EFFECTS OF PACLOBUTRAZOL ON THE GROWTH AND ANATOMY OF STEMS AND LEAVES OF *SYZYGIUM CAMPANULATUM*

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Received April 2006

AHMAD NAZARUDIN, M. R., MOHD FAUZI, R. & TSAN, F. Y. 2007. Effects of paclobutrazol on the growth and anatomy of stems and leaves of *Syzygium campanulatum*. A study was conducted to determine the effects of paclobutrazol on plant height and leaf area of *Syzygium campanulatum*. The height of paclobutrazol-treated (1.25, 2.50 and 3.75 g l^{-1}) plants was found to be inhibited compared with non-treated plants. The treated plants had smaller leaves compared with those of non-treated plants, one month after treatment. However, there was no difference in plant height and leaf area between plants treated with different rates of paclobutrazol. Scanning electron micrographs of stems showed reduction in xylem thickness, while in the leaves, palisade and spongy parenchyma cells were more closely arranged in response to paclobutrazol. There was also an increase in palisade thickness of leaves of plants treated with 3.75 g l^{-1} paclobutrazol. Paclobutrazol was found to effectively inhibit plant height and leaf expansion and alter the stem and leaf anatomy of *S. campanulatum*.

Keywords: Plant growth regulator, plant height, leaf area, scanning electron microscope

AHMAD NAZARUDIN, M. R., MOHD FAUZI, R. & TSAN, F. Y. 2007. Kesan paklobutrazol terhadap pertumbuhan dan anatomi batang dan daun pokok *Syzygium campanulatum*. Kajian dijalankan untuk mengenal pasti kesan paklobutrazol terhadap ketinggian dan luas daun pokok *Syzygium campanulatum*. Pertumbuhan pokok yang dirawat dengan paklobutrazol (1.25, 2.50 dan 3.75 g l^{-1}) direncat berbanding dengan pokok kawalan didapati bertambah. Sebulan selepas rawatan, pokok yang dirawat mempunyai saiz daun yang lebih kecil berbanding dengan saiz daun pokok kawalan. Bagaimanapun, tiada perbezaan ketinggian pokok dan luas daun diperhatikan antara pokok yang dirawat dengan kepekatan paklobutrazol yang berlainan. Mikrograf imbasan elektron menunjukkan penurunan ketebalan xilem, manakala di dalam daun, sel mesofil palisad dan mesofil span didapati tersusun secara padat akibat perencatan paklobutrazol. Sel mesofil palisad pokok yang dirawat dengan 3.75 g l^{-1} paklobutrazol didapati meningkat. Paklobutrazol didapati berkesan merencat ketinggian pokok serta perkembangan daun, dan seterusnya menyebabkan perubahan anatomi batang dan daun pokok *S. campanulatum*.

INTRODUCTION

Syzygium campanulatum, known as kelat paya of the family Myrtaceae, is native to Malaysia and grows naturally in the coastal areas of Terengganu, Kelantan, Pahang and Johore. It is widely planted in urban areas as hedge plant, topiary or as single plant. For such landscaping purpose, this species needs frequent trimming to maintain its shape and aesthetic function. In attempts to control the growth of the landscape plants, the use of plant growth regulators has been explored besides the conventional trimming and clipping practices.

Paclobutrazol is a triazole compound widely used as retardant for controlling the vegetative growth of a wide range of angiosperm (De Jong & Doyle 1984, Quinlan & Richardson 1984, Sterret 1985, Terri & Millie 2000). Lever (1986) showed that paclobutrazol has low toxicity to mammals. He also indicated that the solubility of paclobutrazol in water is low and its half life in soil is usually between three and twelve months. Proper use of plant growth regulators can restrict plant growth without side effects (Rademacher 2000).

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Paclobutrazol inhibits gibberellin biosynthesis, hence, reducing cell elongation and retarding plant growth (Fletcher *et al.* 2000, Rademacher 2000). Many researchers have also reported that paclobutrazol inhibit plant growth. Hamid and Williams (1997) reported that paclobutrazol drenched at 10, 20 and 50 mg/pot effectively reduced shoot growth of *Swainsona formosa*. Ahmad Nazarudin *et al.* (2003) found that non-treated plants of *Ficus microcarpa* gained height approximately ten fold over plants treated with 5 g l^{-1} paclobutrazol, five months after application. In *Chrysanthemum*, paclobutrazol resulted in thicker leaves, reduced stem diameter and roots with an increased diameter (Burrows *et al.* 1992). Berova and Zlatev (2000) reported a reduced height and an increased stem thickness of tomato in response to paclobutrazol treatment.

The objective of the study was to determine the effects of paclobutrazol on the growth of *S. campanulatum* in order to reduce trimming activities. The stem and leaf anatomy studies were carried out to better understand the effects of this compound.

MATERIALS AND METHODS

The study site

The experiment was carried out at the Forest Research Institute Malaysia, Kepong. During the experiment period, the mean daily temperature ranged from 21.1 to 34.2 °C and the annual precipitation was 1914.8 mm. Four rates of paclobutrazol (Cultar-250 formulation, 250 g a.i. paclobutrazol per litre): 0, 1.25, 2.50 and 3.75 g l^{-1} paclobutrazol were applied to *S. campanulatum* plants raised in polythene bags of dimensions 27 cm diameter × 33 cm height respectively. The plants were purchased from a local nursery. The application volume was 1 litre per plant. Each rate was replicated four times in a randomised complete block design (RCBD). A total of 16 plants were used in the experiment. The planting medium was a mixture of top soil, organic matter and sand at a ratio of 3:2:1. The plants were one year old at the start of the experiment.

All plants were first trimmed to conical shape with approximate height of 100 cm. These plants were allowed to produce new shoots prior to the application of paclobutrazol by soil drenching. *Syzygium campanulatum* required 30 days to flush and recover from the trimming effects. The plants were watered twice daily, i.e. in the morning and late afternoon, depending on the weather. Granular compound commercial fertilizer, NPK Green ($15N:15P_2O_5:15K_2O$) was applied at 5 g $plant^{-1}$ $month^{-1}$. Weeds in the polythene bags were controlled manually while a hoe was used to remove weeds in the surrounding area.

Scanning electron microscopy (SEM) studies

Three months after treatment, the first three fully matured leaves were collected for SEM studies. The leaves were cut into rectangular pieces before they were cross-sectioned, fixed in formalin/acetic-acid/alcohol (FAA), post-fixed in 1% cocodylate buffered osmium tetroxide for two hours before being dehydrated in increasing ethanol concentrations (30, 50, 70, 90, 95 and 100%) for 30 min each. The dehydration process using 100% ethanol was repeated twice. Immediately upon the completion of dehydration, all samples were dried in a critical point dryer for 70 min. The samples were then mounted onto stubs using carbon dots and sputter coated with gold-palladium (Bozzola & Russell 1999, Zakaria & Razak 1999). For the stem, the second internode was collected for SEM viewing. A similar procedure was used to prepare the stem specimens.

Data collection and analysis

Plant height (cm) was measured monthly for six months after the application of paclobutrazol. Measurements were taken from the soil surface in the polythene bag to the highest shoot tip using a telescopic height stick. The first three fully developed leaves from each plant were measured for leaf area using a leaf area meter LI-3100. This was done monthly. For the anatomy study, leaf and stem specimens were viewed under the JSM-5610LV SEM at an acceleration voltage of 15 kV. For the leaves, the thicknesses of the palisade and spongy parenchyma were measured, while that of xylem was measured for the stems.

The data obtained were analysed using Statistical Analysis Software (SAS). Analysis of variance (ANOVA) was conducted and the treatment means were then compared using Tukey's studentized range (HSD) test to detect significant difference between treatments.

RESULTS AND DISCUSSION

Plant height and leaf area

Differences in the growth of *S. campanulatum* became apparent one month after application of paclobutrazol (Figure 1). The plant height of treated plants was lower and the crown shape, more compact compared with the control. Analysis of variance and Tukey's studentized range test showed highly significant differences ($p < 0.01$) in terms of plant height between the non-treated and those treated with different rates of paclobutrazol. However, there were no significant differences in plant height between the plants treated with different rates of plant growth regulator. Inhibition effect was noted one month after the application of paclobutrazol, where the non-treated plants had a mean height of 111 cm while the plants treated with the highest concentration of paclobutrazol had a mean height of 100 cm, a reduction of 9.9% (Figure 1).

Five months after the application of paclobutrazol, the difference in height between non-treated plants and plants treated with the highest dosage was about 25.3 cm. At this stage, the height of the control was about 125.5 cm, while those treated with 3.75 g l^{-1} paclobutrazol measured about 100.2 cm, showing a reduction of 20.2% (Figure 1). The uncontrolled lateral growth of the non-treated plants at this stage also affected their crown shape, requiring trimming to restore the previous conical form. In short, these results showed that the vegetative growth of treated plants was effectively retarded by the application of paclobutrazol. This retardation was probably due to the inhibition of paclobutrazol on cell elongation in the stem. Similar reductions in plant height were reported in *Dicentra spectabilis* (Kim *et al.* 1999), *Scaevola* (Terri & Millie 2000) and *Catasetum fimbriatum* (Suzuki *et al.* 2004) in response to paclobutrazol treatment.

The leaves of the treated plants were relatively smaller and more closely arranged as compared with those of the non-treated plants. As a consequence, the treated plants developed a more compact crown shape. The leaf area of plants treated with 3.75 g l^{-1} paclobutrazol was 11.02 cm^2 before application and 7.87 cm^2 one month after application, showing a reduction of 28.6% (Figure 2). At five months after treatment, the mean leaf area of the plants treated with the highest concentration of paclobutrazol was about 4.47 cm^2, while that of the non-treated plants was approximately 11.17 cm^2. This could also be due to the inhibition of paclobutrazol on cell elongation in the leaf. Tonkinson *et al.* (1995) noted that the application of paclobutrazol reduced the length of wheat leaves by reduced cell length rather than cell number. However, there was no significant difference in leaf area between the treated plants in this study.

Fortunately, there were no abnormal leaves produced following treatment with paclobutrazol. Hence, this did not result in reduction in the aesthetic appearance and advantage for a species frequently selected for its appearance in the landscape. Hensley and Yogi (1996) reported that there was no abnormal growth of fronds

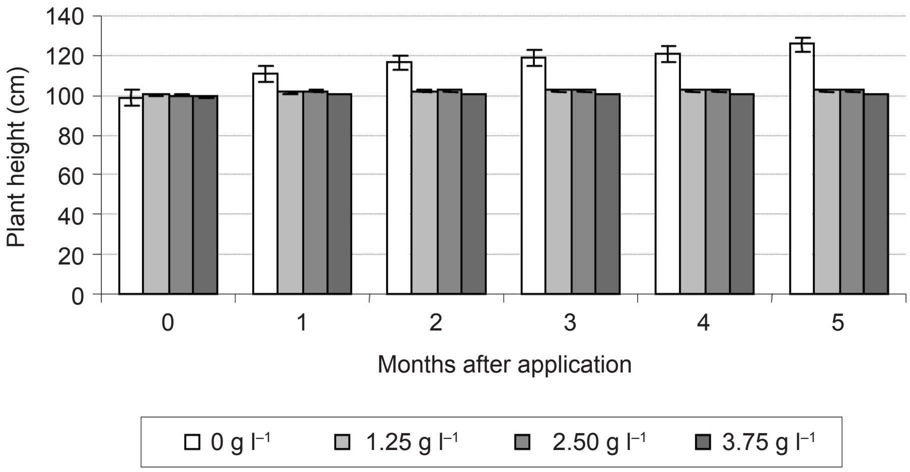


Figure 1 Effects of paclobutrazol on the plant height of *S. campanulatum*. Error bars are standard errors of means.

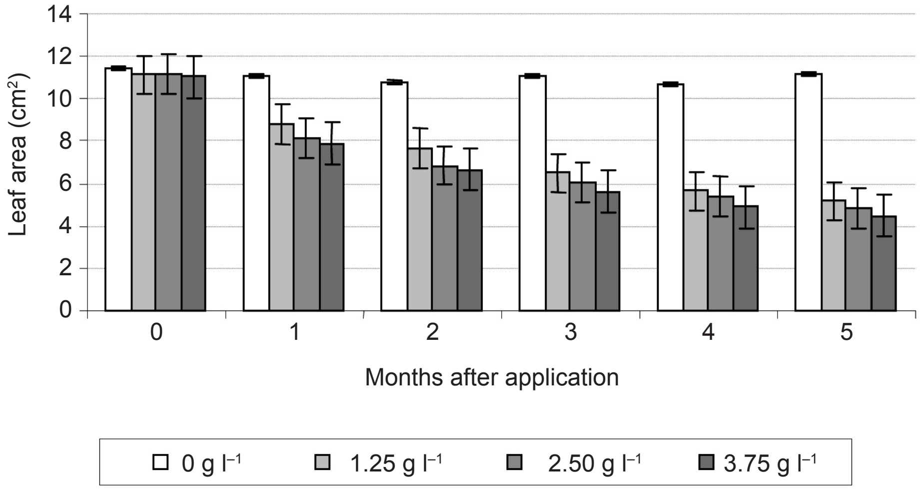


Figure 2 Effects of paclobutrazol on the leaf area of *S. campanulatum*. Error bars are standard errors of means.

in *Syagras romanzoffiana* and *Veitchia merrillii* 11 months after treated with paclobutrazol. Ahmad Nazarudin *et al.* (2003) also indicated that paclobutrazol-treated *F. microcarpa* did not show any abnormal leaf formation four months after application.

Stem and leaf anatomy

SEM observations revealed that the palisade and spongy mesophyll cells of the treated leaves were tightly arranged compared with the non-treated plants (Figure 3). Analysis of variance and Tukey's studentized range test showed that there was a significant increase ($p < 0.05$) in palisade parenchyma thickness of leaves of plants treated with 3.75 g l^{-1} paclobutrazol compared with leaves of non-treated plants (76.05 vs. 49.30 µm respectively, Table 1). On the other hand, the spongy parenchyma thickness of *S. campanulatum* was not affected by the application of this triazole compound. According to Gao *et al.* (1987) triazoles have several morphological effects on leaves, including reduced leaf area, and increased leaf thickness and epicuticular wax. Tekalign (2005) reported that paclobutrazol increased length and width of palisade mesophyll cells of tomato leaves by 32.6 and 41.6% respectively. Burrows *et al.* (1992) reported that increased *Chrysanthemum* leaf thickness in response to paclobutrazol treatment was due to induction of additional layers of palisade parenchyma, although individual cells were shorter, of small diameter and more tightly packed.

Analysis of variance showed a highly significant difference ($p < 0.01$) in the xylem thickness between the stems of non-treated plants and those of treated plants. However, there was no significant difference in xylem thickness between the stems of plants treated with different rates of paclobutrazol. The xylem thickness of the control plants was 164.63 µm while that of plants treated with 3.75 g l^{-1} was about 84.68 µm (Table 2), a reduction of 48.6%. Micrograph images (Figure 3) of the stem cross-sections showed that

Table 1 The palisade and spongy parenchyma thickness of the *S. campanulatum* leaf

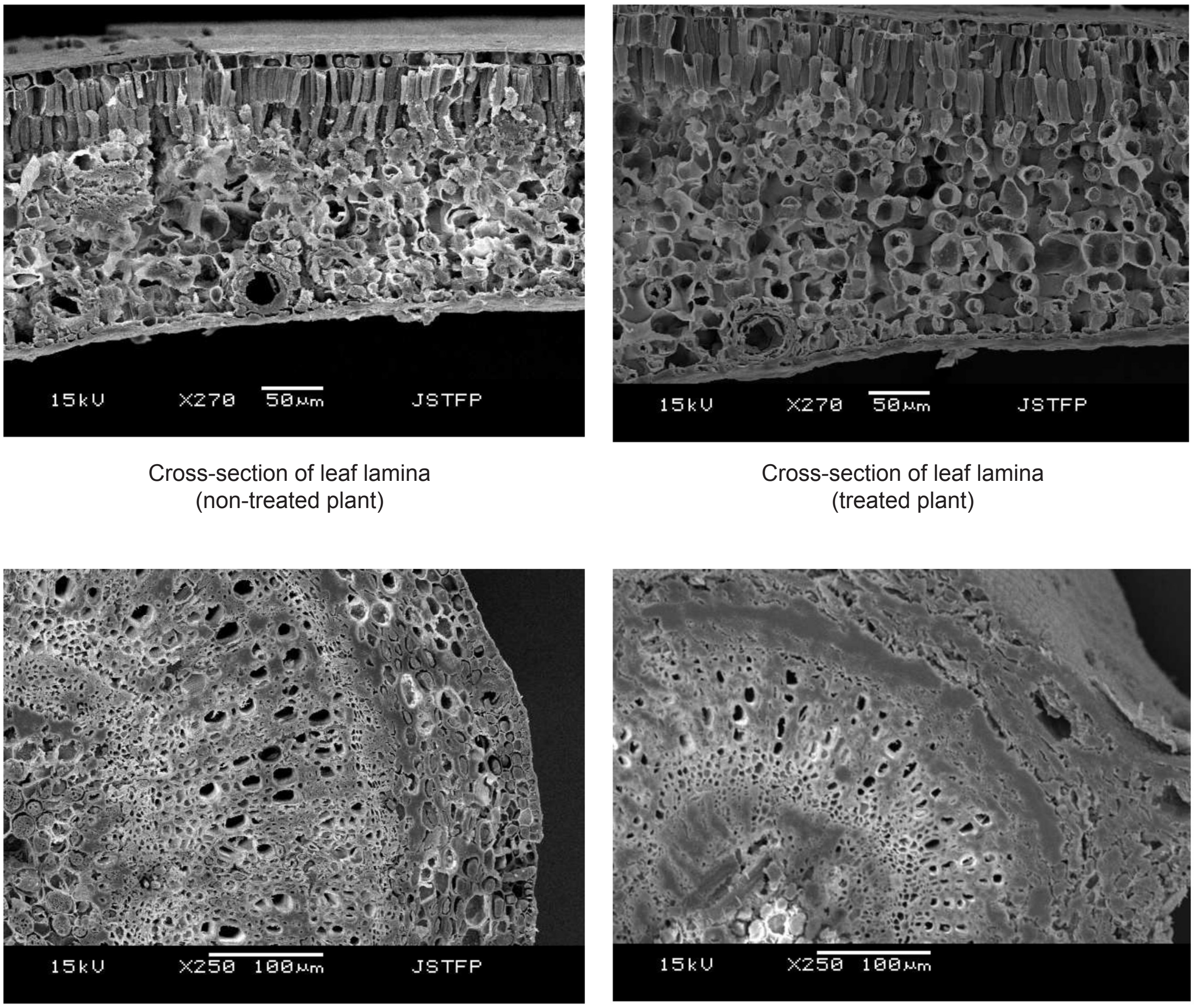
Paclobutrazol (g l^{-1})	Palisade parenchyma thickness (µm)	Spongy parenchyma thickness (µm)
0	49.30 a	109.25 a
1.25	66.00 ab	124.08 a
2.50	65.60 ab	123.33 a
3.75	76.05 b	120.25 a

Means followed by the same letter(s) do not differ ($p < 0.05$) by Tukey's studentized range test.

Table 2 The xylem thickness of *S. campanulatum* treated with paclobutrazol

Paclobutrazol (g l^{-1})	Xylem thickness (µm)
0	164.63 a
1.25	112.03 b
2.50	96.40 b
3.75	84.68 b

Means followed by the same letter(s) do not differ ($p < 0.05$) by Tukey's studentized range test.



Cross-section of leaf lamina (non-treated plant)

Cross-section of leaf lamina (treated plant)

Cross-section of stem (non-treated plant)

Cross-section of stem (treated plant)

Figure 3 Scanning electron micrographs of *S. campanulatum* leaf and stem treated with 3.75 g l^{-1} paclobutrazol

the xylem thickness was reduced with application of paclobutrazol. This finding was in agreement with the results of Aguirre and Blanco (1992) that paclobutrazol reduced the xylem thickness in *Prunus persica*. Murti *et al.* (2001) also reported a reduction in xylem radial width in the shoots of mango cv. Alphonso under paclobutrazol treatment. The reduction in xylem thickness may be due to inhibition of this compound on cell elongation. As a result of the inhibition in thickness and length of xylem cells, water and nutrient uptake in the plant would be affected. Wang and Gregg (1989) reported that restricted water and nutrient supply might occur which could be partially responsible for slow plant growth.

CONCLUSIONS

Paclobutrazol showed rather persistent inhibition on the plant growth of *S. campanulatum*, providing control of plant height up till five months after application. The growth inhibition effects of this compound also resulted in reduction of leaf area and more compact crown but no abnormal leaf formation. It acts as a potential growth maintenance tool in landscape areas. Paclobutrazol increased palisade parenchyma thickness in leaves and reduced xylem thickness in stems of *S. campanulatum*.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to Samsol Bohari, Dairul Haizal Hamidi and Khairul Anuar Idin from FRIM for their technical contributions. Thanks are due to Asiah Othman and Daud Mustam from the Anatomy Laboratory, UPM for providing guidance and necessary facilities in this research. The study was funded by the Malaysian Government (IRPA 01-04-01-0074 EA001).

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