EFFECTS OF DRENCHED-APPLIED PACLOBUTRAZOL AND POTASSIUM NITRATE ON THE LEAF AREA AND PHYSIOLOGICAL RESPONSE OF XANTHOSTEMON CHRYSANTHIS (F. MUELL.) BENTH.

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ABSTRACT
A study was conducted to determine the effects of paclobutrazol (PBZ) and potassium nitrate (KNO₃) on the leaf area and physiological response in Xanthostemon chrysanthus (F. Muell.) Benth., planted in an urban area. A field trial was carried out at Metropolitan Batu Recreational Park, Kuala Lumpur. Nine treatments i.e. T1(0 gl⁻³ PBZ + 0 g KNO₃), T2(0 gl⁻³ PBZ + 100 g KNO₃), T3(0 gl⁻³ PBZ + 200 g KNO₃), T4(0.125 gl⁻³ PBZ + 0 g KNO₃), T5(0.125 gl⁻³ PBZ + 100 g KNO₃), T6(0.125 gl⁻³ PBZ + 200 g KNO₃), T7(0.25 gl⁻³ PBZ + 0 g KNO₃), T8(0.25 gl⁻³ PBZ + 100 g KNO₃) and T9(0.25 gl⁻³ PBZ + 200 g KNO₃) with nine replicates were arranged in a Completely Randomized Design. Monthly, leaf area was measured, while the changes in photosynthetic rate, transpiration rate and stomatal conductance were recorded at sixth and twelfth month after the treatments. Reduced leaf area due to inhibition effect of PBZ was observed. Photosynthetic rate, transpiration rate and stomatal conductance were also decreased with the existence of PBZ. Reduced stomatal conductance might be helpful in controlling water lost from the leaves of the treated trees and beneficial for trees grown in a harsh urban environment.

Keyword: Plant growth regulator, ornamental tree, urban forestry, environmental stress

Introduction
Physiological dysfunctions in plants are often occurring as a response to abiotic interferences. Although plants are able to adapt to their environment accordingly, plant death due to physiological dysfunctions is a result of severe exposure to extreme environmental stress (Kozlowski et al., 1991). Soil compaction, air and soil pollutants (oxides of S and N, SO₂, polychlorinated biphenyls), heavy metals (Zn, Cu, Cd), atmospheric particulates, ozone, drought or water logging, retard tree growth through reduced translocation of photosynthates to roots, impaired stomatal function, premature leaf senescence, clogged lenticels, branch dieback, stem or trunk lesions, leaf scorch and eventual tree death (Percival et al., 2006). Urban area is often associated with compact and infertile soil, lack of nutrients and exposure to heat stress, resulting in poor growth quality of plants (Lorenz and Lal, 2009; Pickett and Cadenasso, 2009; Rahman et al., 2011; Weltecke and Gaertig, 2012). Previous study showed that urban soil within Kuala Lumpur had unpredictable substances which contained more than 65% of sand, insufficient nutrients and water, and high penetration resistance (Ahmad Nazarudin et al., 2014a). Soil containing great amount of sand has high permeability which affects the growth performance of plants.

Plant growth regulators (PGRs) have been widely used in many aspects of plant growth and development. This triazole is used for controlling excessive growth, improving flower and increasing drought and salinity tolerance of various plant species. One of the most effective PGRs is paclobutrazol (PBZ), (2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl) pentan-3-ol. PBZ has growth-regulating effects on plant such as inhibition of gibberellins (GAs) biosynthesis, reduction of cell elongation, stimulation of rooting, increment of chlorophyll content, and amelioration of phytohormone such as abscisic acid (ABA) and cytokinin (Kamounitis and Chronopoulou-Sereli, 1999; Fletcher et al., 2000; Tekalign and Hammes, 2004; Jaleel et al., 2007; Urban et al., 2008). PBZ was also effective in protecting plants from various abiotic stresses that interfere with their normal physiological processes (Fernandez et al., 2006; Sankar et al., 2007).

Potassium (K) is an essential nutrient and is required for desirable growth and reproduction of plants. K has indirect role in plant growth, meaning that it does not make up any plant part (Zubair et al., 2006). However, it involves in both uptake of water through plant roots and release of water via stomata. K element is also known to enhance plant tolerance to drought conditions (Thomas and Thomas, 2009; Kim et al., 2010). It is also pivotal in activating enzymes responsible for protein and starch synthesis, as well as many growth related enzymes in plants (Mengel, 2007; Patil, 2011).

Thus, this study was conducted to determine the effects of PBZ and potassium nitrate (KNO₃) on the leaf area and physiological response in a landscape tree, Xanthostemon chrysanthus (F. Muell.) Benth (Myrtaceae). This species is native to tropical northern Australia, New Guinea, Indonesia and the Philippines (Sosef et al., 1998). It is locally known as jambu kuning and planted widely as ornamental tree in Malaysian cities due to its morphologically unique yellow florals.

Materials and Methods

Study Site and Plant Materials
A study site consisted of 81 *X. chrysanthus* tree was established at Metropolitan Batu Recreational Park, Kuala Lumpur (3° 12' 49" N; 101° 40' 43" E). During the observation period of 12 months (April 2012 till March 2013), the total precipitation received was 3,181.7 mm with temperature of about 22.9-33.3 °C and 76.4% relative humidity.

These trees were six years after planting at the start of the study, with the average height and average stem diameter at breast height of about 6 m and 10 cm, respectively. They were planted as roadside trees in the park. Nine treatments i.e., T1(0 g l−1 PBZ + 0 g KNO3), T2(0 g l−1 PBZ + 100 g KNO3), T3(0 g l−1 PBZ + 200 g KNO3), T4(0.125 g l−1 PBZ + 0 g KNO3), T5(0.125 g l−1 PBZ + 100 g KNO3), T6(0.125 g l−1 PBZ + 200 g KNO3), T7(0.25 g l−1 PBZ + 0 g KNO3), T8(0.25 g l−1 PBZ + 100 g KNO3) and T9(0.25 g l−1 PBZ + 200 g KNO3), with nine replicates were arranged in a Completely Randomized Design. PBZ was soil drenched at an application volume of 1 l per tree, while the control plants were treated with plain water at the same amount. Application of PBZ was carried out one time at the start of the study. KNO3 (15.7:0:38.4) was applied at three months intervals.

**Data Collection**

Randomly, the first three fully expanded leaves of five trees of each treatment were selected for the measurements of photosynthetic rate (A), transpiration rate (E) and stomatal conductance (g). These measurements were recorded at sixth and twelfth month after treatment (MAT) using a Portable Photosynthesis System, Li-6400 XT (LICOR Nebraska, USA). A, E and g, were recorded between 9.00 a.m. to 11.30 a.m. in sunny condition. The measurement was made at ambient humidity at 28 °C. The measurements were taken at 1200 μmol photon m−2 s−1 of quantum flux and the concentration of CO2 was between 360 to 400 μmol m−2 s−1. The A was measured in μmol m−2 s−1, while E and g were measured in mmol m−2 s−1 and mol m−2 s−1, respectively.

Changes in leaf area (LA) were recorded monthly. The first fully expanded leaves were plucked from three randomly selected developing branches of each tree. The LA was measured using a Leaf Area Meter (Cl 202, CID. Inc., USA). LA was measured in squared centimeter (cm2). The relative growth rate (RGR) of LA was then calculated using the formula by Hunt (1982) as below:

\[
RGR = \frac{(\ln V_t - \ln V_i)}{t - t_0}
\]

Where:

\[
\ln V_c: \quad \text{natural logarithm of parameter measurement at initial time} \\
\ln V_t: \quad \text{natural logarithm of parameter measurement at t time} \\
t_c: \quad \text{initial time} \\
t: \quad t \text{ time}
\]

**Data Analysis**

All data were subjected to ANOVA and the treatment means were then compared using Duncan’s Multiple Range Test (DMRT). The Pearson correlation was performed to investigate the degree of association between leaf area and the physiological attributes.

**Results and Discussion**

**Physiological Changes**

A of the first fully developed leaf differed significantly between the control and PBZ treatments at sixth MAT (Table 1). Second cycle of physiological measurement at twelfth MAT found that the control tree had higher A than all the other treatments. Trees treated with PBZ alone and combination of PBZ and KNO3 gave lower A compared to the untreated control tree. At sixth MAT, A of the control tree was 5.61 μmol m−2 s−1, while T7-treated tree had 3.06 μmol m−2 s−1, showing a difference of 45.45%. At twelfth MAT, there was no difference in A among the treatments where PBZ existed. However, the A values were lower than those recorded with T1, T2 and T3. It shows that PBZ influenced the physiological performance of the tree. Reduced A were also reported in Mangifera indica (Shivashankara and Mathai, 2000; Urban et al., 2004). According to Urban et al. (2004), reduced A in *M. indica* was due to neither a decreased in g, and the associated decreased in intercellular partial pressure of CO2. PGR-treated plants normally had a moderate restraining consequence on CO2 exchange rate, thus minimizing the A (Gaussian et al., 1997). The highest E was observed in T1 compared to other treatments (Table 1). Significant differences were also recorded among the control and other treatments. For example, at sixth MAT, E of the control tree was 2.46 m3 mol−1 s−1 whereas T9 had E of 0.73 mmol m−2 s−1, giving a great difference of approximately 70.33%. The difference of E between T1 and other treatments was only recorded between T1 and treatments where PBZ was present (Table 1). It reveals that PBZ influenced the physiological performance of the tree.

Significance difference in g was only recorded between T1 and treatments where PBZ was present (Table 1). It reveals that PBZ was able to control the stomata aperture which further influenced the gas exchange in the leaf. Hence, trees treated with PBZ usually had lower rate of biochemical processes as compared to other treatments without PBZ. However, KNO3 showed no effects on g, as it gave similar results to T1. The reduction in A would also cause reduction in E as both processes are closely connected with stomata behaviour and leaf size (Salisbury and Ross 1992; Abod and Jeng 1993). The decline in E would subsequently decrease the amount of water release through stomata. According to Olsen and Andersen (1995), reduction in E would prevent the plant against abiotic stress due to water limitation or drought episode. Besides affecting GAs, PBZ also increased other phytohormone, ABA and cytokinins (CK) contents concomitant with leaf water potential change in *M. indica* to elicit flowering responses (Upeti et al., 2013). Increased ABA, on the other hand, has frequently been associated with the level of plant tolerance towards various stresses (Nambara and Marion-Poll, 2005). In potted Syzygium myrtifolium, PBZ reduced A and E but the g was not affected (Ahmad Nazarudin et al., 2012). PGRs-treated plants showed reduction in E, used less water
and may be able to survive drought better than untreated plants (Fletcher et al., 2000). In this study, the existence of PBZ reduced A, E, and g_s which benefit the trees planted in extreme environment such as urban areas.

Table 1: Physiological changes in X. chrysanthus at sixth and twelfth months after treatment with PBZ and KNO_3

<table>
<thead>
<tr>
<th>Trt</th>
<th>Photosynthetic rate (μmol m^{-2}s^{-1})</th>
<th>Transpiration rate (mmol m^{-2}s^{-1})</th>
<th>Stomatal conductance (mol m^{-2}s^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6th Month</td>
<td>12th Month</td>
<td>6th Month</td>
</tr>
<tr>
<td>T1</td>
<td>5.61 a</td>
<td>6.54 a</td>
<td>2.46 a</td>
</tr>
<tr>
<td>T2</td>
<td>5.11 a</td>
<td>5.27 b</td>
<td>2.02 b</td>
</tr>
<tr>
<td>T3</td>
<td>5.11 a</td>
<td>5.24 b</td>
<td>1.99 b</td>
</tr>
<tr>
<td>T4</td>
<td>3.88 b</td>
<td>3.55 c</td>
<td>0.77 c</td>
</tr>
<tr>
<td>T5</td>
<td>3.97 b</td>
<td>3.67 c</td>
<td>0.77 c</td>
</tr>
<tr>
<td>T6</td>
<td>3.87 b</td>
<td>3.77 c</td>
<td>0.75 c</td>
</tr>
<tr>
<td>T7</td>
<td>3.06 c</td>
<td>3.47 c</td>
<td>0.73 c</td>
</tr>
<tr>
<td>T8</td>
<td>3.41 bc</td>
<td>3.39 c</td>
<td>0.75 c</td>
</tr>
<tr>
<td>T9</td>
<td>3.26 c</td>
<td>3.35 c</td>
<td>0.73 c</td>
</tr>
</tbody>
</table>

Means followed by the same letter(s) within column do not differ (p<0.05) by DMRT; Trt=treatment; n=15/treatment

Leaf Area Changes

Differences in LA were initially noted in June 2012, where the highest and the lowest LA were measured in the control and tree treated with T9, respectively (Table 2). At this stage, LA of the control tree was about 40.2 cm^2 while it was only 34.56 cm^2 for trees treated with T9, indicating a difference of approximately 14.03%. Results also showed that treatment with PBZ, or combination of PBZ and KNO_3, had a persistent inhibition effect on leaf expansion of the species. These treatments also resulted in a smaller LA than those treated with T1, T2 and T3 from August 2012 onwards until the end of the study period.

Table 2: Effects of paclobutrazol and potassium nitrate on leaf area of X. chrysanthus

<table>
<thead>
<tr>
<th>Trt</th>
<th>Apr '12</th>
<th>May '12</th>
<th>Jun '12</th>
<th>Jul '12</th>
<th>Aug '12</th>
<th>Sep '12</th>
<th>Oct '12</th>
<th>Nov '12</th>
<th>Dec '12</th>
<th>Jan '13</th>
<th>Mar '13</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>41.67 a</td>
<td>40.65 a</td>
<td>40.20 a</td>
<td>38.80 ab</td>
<td>41.11 a</td>
<td>41.97 a</td>
<td>43.39 a</td>
<td>44.64 a</td>
<td>45.52 a</td>
<td>46.06 a</td>
<td>45.59 a</td>
</tr>
<tr>
<td>T2</td>
<td>37.22 a</td>
<td>36.49 a</td>
<td>35.96 abc</td>
<td>37.33 abc</td>
<td>37.70 a</td>
<td>38.26 b</td>
<td>39.83 b</td>
<td>41.24 b</td>
<td>42.43 b</td>
<td>43.47 a</td>
<td>43.33 a</td>
</tr>
<tr>
<td>T3</td>
<td>40.42 a</td>
<td>40.12 a</td>
<td>39.74 ab</td>
<td>39.63 a</td>
<td>40.48 a</td>
<td>41.02 ab</td>
<td>42.78 ab</td>
<td>43.79 ab</td>
<td>45.01 ab</td>
<td>45.84 a</td>
<td>44.65 a</td>
</tr>
<tr>
<td>T4</td>
<td>41.24 a</td>
<td>38.92 a</td>
<td>36.76 abc</td>
<td>34.77 bc</td>
<td>33.50 b</td>
<td>32.79 c</td>
<td>31.75 c</td>
<td>30.75 c</td>
<td>29.83 c</td>
<td>28.51 c</td>
<td>27.59 b</td>
</tr>
<tr>
<td>T5</td>
<td>41.99 a</td>
<td>39.85 a</td>
<td>37.89 abc</td>
<td>34.99 bc</td>
<td>34.11 b</td>
<td>33.53 c</td>
<td>32.34 c</td>
<td>31.58 c</td>
<td>30.69 c</td>
<td>29.96 b</td>
<td>29.19 b</td>
</tr>
<tr>
<td>T6</td>
<td>39.08 a</td>
<td>36.73 a</td>
<td>34.94 c</td>
<td>33.69 abc</td>
<td>32.90 b</td>
<td>32.30 c</td>
<td>31.28 c</td>
<td>30.43 c</td>
<td>29.56 c</td>
<td>28.78 c</td>
<td>27.91 b</td>
</tr>
<tr>
<td>T7</td>
<td>39.58 a</td>
<td>37.44 a</td>
<td>35.73 bc</td>
<td>34.19 a</td>
<td>33.02 b</td>
<td>32.30 c</td>
<td>31.46 c</td>
<td>30.53 c</td>
<td>29.49 c</td>
<td>28.37 b</td>
<td>27.55 b</td>
</tr>
<tr>
<td>T8</td>
<td>39.96 a</td>
<td>36.59 a</td>
<td>34.66 c</td>
<td>34.11 c</td>
<td>33.19 b</td>
<td>32.56 c</td>
<td>31.47 c</td>
<td>30.91 c</td>
<td>30.10 c</td>
<td>29.29 c</td>
<td>28.39 b</td>
</tr>
<tr>
<td>T9</td>
<td>39.48 a</td>
<td>36.39 a</td>
<td>34.56 c</td>
<td>34.05 c</td>
<td>33.23 b</td>
<td>32.51 c</td>
<td>31.68 c</td>
<td>30.86 c</td>
<td>30.11 c</td>
<td>29.20 c</td>
<td>28.26 b</td>
</tr>
</tbody>
</table>

Means followed by the same letter(s) within column do not differ (p<0.05) by DMRT; Trt=treatment; n=9/treatment

Reduced size in the newly developed leaves was observed for the first four months after application of PBZ, singly or in combination with KNO_3 of up to 200 g per tree (Figure 1). Smaller LA of the newly expanded leaves in those treatments was continuously observed until March 2013. Nonetheless, bigger LA was observed in trees treated with T1, T2 and T3 as compared to PBZ treatment from September 2012 onwards until the end of the study period. These results show that K element has a great influence in promoting plant growth and development. However, the effects of K in this study were overtaken by PBZ, in all treatments with combination with PBZ, resulting in reduced LA due to cell inhibition effect by this compound.

PBZ was reported to suppress leaf expansion in other plants for example, Dianthus caryophyllus (Sebastian et al., 2002), M. indica (Yeshitela et al., 2004), S. myrtifolium (Ahmad Nazaruding et al., 2014b) and Lantana camara (Matoukis et al., 2014). Fortunately, PBZ dosages used in this study did not give any extremely bad effects on the leaf morphology. There was no formation of abnormal leaves such as curly leaves which could reduce the aesthetic value of the landscape tree although LA reduction was evident with PBZ treatments. In other study, potted ornamental plant of S. myrtifolium also demonstrated similar effects on the morphological appearance of the leaves (Ahmad Nazaruding et al., 2012). Thus, K in this study may be useful to
improve plant tolerance to drought condition as reported in previous reports by Thomas and Thomas (2009) and Kim et al. (2010) rather than to increase the LA of \( X. \) chrysanthus.

Figure 1: Leaf area increment of \( X. \) chrysanthus after treated with PBZ and KNO₃

Correlation Analysis

Coefficient determination, \( R^2 \) of the physiological attributes shows positive relationships with LA (Figure 2, 3 and 4). It implies that as the LA increased the \( A, E \) and \( g \) were also increased. This is because bigger leaf surface would enhance the capability of the tree to capture the light for its biochemical processes. The existence of PBZ however, modified the morphological of the leaf by reducing the leaf size, which then reduced the physiological performance of the tree. Smaller LA in PBZ-treated \( S. \) myrtilifolium contributed to the reduction of the total leaf surface that absorbs the sunlight for photosynthesis (Ahmad Nazarudin et al., 2012). Reduction of \( E \) and \( g \) were also associated with the decreased LA as shown in Figure 3 and 4.

Anatomical studies showed that PBZ also affected the cells arrangement in the leaves of \( S. \) myrtilifolium (Ahmad Nazarudin et al., 2007), Catharanthus roseus (Jaleel et al., 2009) and \( X. \) chrysanthus (Ahmad Nazarudin et al., 2015). The palisade parenchyma of these plants was tightly packed following PBZ treatment. This could be due to the reduced leaf size forcing the tissues in such arrangement. This kind of cells arrangement would also reduce the intercellular \( CO_2 \) in the leaves which further affect the physiological performance of the treated plants as observed in this study.

Figure 2: Relationship between leaf area and photosynthetic rate

\[ y = 3.5232x + 21.522 \]
\[ R^2 = 0.5311 \]
Conclusion

The existence of PBZ reduced the A, E and gs in *X. chrysanthus* grown at Metropolitan Batu Recreational Park, Kuala Lumpur. In other words, PBZ was able to enhance the plants ability to control water lost via stomata. The ability of controlling the water content is important for plants planted in urban areas which usually exposed to extreme temperature and drought condition. Meanwhile, K in this study may be useful to enhance enzymatic functions in the plant rather than improving the LA and physiological performance. Further experiments on the appropriate application frequencies of this treatments on the species as well as the cost effectiveness of using the treatments are needed. Experiments involving this triazole and other landscape tree species are also required in order to implement this approach as one of the urban tree management strategies under local climate condition.

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