards[32] working on *Camellia*× *williamsi* also showed

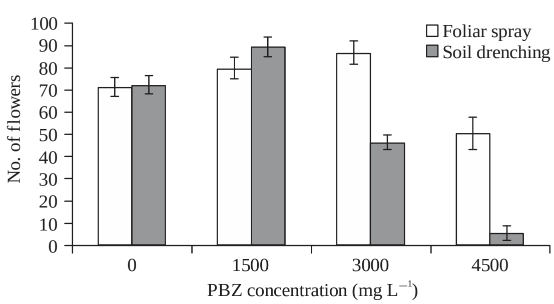

Fig. 7: Effects of paclobutrazol application technique and concentration on the number of flowers of *Lagerstroemia indica*. Vertical bars indicate standard of means

that the plants treated with paclobutrazol as a foliar spray promoted flowering, however soil drench treatments reduced the number of opened flowers.

The promotion of flowering following application of PBZ was observed in many plant species, especially fruit trees. Apart from inducing more flowers, PBZ was also found to reduce juvenility of the plants and the mechanism leading to the early flowering episode of the plants are not fully understood. Paclobutrazol has been found predominantly effective in the induction of early flowering. It may involve hormonal relationship and/or interplay between C:N ratio in plant tissues as reported by Upreti *et al.*[33] who observed that there was increase in C:N ratio and leaf following PBZ application and these were followed with a drastic increase of C:N at the bud break. They also noticed that C:N ratio in shoot of PBZ treated mango was positively related to ABA and cytokinins content prior to floral bud break.

The effects of PBZ on plants is confounded with other growth factors. At low concentration, lack of significant differences observed between non-treated versus PBZ-treated plants could be explained by higher day and night temperatures that experienced by plants grown in the tropics. Under higher growing temperatures, a higher concentration of the PGR is generally required to produce the effects[26].

The effects of PBZ on plant growth and flowering may be modified by other chemicals imposed on the plants. Nazarudin *et al.*[34] reported that the thickness of palisade parenchyma and xylem of leaves of *Xanthostemon chrysanthus* varied when treated with different combinations of PBZ and KNO_3. It is concluded that the use of a right combination of the two chemicals may generate synergistic effects in enhancing plant growth and development[34]. Furthermore, the reduction in growth and development by PBZ is expected to be more pronounced with a longer time of exposure. Working with cycocel, another type of gibberellin blocker, North *et al.*[35] observed that severity of reducing effects of the PGR on *Dombeya burgessiae* was more obvious as the growing period extended.

CONCLUSION

Paclobutrazol applications at 1500 mg L^{-1} to *Lagerstroemia indica* plants significantly inhibited extension growth of stem thus reduced the overall height of plants which is linked to shortened internode but at the same time increased branch, leaf and flower number. These effects could be regarded as positive effects as the treatments would produce shorted statured plants which are normal desirable to landscape enthusiasts. Excessive concentration (4500 mg L^{-1}), especially when given as soil drenching have proved to have more negative effects as it reduced leaf photosynthesis and caused deformation of leaves and reduced flower number.

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SIGNIFICANCE STATEMENTS

- *Lagerstroemia indica*, a landscape shrubs is a fast growing plants that requires frequent pruning and this can be done efficiently with the help of an effective plant dwarfing growth regulator (PGR)
- One of the possible PGR is paclobutrazol (PBZ). Research on the effects of dwarfing effect of PBZ on *L. indica*, a woody shrub is never been done
- Results obtained from this study is useful in providing physiological basis that underpinning the plant responses to PBZ and helpful in practical application towards efficient management of the landscape shrubs
- Unlike most herbaceous plants, the effect of PBZ on *L. indica* was found effective only at high PBZ concentration (3000 and 4500 mg L^{-1})

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