Influence of Paclobutrazol on Vegetative Growth, Nutrient Content, Flowering and Yield of Mango (Mangifera indica L.) and Its Residual Dynamics

K. Kishore1*, H. S. Singh2, D. Sharma3, T. R. Rupa3, R. M. Kurian3, and D. Samant1

ABSTRACT

Present study aimed to assess the efficacy of Paclobutrazol (PBZ) in mango in terms of vegetative growth, leaf nutrient status, flowering, yield, and fruit quality. Moreover, residual dynamics of PBZ in soil and plant parts was also assessed. Studies were conducted under tropical hot and humid climatic conditions of eastern India during 2013-2017 on 15-year-old trees of mango var. Arka Neelachal Kesari. Paclobutrazol was applied at 0.25–1.0 gram active ingredient (g ai m-1) canopy spread in soil during September. Results indicated that PBZ significantly reduced Trunk Cross Sectional Area (TCSA), shoot length, and leaf area. There was a reduction in leaf N and K contents, whereas the levels of Ca, Mg, Cu and Zn were increased in PBZ-treated plants. PBZ advanced floral bud break and increased flowering intensity, percentage of bisexual flowers, fruit yield, and yield efficiency. Higher concentration of PBZ aggravated shoot and panicle compaction. PBZ tended to increase Total Soluble Solids (TSSs) but pulp content and pulp/stone ratio were unaffected. PBZ residues in soil persisted for 9 months at higher rate of application, whereas at lower rate residues reached non-detectable level within 5-6 months after application. Fruits were free from PBZ residue, irrespective of dose. Application of PBZ at lower dose (0.25 g ai m-1 canopy spread) was not only efficacious in enhancing flower induction and yield without affecting plant growth but also exhibited high rate of depletion in soil.

Keywords: Flowering intensity, Fruit yield efficiency, Leaf nutrient status.

INTRODUCTION

Crop periodicity is markedly evident in most of the commercially important mango varieties of India. The ‘on’ year of mango is characterized by the prominence of reproductive shoots, whereas ‘off’ year is marked by vegetative shoots. The cyclic variation in flowering intensity and yield in mango may be attributed primarily to crop load and physiological roles played by hormones (Remirez and Davenport, 2010). Under subtropical condition, low temperature acts as a stimulus for floral induction, whereas in tropics, wherein cold inductive temperature is brief, shoot age is considered to be critical for floral induction. Hormones play central role in regulation of reproductive and vegetative phases of plants (Wilkie et al., 2008). Gibberellins (GAs) promote vegetative growth, whereas the ratio of auxin and cytokinin regulates flower induction in mango. It has been observed that reduction in the biosynthesis of GAs by inhibitors induces flowering in mango. Paclobutrazol, a gibberellin inhibitor, has exhibited its efficacy in regulating vegetative growth, flowering, and

1 Central Horticultural Experiment Station (ICAR-IIHR), Bhubaneswar -751019, Odisha. India.
* Corresponding author; e-mail: kkhort12@gmail.com
2 ICAR- National Center for Integrated Pest Management, New Delhi, India.
3 ICAR-Indian Institute of Horticultural Research, Bengaluru, India.
yield in perennial fruit crops. However, efficacy varies with climatic conditions, crop species, rate and methods of application (Nartvaranant et al., 2000). Paclobutrazol (PBZ) has been commonly used in mango to manipulate flower induction and vegetative growth (Upreti et al., 2013; Protacio et al., 2000; Oliveira et al., 2017; García et al., 2014). Apart from influencing intensity of flowering, PBZ has also been effective in influencing sex ratio, fruit set, yield, and fruit quality in mango (Singh, 2000; Burondkar et al., 2013).

Although PBZ has been efficacious in regulating physiological behaviour of plants, its residual aspect has been a matter of investigation, as the retardant has been considered moderately hazardous for human being (WHO, 2010). Concerns have also been expressed over the use of paclobutrazol as it inhibits gibberellin bio-synthesis, which is responsible for cell elongation and internode extension. It has been reported that continuous application of PBZ causes stunting and produces compressed panicles in mango. Moreover, residual influence was also recorded in soil and fruits (Reddy and Kurian, 2008; Sharma and Awasthi, 2005). However, in some of the cases, residues were not detected above quantifiable levels in soils and fruits even with continuous application of PBZ (Sharma et al., 2008).

Though considerable work has been carried out to assess the efficacy of PBZ in fruit crops under different climatic conditions, yet variation in its effectiveness in yield-contributing parameters has been observed. Moreover, residual fate of PBZ is debatable. Considering the importance of growth retardant in regulating vegetative and reproductive traits under tropical eastern region of India, this study aimed to assess the effectiveness of PBZ in mango and to study the residual dynamics of PBZ to optimise the application rate of PBZ in mango.

**MATERIALS AND METHODS**

Studies were conducted during 2013-2017, at the research farm of Central Horticultural Experiment Station, Bhubaneswar, India (Elevation: 45 m amsl; Latitude: 20° 27’ N; Longitude: 85° 40’ E). The site is located in the eastern coastal region of India, which is characterized by tropical, hot and humid climate (T<sub>max</sub>: 33.7°C, T<sub>min</sub>: 22.2°C, Rainfall: 1,550 mm, RH: 76%). Soil was sandy loam, strongly acidic in reaction, and had low organic carbon (< 0.5%) and N content (< 200 kg ha<sup>-1</sup>).

Fifteen-year-old ‘Arka Neelachal Kesari’ mango trees, planted at a density of 100 trees ha<sup>-1</sup> and uniform in vigour and canopy spread were selected for study. All the trees were provided with standard orchard management practices including nutrient and pest management. The experiment was laid out in randomized block design with four treatments i.e. T1- Control, T2– PBZ at 0.25 g, T3– PBZ at 0.50 g, and T4– PBZ at 0.75 g ai per meter canopy spread. The average canopy spread of tree was 8 meters and the dose of paclobutrazol was worked out accordingly. There were six replications each with a single tree unit. The quantified amount of PBZ (Lustar- 28% w/w) was dissolved in 15-20 litres of water and applied around the root zone by making a ring with a radius of 1.5-2.0 m in mid-September. The control trees were treated with water.

In order to study vegetative growth parameters (shoot growth and intermodal length), 200 shoots were randomly tagged in different directions (under each treatment) at the time of imposition of treatments. Average length of new shoots and intermodal length during the course of experiment was presented as shoot growth and intermodal length, respectively. Trunk Cross Sectional Area (TCSA) of mango trees was calculated by using the formula πr<sup>2</sup> considering the cross sectional area of trunk as a circle and expressed in cm<sup>2</sup>. Annual increment in TCSA under different treatments was determined on the basis of annual growth and expressed in percentage. Leaf area of 30 recently matured leaves per tree from the tagged branches was measured following the method suggested by Pandey and Sharma (2011). Days required for floral bud break after imposition of treatment were recorded when emergence of floral buds
Influence of Paclobutrazol on Mango

Flowering intensity (%)= (No. of flowering shoots/Total no. of shoots)×100

Intensity of shoot and panicle compaction was calculated based on the number of compact shoots (shortened internodes) and panicles in 200 tagged shoots and expressed in percentage. Per cent hermaphrodite flower was determined on the basis of number of stamate and hermaphrodite (bisexual) flowers counted at full bloom (> 75% flowers were open) in twenty tagged panicles under each replicate. Fruit set percentage was recorded on the basis of number of fruits retained at pea stage (7-8 mm) and number of bisexual flowers. Fruits were harvested separately in each replicate and average was worked out to express the yield in kg tree\(^{-1}\).

Yield efficiency was computed by dividing the yield by TCSA and expressed in kg cm\(^{-2}\) (Stern and Doron, 2009).

Soil samples were collected from the site of application (collar region) at different intervals i.e. 3, 5, 7 and 9 Months After Application (MAA) using a soil auger. A homogeneous soil sample was drawn from each of the treated or control tree basins at 10–15 cm depth. The soil collected from each tree basin (500 g) was pooled together, air-dried, mixed thoroughly and sieved through a 2 mm sieve and 50 g sample was taken for estimation. Hundred fully mature leaves (3 MAA), 50 panicles, and 20 mature fruits were randomly sampled under each treatment. Fruit pulp were cut into pieces and mixed thoroughly to prepare a homogenous sample. Leaves, panicles and fruit pulp were oven dried (70°C for 48 hours), powdered and sieved. All the samples were processed immediately and analyzed within 7 days.

PBZ residue was estimated by LC-MS/MS using QuEChERS method (Anastassiades et al., 2003) of sample preparation with slight modification. Three g powdered sample of leaf or panicle, 10 g soil sample, and 15 g homogenized fruit sample were taken separately in 100 mL centrifuge tube. Fifteen mL 1% acetic acid in acetonitrile, 5 g anhydrous magnesium sulphate and 1.5 g sodium acetate were added in the tube and the contents were mixed thoroughly for 2 minutes by using a vortex mixer. The mixture was centrifuged at 4,000 rpm for 10 minutes, and 4 mL of supernatant was taken in another centrifuge tube and 50 mg PSA and 150 mg anhydrous magnesium sulphate were added. The mixture was again vortexed for 1 minute and then centrifuged and the supernatant was analyzed directly using LC-MS/MS equipment with positive ESI ion source. LC-MS/MS
analysis for paclobutrazol residues was performed with an Agilent (California, USA) 1290 HPLC hyphenated to Agilent 6460 C triple quadrupole mass spectrometer with ESI probe in the positive mode. The analytical column used was a Zborax (CA, USA) make Eclipse plus C-18, 100×3 mm id, 1.8 μm particle size. The column temperature was 40°C, injection volume was 1 μL and flow rate was 0.5 mL min⁻¹. The mobile phase consisted of 5 mM ammonium formate and 0.01% formic acid in water (A) or methanol (B). The gradient programme used was as follows: Time 0 min= 15% B, 1.0 min= 15% B, 6.0 min= 50% B, 12.0 min= 95% B, 17.5 min= 95% B, 18.0 min= 15% B. Under the above conditions, paclobutrazol eluted out at a retention time of 4.5 min. The MS conditions were, Nebulizer- 30 psi, Sheath gas flow- 11 L min⁻¹, Capillary voltage- +3000V, Sheath gas temperature– 375°C. The fragmentor voltage for paclobutrazol standard was optimized to produce the greatest signal for the precursor ion. The protonated molecule (m/e= 294.1) was used as the precursor ion. Paclobutrazol residues in the samples were analyzed using matrix matched calibration standards to remove the effect of matrix.

Statistical Analysis

Differences between treatments were determined with Analysis Of Variance (ANOVA) by using OPSTAT (HAU, Hisar) and Critical Difference (CD) and standard error of mean were calculated. Whenever significant differences were observed, means were separated using Least Significant Difference (LSD) test at the 5% level of significance.

RESULTS AND DISCUSSION

Vegetative Growth

Paclobutrazol tended to affect plant growth by reducing annual increment in TCSA, shoot length, and leaf area (Table 1). There was a gradual reduction in the annual increment of TCSA with the increase of PBZ application rate. There was a significant reduction in the internodal length of new flush, which consequently reduced the length of new shoots (20-30%) in PBZ treated plants. It was evident from the findings that the rate of reduction in vegetative growth was proportional to the rate of PBZ application. There was a significant reduction in the leaf area (25-35%) of treated trees, which followed progressive dose response (Table 1). PBZ significantly influenced the intensity of shoot compaction, which increased with the rate of application (Table 1). It was also observed that compact shoots were either vegetative or produced compact panicles retaining no fruit. The efficacy of PBZ in influencing vegetative growth has been reported by many researchers; however, climatic conditions, varieties, and rate of application play important roles in influencing its efficacy (Arzani and Roosta, 2004). Significant reduction in the vegetative growth and leaf area may be due to the reduction in the content of the endogenous Gibberellins (GAs) influenced by PBZ (gibberellins-inhibitor). Bioactive GAs regulate the natural developmental processes including cell elongation and cell division by inducing transcription of genes involved in these processes which culminate in stem growth in plants (Sun, 2010). Since GA stimulates elongation of cell and internode, reduction in biosynthesis could have affected the shoot growth, leaf area, and TCSA (Wang and Irving, 2011).

Leaf Mineral Composition

Leaf nutrient content, an indicator of root nutrient uptake, was influenced by PBZ resulting in decreased levels of N and K and increased levels of Ca, Mg, Cu, and Zn, however, the level of P was unaffected (Table 3). There was a linear relation between leaf Ca content and PBZ.
concentration suggesting a progressive dose response. On the other hand, PBZ had inverse relationship with N and K contents. Leaf Mg, Cu, and Zn contents didn’t exhibit progressive linear relationship with PBZ concentration. Reduction in the leaf N and K contents in PBZ-treated trees could be due to reduced root hydraulic conductivity and root length, which in turn reduces water flux responsible for passive uptake of mobile nutrients like N and K (Reiger and Scalabrelli, 1990). On the other hand, the increase in the leaf Ca, Mg, Cu, and Zn contents suggests their high rate of absorption either through diffusion or root interception. Arzani et al. (2009) reported that PBZ enhanced the Ca and K concentrations in leaves without influencing N and P levels. On the other hand, Singh et al. (2005) observed that soil application of paclobutrazol for two consecutive years increased the levels of P, K, and Ca at lower doses but decreased the levels at higher dose. The influence of paclobutrazol on leaf nutrient status lacks consistency as the level of nutrient varies differently with the application rate and soil conditions.

Flowering Behaviour and Fruit Set

Paclobutrazol was efficacious in advancing floral bud break and, in turn, flowering. Trees treated with high concentration of PBZ took the lowest number of days for attaining floral bud break stage. There was an advancement of flowering by about two to three weeks in PBZ-treated trees (Table 1). Flowering intensity was also significantly influenced by the application of PBZ, however, year-wise variation was evident (Figure 1). The effect of PBZ was more pronounced (increased by 2-3 folds) when untreated plants had low flowering intensity (off-year). In contrary, the effect became less pronounced in ‘on’ year (when untreated trees had moderately high flowering intensity). Floral induction is considered to be the result of elevated levels of up-regulated Florigenic Promoter (FP).
Table 2. Effect of paclobutrazol on yield efficiency and fruit quality of mango.

<table>
<thead>
<tr>
<th>Rates of PBZ (g ai m⁻¹ canopy spread)</th>
<th>Yield efficiency (kg cm⁻²)</th>
<th>Fruit weight (g)</th>
<th>Pulp (%)</th>
<th>Pulp/Stone ratio</th>
<th>TSS (°Brix)</th>
<th>Titratable acidity (%)</th>
<th>Acid:Acid ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Control)</td>
<td>0.052ab</td>
<td>183.52c</td>
<td>68.12a</td>
<td>4.03a</td>
<td>16.85bc</td>
<td>0.28b</td>
<td>55.12c</td>
</tr>
<tr>
<td>0.25</td>
<td>0.075bc</td>
<td>174.28a</td>
<td>67.85a</td>
<td>3.99a</td>
<td>17.64a</td>
<td>0.32c</td>
<td>54.73c</td>
</tr>
<tr>
<td>0.50</td>
<td>0.115a</td>
<td>170.84c</td>
<td>67.74a</td>
<td>3.98a</td>
<td>17.91a</td>
<td>0.33c</td>
<td>54.27c</td>
</tr>
<tr>
<td>0.75</td>
<td>0.090bc</td>
<td>171.66c</td>
<td>67.87a</td>
<td>3.97a</td>
<td>17.60a</td>
<td>0.34c</td>
<td>51.76b</td>
</tr>
<tr>
<td>SED</td>
<td>0.10</td>
<td>2.31</td>
<td>1.35</td>
<td>0.22</td>
<td>0.66</td>
<td>0.03</td>
<td>2.11</td>
</tr>
</tbody>
</table>

* Means followed by the same superscripted letters in a column are significantly different by LSD test at P< 0.05.

Table 3. Effect of paclobutrazol on leaf nutrient status of mango.

<table>
<thead>
<tr>
<th>Rate of PBZ (g ai m⁻¹ canopy spread)</th>
<th>N (%)</th>
<th>P (%)</th>
<th>K (%)</th>
<th>Ca (%)</th>
<th>Mg (%)</th>
<th>Cu (ppm)</th>
<th>Zn (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Control)</td>
<td>0.62c</td>
<td>0.10a</td>
<td>0.90c</td>
<td>3.01c</td>
<td>0.38c</td>
<td>8.90c</td>
<td>19.20c</td>
</tr>
<tr>
<td>0.25</td>
<td>0.54b</td>
<td>0.11a</td>
<td>0.81b</td>
<td>3.68b</td>
<td>0.43b</td>
<td>10.30a</td>
<td>25.90a</td>
</tr>
<tr>
<td>0.50</td>
<td>0.52b</td>
<td>0.10a</td>
<td>0.78b</td>
<td>3.82b</td>
<td>0.48b</td>
<td>9.62b</td>
<td>22.10b</td>
</tr>
<tr>
<td>0.75</td>
<td>0.46c</td>
<td>0.10b</td>
<td>0.71c</td>
<td>4.32c</td>
<td>0.43b</td>
<td>9.54b</td>
<td>21.24c</td>
</tr>
<tr>
<td>SED</td>
<td>0.06</td>
<td>0.03</td>
<td>0.10</td>
<td>0.45</td>
<td>0.03</td>
<td>0.52</td>
<td>0.74</td>
</tr>
</tbody>
</table>

* Means followed by the same superscripted letters in a column are significantly different by LSD test at P< 0.05.

**Figure 1.** Temporal variation in flowering intensity of mango as influenced by paclobutrazol.

**Figure 2.** Temporal variation in fruit yield as influenced by paclobutrazol.
Influence of Paclobutrazol on Mango

and down-regulated Vegetative Promoter (VP), primarily gibberellins (Davenport, 2007). Paclobutrazol tends to reduce the level of gibberellins (vegetative promoters) and thereby stimulates flower induction in weakly inductive shoots of fruit crops (Adil et al., 2011). It seems that by affecting biosynthesis of GAs, PBZ demonstrates two pronged action i.e. induction of flower and regulation of vegetative growth. The inhibitory role of GAs in flowering of perennials at the expense of reproductive development has been reported in many fruit crops (Wilkie et al., 2008).

When influence of PBZ on panicle compaction was taken into account, a corresponding increase in the intensity of compaction was observed with the rate of application (Table 1). Substantially high number of panicles exhibited compaction when PBZ was applied at higher dose, whereas the intensity was reduced at low rate of application. Increase in the intensity of panicle compaction is due to the reduction in internodes of panicle, which could be attributed to the inhibition of gibberellin activity at higher dose of PBZ. Application of PBZ significantly increased the percentage of bisexual flowers. However, the increase was not in correspondence with the rate of PBZ application. The highest percentage of bisexual flowers were recorded when PBZ was applied at 0.50 g ai m⁻¹ canopy spread. Singh (2000) reported that, apart from enhancing flowering intensity, PBZ was also effective in increasing sex ratio in mango. It may be presumed that reduction in level of GAs due to PBZ could have stimulated the biosynthesis of ethylene, which is responsible for induction of femaleness in many plants. Moreover, ethylene signalling pathway also mediates the arrest of stamen primodia and in turn reduces the production of male flowers (Weiss and Ori, 2007; Yamasaki et al., 2005).

Regardless of the concentrations applied, PBZ-treated trees had lower fruit set. The minimal fruit set was recorded when PBZ was applied at higher rate (Table 1). Fruit set is primarily determined by the transfer of viable pollen on the stigma, pollen germination, and fertilization, and in all the physiological events gibberellins play important role. During fruit set and development rapid cell division and cell expansion occur, which are primarily regulated by gibberellins. It has been reported that fruit set and fruit growth are reduced significantly if biosynthesis of gibberellins is inhibited by gibberellins inhibitor like paclobutrazol, however, inhibitory effect may be fully counteracted with the application of GA₃ (Serrani et al., 2007). Yield and yield efficiency were influenced by PBZ; however, temporal variation was evident. Application of PBZ at 0.25 g ai m⁻¹ canopy spread was found to be the most effective in enhancing yield and yield efficiency (Table 2). On the other hand, high dose of PBZ (T4) was relatively less efficacious in enhancing fruit yield in spite of inducing high flowering intensity. High intensity of panicle compaction and low fruit set may be attributed for low yield under T4. It has been observed that the influence of PBZ on fruit yield was significantly more in 2014 than 2015 and 2016 (Figure 3). In 2014, the increase in yield in PBZ treated trees was significantly higher than the control, whereas in 2015 and 2016 increase was comparatively low. It may be interpreted that PBZ was more efficacious in enhancing flowering intensity and fruit yield when the control plants exhibited low yield potential. In 2015, the influence of PBZ in enhancing fruit yield was minimal when untreated plants had moderately high yield.

Influence of PBZ on physico-chemical attributes of mango indicated that fruit weight was significantly reduced in treated trees as minimal fruit weight was recorded when PBZ was applied at higher dose (Table 2). On the other hand, pulp content, stone percentage and pulp stone ratio were minimally affected by PBZ. An increase in TSS and titratable acidity and reduction in TSS: Acid ratio was recorded in treated plants; however, increase was not in
correspondence with the rate of PBZ application. Burondkar et al. (2013) and Singh (2000) also reported improvement in fruit quality in terms of TSS and acidity with paclobutrazol application. On the other hand, some of the findings indicate no improvement in fruit quality with the application of PBZ (Lolaei et al., 2012; Arzani et al., 2004).

**Residual Dynamics**

The residue of PBZ in soil varied significantly with the rate of application (Figure 4). The high concentration of residues were recorded when PBZ was applied at higher rate and there was a corresponding decrease in residue with the reduction in PBZ level. Initially, the residue level in soil was significantly high, which was followed by temporal depletion. Data indicated that within five months of PBZ application, soil residue was reduced by more than 70% under different treatments. Furthermore, the residue reached non-detectable level five months after application of PBZ at relatively lower rate (0.25 g ai m⁻¹ canopy spread), whereas the residue persisted for nine months when PBZ was...
applied at higher rate (0.75 g ai m⁻¹ canopy spread). The results clearly indicate that there is no carry over effect of PBZ. The slow rate of PBZ degradation in soil may be due to its slow rate of metabolism and low vapour pressure, which make it an environmentally stable compound (USEPA, 2007; Vaz et al., 2015). Sharma and Awasthi (2005) detected residues of paclobutrazol in soil at the end of each season and reported that continuous application of PBZ tended to increase residues in soil. Reddy and Kurian (2008) also observed residual influence of PBZ in soil with continuous application. On the other hand, Sharma et al. (2008) could not detect paclobutrazol residues above quantifiable levels in soil even after continuous application and suggested low rate of PBZ application. PBZ residue was also quantified in plant parts, but it was observed that leaves and panicles of treated plants had no residue even three months after application (Figure 5). Moreover, mature fruits were also free from residue. The results comply with the findings of Costa et al. (2012) who reported non-translocation of PBZ in mango fruits.

CONCLUSIONS

Paclobutrazol was efficacious in advancing floral bud break and in increasing flowering and fruit yield, whereas vegetative growth, leaf area, leaf N and K contents and fruit set were affected negatively. The persistence of soil residues followed progressive dose response as residues persisted for longer duration at higher application rate. Importantly, no residue was detected in mature fruits of PBZ-treated trees. It has been observed that high rate of application (0.75 g ai m⁻¹ canopy spread) not only promoted shoot and panicle compaction but also left residues for longer period in the soil. It may be concluded that application of PBZ at 0.25 g a. i. m⁻¹ canopy spread may be optimised to regulate growth, flowering, and yield of mango. Nevertheless, comprehensive work on residual dynamics is required to optimize the dose of PBZ under different agro-climatic conditions.

ACKNOWLEDGEMENTS

Authors are thankful to the Director, ICAR-Indian Institute of Horticultural Research, Bengaluru for extending support during studies.

REFERENCES


اثر پاکلووتوترازول روی رشد سبزینه ای، محتواعنصار غذایی، گلدهی، و عملکرد مانگو (Mangifera indica L.)

ک. کیشور، ه. س. سینگ، د. شارما، ت. ر. روپا، ر. م. کوریان، و د. سامانت

چکیده


