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Gaseous exchange, biochemical parameters and yield as affected by application techniques and doses of Paclobutrazol in litchi tree

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ABSTRACT

An experiment was conducted to see the effect of leaf gaseous exchange parameter, biochemical status and fruit quality of litchi cultivars China and Shahi during 2016-17 in subtropical climate of northern Bihar. Two methods of paclobutrazol (PBZ) application i.e. ring basin (RB) and trunk soil line pore (TSLP) were used with various doses of paclobutrazol (1.00-4.00 g per m canopy diameter) during September month. There was non-significant effect recorded for net photosynthetic rate during August and October but it diminishes during flowering stage (3.3 to 4.2) over control (4.96). The highest rate of transpiration was recorded during flowering phase in trees treated with 3.0 g PBZ whereas 2-3 times more stomatal conductance was recorded in PBZ applied trees @ 1 or 2.0 g through TSLP over control. During pre-FBD (i.e. October-November), leaf chlorophyll content was improved due to RB method over control. PBZ also reduced leaf Nitrogen content during October, which was conducive for flower bud differentiation in litchi trees. 4.0 g PBZ applied through TSLP method assured highest yield ((2567.66 number of fruits per tree), while in control it was only 32-140. The total sugar, vitamin 'C' in pulp was also recorded highest in tree treated with 4.0 g PBZ with any method of PBZ application. However same dose of PBZ with TSLP method resulted in higher Vitamin 'C' and total anthocyanin (28.51 mg per 100 g peel) content. Although, paclobutrazol had only small effect on gas exchange parameters but it significantly improved chlorophyll content, reduced leaf N content (just prior to FBD) and improved yield (by 10-15 fold), pulp vitamin C and anthocyaninin content.

Key words: *Litchi chinensis*, nitrogen, photosynthesis, ring basin.

INTRODUCTION

The flushing habit of litchi varieties is intimately connected with irregular bearing and any vegetative growth prior to panicle emergence and flowering, eliminates the crop completely. In India, litchi produces two growth flushes i.e., first during August followed by the second one during November, apart from the initial flushing just [after fruit harvest] after monsoon. Several research workers advocated the use of various growth retardants as an alternate approach in litchi to restrict vegetative growth before panicle emergence (Chapman *et al*., 5). Some trees vegetatively flush too close to flowering time. Not all branches will flush and those that don't, flower normally, the others will tend to flower over an extended period as they harden in the colder winter temperatures, and a normally early variety may then appear to be a later variety. Reports from China of some varieties tending to fruit only in alternate years is probably because they are being grown away from their best natural climate (Marshal, 11). One method to manipulate flowering is to use growth retardants like, paclobutrazol. The post-flushing application of a small amount of paclobutrazol to the soil significantly promotes flowering and fruiting in the following year. Typically, leaves that formed after paclobutrazol (PBZ) treatment are smaller and darker green than leaves that developed before treatment or on control plants. Paclobutrazol, which is a broad spectrum gibberellins (GA) biosynthesis inhibitor that [belongs to triazole plant growth regulator] have capacity of inhibiting GA biosynthesis by interfering with the *ent*-kaurene oxidase, which catalyzes the sequential oxidations from *ent*-kaurene to *ent*-kaurenoic acid. It has been effectively used to induce and manipulate flowering, fruiting and tree vigour in several perennial fruit crops. However, its use in mango is quite common but not in litchi. Besides reducing gibberellins level, PBZ increases cytokinin contents, root activity and C: N ratio, whereas its influence on nutrient uptake that lacks consistency (Kishore *et al*., 9). PBZ also affects microbial population and dehydrogenase activity in soil. Thus, paclobutrazol holds considerable promise in manipulation of flowering, yield and vigour in fruit crops (Kishor *et al*., 9). Therefore, an investigation was conducted to see how paclobutrazol dose and application techniques affect leaf gaseous exchange attributes, biochemical status, fruit yield and quality parameters in Litchi (*Litchi chinenesis* Sonn.)" cultivars with an objective to ascertain regular

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flowering in irregular bearing litchi varieties under north Indian condition.

MATERIALS AND METHODS

The experiment was conducted during 2016-2017 at Experimental Farm of ICAR-National Research Centre on Litchi, Mushahari, Muzaffarpur, Bihar, India. The experimental site is situated at 26^0 5' 87'' N latitude 85^0 26' 64'' E longitudes at an elevation of 210 m above the mean sea level having warmer/ tropical fringe of subtropical climate with average annual rainfall of about 1180 mm. The experimental soil was alluvial with sandy loam texture and are calcareous having pH 7.5 - 8.0. The chosen design was factorial Randomized Block Design (RBD) with spacing of the trees at 8.0 m × 8.0 m with 12 numbers of treatments viz. Two methods of application [(M1: Ring basin (1.5-2.0 m away from the trunk) and, M2: Trunk Soil Line Pore (TSLP) method (near collar region, 2.5 feet away from the tree trunk)] with 4 doses of paclobutrazol [(i.e. T1: 1.0 g a.i. per m^2 canopy diameter, T2: 2.0 g, T3: 3.0 g, T4: 4.0 g] and two control [T5: 0.0 g (China) and, T6: 0.0 g (Shahi)]. There was no any treatment was imposed in litchi cv. Shahi but a tree was used to compare with untreated 'China' tree. The Burondkar and Gunjate (4) method of soil drenching was followed for paclobutrazol ('Cultar'' from Indonesian Operations, Jakarta) application in which 10 small holes (10–15 cm depth) was made in the soil around the collar region (2.5 feet away from the tree trunk) and 500 ml of this solution was drenched in each hole during the first week of September month of first year of experiment. Ten, 12- 15 year old uniform sized litchi cv. 'China' and two trees of 'Shahi' trees were randomly selected for the experiment. Each treatment was replicated thrice with a single tree as a replication unit. For estimation of various parameters, the leaf samples from 3rd and 4th leaf position were collected from the mid height of the plant in the month of August, October and February months. Sample were collected uniformly to study the suitability of index, tissue to forecast nutritional standards, relative water contents and chlorophyll contents (a, b and total) in litchi leaf.

The measurements (during August, October and February) of the gas exchange parameters like net photosynthetic rate (*Pn*) [μ mol CO_2 $m^{-2}s^{-1}$], transpiration rate (*E*) [m mol H_2O $m^{-2}s^{-1}$], and stomatal conductance (*gs*) [m mol H_2O $m^{-2}s^{-1}$] was done using a portable gas exchange system (CIRAS-2; PP Systems, Made in USA) with a PLC 6(U) universal leaf auto cuvette in closed-circuit mode. All measurements were taken on attached mature leaves that were 3-4 months old without visible injury symptoms and with an apparent homogeneous green colour.

The chlorophyll contents (*a, b and total chlorophyll*) of the leaves were analyzed during August, October and February, following the method as suggested by Barnes *et al*. (3). The leaf relative water content (RWC) in the recently mature leaves was determined using the method suggested by Weatherley (17) using following formula:

$$\text{RWC (\%)} = \frac{\text{Fresh weight} - \text{Oven dry weight}}{\text{Turgid weight} - \text{Oven dry weight}} \times 100$$

Leaf Nitrogen Content (%) was assessed by micro-kjeldahl method, After collection of fresh leaves and were decontaminated by tap water to remove dust particles, and then with N/10 HCl to remove metallic contaminants and finally with deionised water to wash off the previous solution. After washing, the extra moisture was wiped and samples were kept in paper bags for drying in an electric oven at 60 ºC till a constant weight was achieved. Dried leaf tissue (0.5 g) was then transferred to a 100 ml kjeldahl flask and 20 ml of sulphuric-salicylic acid mixture was added with gentle swirling and left overnight. Next day, 5 g sodium thiosulfate and 1g digestion mixture (20 parts K_2SO_4 and 1 part catalyst mixture i.e. 20 parts $CuSO_4$ + 1 part selenium powder) was added and the contents were digested till a clear solution was obtained. The digests were distilled in the presence of 40 % NaOH and liberated ammonia was collected in 4% boric acid solution containing bromo-cresol green and methyl red indicator. After distillation, the boric acid was back titrated with N/200 H_2SO_4. The results were expressed as percent N on dry weight basis.

No. of fruit per panicle was estimated by counting number of fruits set on tagged panicle and average of 5 panicle was taken for final value.The data on yield was recorded by weighing all the fruits under each treatment and replication with the help of a top pan balance (10 kg capacity) at the time of harvesting. The titrable acidity of fruits was determined by the titration method as suggested by Ranganna (12). Total soluble solids (TSS) of fully ripened litchi fruit was recorded by putting a drop of juice on hand refractometer and the value was corrected at 20 ^{0}C with the help of temperature correction chart and expressed in ^{0}Brix. Sugars were determined as per "Lane and Eynon" as described by Ranganna (12).

Ascorbic acid content of litchi fruits was determined by the reduction of 2, 6, dichlorophenol-indophenol (dye) by ascorbic acid as method described by Ranganna (12). For estimation of anthocyanin, 10 g of the sample was blended with 10 ml of ethanolic HCl with the help of pestle and mortar and transferred into 50 ml conical flask by using 10 ml ethanolic HCl for washing. The solution was stored overnight at 4°C. Afterwards solution was filtered with Whatman No. 1 filter paper. Final volume was made up to 100 ml and stored in the dark for 2 h. Absorbance was taken at 535 nm.

$$\text{Total anthocyanin (mg /100g)} = \frac{\text{OD} \times \text{dilution} \times \text{total volume made up} \times 100}{\text{Weight of sample taken} \times e}$$

Where e = 98.2 (absorbance of a solution containing 0.1 mg/ml anthocyanin)

The mean was computed for the data on various attributes, whereas a two-factor analysis of variance (ANOVA) using a randomized block design (RBD) was conducted with SAS® 9.2 statistical software for the data on quality parameters. The least significant differences (LSD) between means at $P_{0.05}$ were computed.

RESULTS AND DISCUSSION

The net photosynthetic rate (*Pn*), transpiration Rate (*E*) and stomatal conductance (*gs*) in the experiment were recorded during August (flushing stage), October (final flushing stage) and February (pre-bloom stage) in the experimental plant.

The net photosynthetic rate (*Pn*) (m mol CO_2 m^{-2} s^{-1}) did not influenced by method of application of PBZ (Fig. 1). However, *Pn* was higher during October than August and February (flowering period) month. This response could be linked with the increase in chlorophyll concentration. In contrast, Roseli *et al.* (13) showed reduced photosynthetic rate than control after three-month of application of paclobutrazol. 4.0 g PBZ with TSLP and 1.0 g PBZ with RB method led to higher *Pn* during flowering phase (February) over vegetative growth. There was enhancement of *Pn* due to PBZ at lower rate (1.0 or 2.0 g) with ring basin method during flowering time. The *Pn* was found to increase from August to October and again got reduced during February in both the cultivars but *Pn* was reduced with higher rate in 'Shahi' than 'China' litchi. In Sweet orange, Joseph and George (8) also found reduced leaf photosynthetic rate, ribulose bisposphate carboxylase activity, total non-structural carbohydrate and dark respiration (70 to 80 per cent of the control plant) with paclobutrazol (250 and 500 mg per plant) contributing to biomass reduction in treated sweet orange.

The transpiration rate (*E*), reduced due to lower dose of PBZ (1-2.0 g PBZ) and found to be highest (2.10 mmol m^{-2} s^{-1}) with 3.0 g PBZ with TSLP method during flowering stage (Fig. 2). The transpiration rate was higher in Shahi, than China litchi and *E* got reduced during flowering than at vegetative phase. 2.0 g PBZ with TSLP method of application was better (4.63 mmol m^{-2} s^{-1}) to keep higher leaf transpiration rate during flowering time. Roseli *et al*. (13) observed that paclobutrazol at 3.75 g L^{-1} significantly reduced the transpiration rate as compared with the control plants in *Syzygium myrtifolium*.

The stomatal conductance (*gs*) insignificantly affected by method of application of PBZ in cv. China. In tree receiving 2.0 g PBZ, has recorded the highest value (278.33 m mol H_2O) with RB method. The lowest *gs* during February (67.00 m mol H_2O) in RB method with 2.0 g PBZ and in 52.66 m mol H_2O with 3.0 g PBZ has been recorded (Fig. 3). This reduction correlated well with reduced stomatal activity and decreased leaf area (Abode and Jeng, 1). Thus 1-2.0 g PBZ applied though TSLP recorded highest *gs* (up to 2-3 times) over control and an increase in dose of PBZ further reduce the *gs* during flowering. Abod and Jeng (1) also observed transpiration and stomatal conductance of PBZ treated plants were lower than the control throughout the 12 weekly measurements in *Acacia mangium* seedlings when applied to the soil. This may be attributed to the chemical being readily absorbed by the roots and translocated almost exclusively in the xylem acropetally to the meristematic regions.

During October- November leaf chlorophyll (*Chl a* and *Chl b*) content improved over control by dose of PBZ, irrespective of method of application (Table 1). However total chlorophyll content although reduced during August and October but was higher in all PBZ treated trees over control (6.75 mg per g FW). Doses of PBZ also influenced leaf *Chl a, Chl b*, and *total Chl* content (Table 1) and higher the dose of PBZ found to be better for improvement in concentration during November month. Ring basin method was found to be better for improving *Chl a*, *Chl b*, and *total Chl* content over TSLP method after two month of application of PBZ *i.e.* RB method is able to retained more greenness of leaves over TSLP method during October month. The increment of chlorophyll content is due to enhanced chlorophyll biosynthesis or is simply a "concentrating effect" due to the decrease leaf expansion or the increase of leaf thickness due to PBZ. Hu *et al*. (7) also found that application of PBZ increased relative water content, proline content, chlorophyll (*a*+*b*) content, and antioxidant enzyme activities in Chinese bayberry [*Myrica rubra* (*Lour.*) *Zucc.*], resulting in a better acclimation to salt stress and an increase in dry matter production. Singh and Sharma (14) recorded increase in C:N ratio, leaf water potential, chlorophyll content, total sugar, total protein, nitrate reductase activity, ABA and cytokinins – zeatin (Z), zeatinriboside (ZR) and dihydrozeatinriboside (DHZR) in paclobutrazol treated mango.

The leaf relative water content (RWC) which was also affected by method of application and

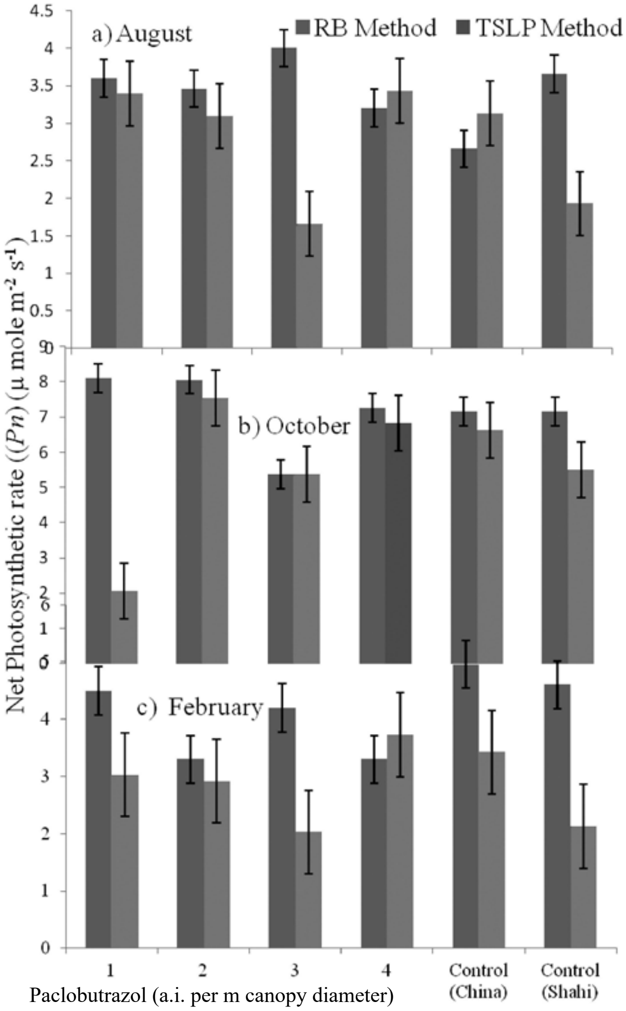

Fig. 1. Effect of methods of application and doses of paclobutrazol on net photosynthetic rate.

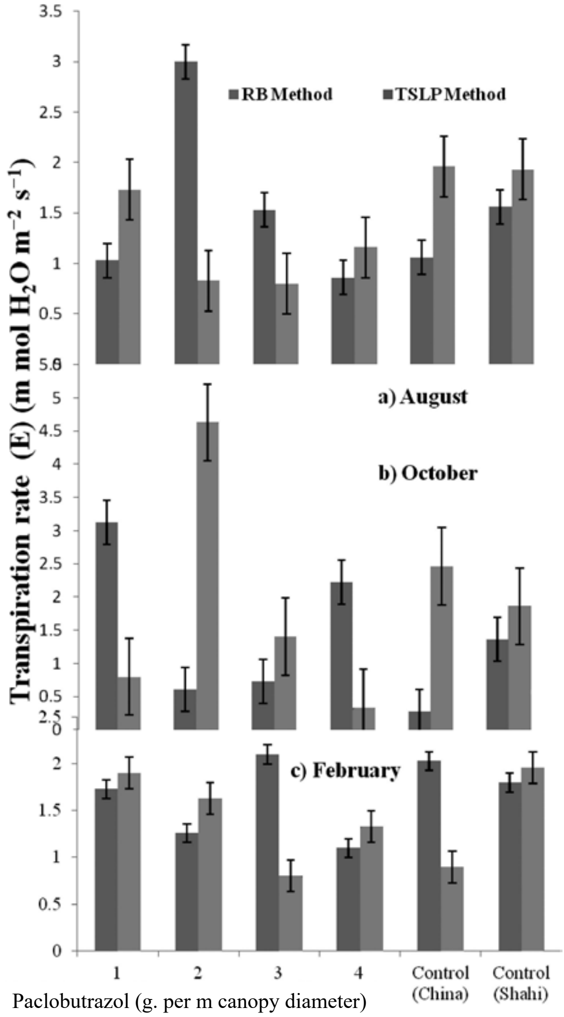

Fig. 2. Transpiration rate in litchi cv. China.

Values are expressed as means ± SD.

dose of PBZ and recorded maximum in February month in the tree receiving PBZ @ 1.0 g with RB method, however with increase in dose of PBZ, the RWC decreased. During October also lower value of RWC was observed in PBZ treated plant. During flowering, lower doses of PBZ through RB method demonstrated higher RWC over control plant but higher doses of PBZ showed low RWC. Thus, during August and October month, TSLP methods recorded highest RWC with PBZ @ 2-3.0 g but it got reduced during February month. Werner (16) reported that plants treated with PBZ concentrations (0 and 2.0 µmol L^{-1}) increased a relative water content, proline content, chlorophyll (a+b) content and antioxidant enzyme activities in Chinese bayberry seedlings.

Leaf Nitrogen (N) has also improved due to application of PBZ but method of application was found to be insignificant during flowering stage. From October to February, PBZ application led to decrease in leaf N (1.51-1.57 %) while increased in control trees (1.61 %) (Table 1). 4.0 g PBZ applied through RB method recorded high N content (1.57 %) and with increase in dose of PBZ, leaf N content increased in RB method. In October, the leaf N was observed lower in PBZ treated plant than the control plant. But in February, RB method recorded higher value of N over control. Contrary to this, PBZ treated plant demonstrated lower value of leaf N through TSLP in February month.

As per Table 2, TSLP helps the litchi trees to record higher yield (i.e up to 70.0 kg fruit per tree due to PBZ @ 4.0 g per m canopy diameter followed by 3.0 g PBZ), lesser dose of PBZ (1.0 g per m canopy diameter) applied through ring basin method

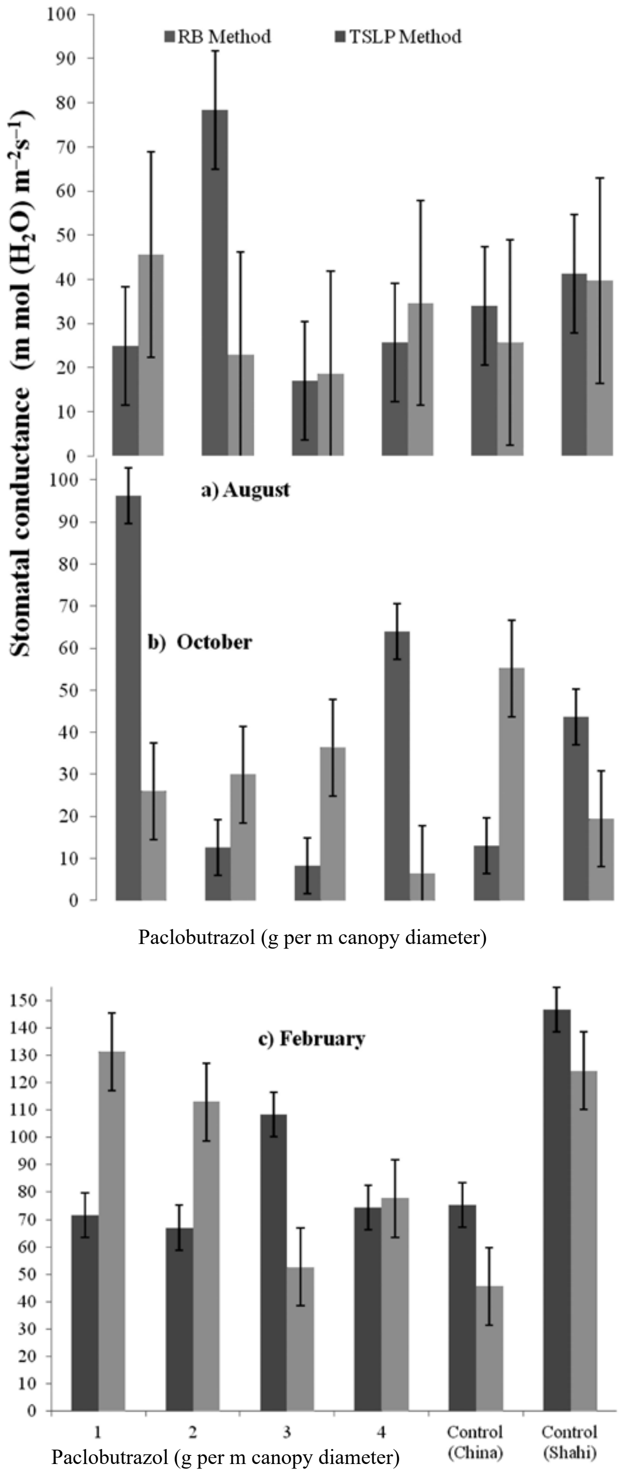

Fig. 3. Effect of methods and dose of paclobutrazol application on stomatal conductance in litchi cultivars.

was found to significant for improvement in yield which had higher no. of fruits per panicle (22.00) or per tree (330.00). Exceptionally, highest no. of fruit per tree (2567 fruit per tree) was recorded with TSLP application of PBZ @ 4.0 g which had also highest no. of fruit per panicle (22.00). Davis *et al*. (6) also applied PBZ to the soil and found that a continuous supply of paclobutrazol might have taken up by the roots which are translocated acropetally via the xylem, thus maintaining the concentration of paclobutrazol above the threshold required for the inhibition of gibberellins biosynthesis and subsequently yield was enhanced. Control trees of 'China' and 'Shahi' recorded very meager yield (0.81, 0.74 kg per tree) (Table 2).

Our results were in congruence with findings of Anusuya and Selvarajan (2) who found that the paclobutrazol at 0.75 g a.i. applied during September was the best with the highest yield (248.50 per tree). PBZ also increased the yield in all cultivars of mango (Alphonso, Kesar and Rajapuri) (Tandel and Patel, 15). Lolaei *et al*., (10) also reported increase in yield of fruit trees due to paclobutrazol. The reason attributed to increased yield with paclobutrazol may have been due to its effect on shifting of assimilates, chlorophyll, mineral elements and soluble proteins in leaves, stems and roots.

The fruit acidity content in treated plant do not varied significantly in different treatment by either method of application or dose of paclobutrazol. The total sugar (TS) was also improved due to application of PBZ applied though RB method and 4.0 g PBZ recorded highest total sugar content (15.80 %) over control (15.21 %); TSLP method also recorded highest total sugars in the same treatment. Likewise total sugar, vitamin 'C' was also recorded highest in tree with 4.0 g PBZ with any method of PBZ application, however TSLP method recorded higher vitamin 'C' content in pulp over RB method. The dose of PBZ affected significantly on peel anthocyanin content and highest value of peel total anthocyanin (28.51 mg per 100 g peel) was recorded with 4.0 g PBZ applied through TSLP. Fruit quality in paclobutrazol treated trees may have improved due to diversion of photosynthates towards the fruit.

Although, PBZ is a potent plant growth regulator and it is required in relatively low concentration to induce physiological, anatomical and morphological changes in plants but it is useful to reduce N content during pre-bloom stage, increasing yield and enhancing anthocyanin content, total sugars. PBZ @ 4.0 g per m canopy diameter with TSLP method is helpful in enhancing fruit production in litchi cv. China. Although, paclobutrazol was effective

Table 1. Paclobutrazol doses and application techniques influencing leaf chlorophyll *(a, b and total)*, leaf relative water contents (RWC) and nitrogen status contents in litchi cv. China.

Treatments	Dose of PBZ	Chl a			Chl b			Total Chl			Relative Water Content (%)			Leaf N (%)		
		I*	II**	III***	I	II	III	I	II	III	I	II	III	I	II	III
Ring Basin Methods	1.0	4.48	3.28	3.54	1.83	2.86	3.22	6.31	6.14	6.76	82.23 (65.04)	82.23 (57.69)	96.45 (79.23)	1.61	1.54	1.510
	2.0.	4.24	3.72	3.73	2.06	2.84	3.05	6.42	6.10	6.83	80.33 (63.66)	80.33 (56.51)	87.30 (69.10)	1.52	1.54	1.530
	3.0	4.12	3.68	3.73	1.78	2.94	3.13	6.02	6.66	6.86	91.20 (72.73)	91.20 (62.43)	81.86 (64.77)	1.54	1.57	1.550
	4.0	4.36	3.68	3.70	2.39	2.94	3.07	6.62	6.64	6.82	91.15 (72.68)	91.15 (61.08)	74.36 (59.55)	1.51	1.57	1.570
	Control (China)	3.61	3.64	3.38	2.38	3.00	3.01	6.50	6.68	6.74	92.43 (74.01)	92.43 (61.71)	83.76 (66.21)	1.53	1.59	1.610
	Control (Shahi)	3.36	3.54	3.42	2.19	2.9	2.83	6.83	7.28	7.20	77.39 (61.58)	77.39 (63.51)	81.70 (64.64)	1.56	1.61	1.620
TSLP	1.0	4.36	3.30	3.78	2.38	3.00	3.03	6.64	6.68	6.73	77.58 (61.71)	77.58 (60.37)	82.51 (65.25)	1.55	1.51	1.530
	2.0.	4.23	3.70	3.75	2.52	2.53	3.02	6.28	6.20	6.76	92.97 (74.61)	92.97 (61.76)	81.72 (64.66)	1.53	1.54	1.543
	3.0	4.68	4.38	4.37	2.55	2.60	2.75	6.16	6.24	6.15	91.30 (72.84)	91.30 (62.10)	78.86 (62.60)	1.58	1.56	1.570
	4.0	3.76	3.67	3.74	2.52	2.64	2.78	6.325	6.30	6.18	83.77 (66.22)	83.77 (61.65)	83.10 (65.70)	1.61	1.58	1.523
	Control (China)	3.80	3.66	3.40	2.66	3.17	2.82	6.02	6.71	6.24	82.44 (65.20)	82.44 (62.70)	82.38 (65.15)	1.55	1.61	1.610
	Control (Shahi)	4.36	4.32	4.96	2.40	2.99	2.50	6.76	7.31	7.46	91.38 (72.91)	91.38 (62.10)	84.28 (66.62)	1.57	1.53	1.560
$CD_{0.05}$	A	0.112	0.061	0.142	0.050	0.077	NS	NS	NS	0.010	0.722 (NS)	0.473 (0.327)	0.490 (0.487)	0.008	0.011	NS
	B	0.064	0.035	0.082	0.086	0.134	NS	NS	0.058	0.017	1.251 (1.096)	0.819 (0.566)	0.849 (0.843)	0.014	0.020	0.042
	A × B	0.158	0.086	0.201	0.122	0.189	NS	NS	0.082	0.024	1.769 (1.550)	1.158 (0.800)	1.200 (1.192)	0.020	0.028	0.059

*Stages of observation taken (I: August; II: October and III: February); TSLP: Trunk Soil Line Pore; RB: Ring Basin; NS: Non-Significant at $P_{0.05}$
Values in parenthesis are arc sine converted values.

Table 2. Effect of methods of application and doses of paclobutrazol on yield and fruit quality attributes in litchi cultivars.

Treatments	Dose of PBZ	No. of Fruits per panicle	No. of Fruit per tree	Fruit Yield (kg per tree)	TSS (^{0}B)	Total sugar (%)	Vitamin C (mg per 100 g FW)	Anthocyanin (mg per 100 g peel)
Ring Basin Methods	1.0	22.00	330.00	8.29	19.97	15.20	36.20	27.20
	2.0.	5.00	20.00	0.48	20.10	15.60	37.50	27.70
	3.0	5.00	23.00	0.58	19.80	15.30	36.50	28.20
	4.0	6.00	26.00	0.61	19.30	15.80	38.25	27.80
	Control (China)	21.00	140.00	5.13	20.20	15.21	37.20	28.30
	Control (Shahi)	18.00	120.00	2.46	20.00	16.32	50.36	36.17
TSLP	1.0	7.00	28.00	0.70	19.90	15.63	38.00	28.21
	2.0.	6.00	30.00	0.76	20.10	15.64	38.20	28.34
	3.0	8.00	736.00	19.35	20.30	15.74	38.15	28.48
	4.0	22.00	2,567.66	70.00	20.40	15.76	38.56	28.51
	Control (China)	12.67	32.00	0.81	20.10	15.72	37.50	28.20
	Control (Shahi)	27.00	35.00	0.74	19.50	16.25	50.30	36.00
$CD_{0.05}$	A	0.43	0.57	0.20	0.012	0.033	0.037	0.269
	B	0.74	0.98	0.35	0.021	0.057	0.063	0.465
	A × B	1.05	1.39	0.50	0.030	0.081	0.090	NS

in controlling vegetative growth in litchi but these finding must be ascertained by further orchard trials and overall health assessment of the trees to reach up to coherent recommendation.

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