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Regulatory Effect of Prohexadione Calcium on Mango: Enhances Early Floral Induction and Flowering Physiology

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ABSTRACT: India possesses a competitive advantage over other nations in mango production due to its favorable natural resources and climactic circumstances. Climate change negatively impacts mango cv. Banganapalli cultivation by altering its ideal growing conditions, increasing pest and disease outbreaks and potentially changing suitable growing areas. Irregular flowering, limited fruit set and inadequate retention resulted in diminished output and inferior fruit quality in mango production. The efficiency and production of mango orchards are influenced by various factors such as biennial bearing, significant fruit drop during the early phases of fruit growth and adverse environmental circumstances that lead to reduced fruit set. The main aim of this study was to examine the effects of Prohexadione calcium on the early floral induction and flowering physiology of the mango cultivar Banganapalli. The chosen trees were treated with different treatments *viz.*, Absolute control (T_1), Control (Water spray) (T_2), Prohexadione calcium 150 ppm (T_3), Prohexadione calcium 200 ppm (T_4), Prohexadione calcium 250 ppm (T_5), Prohexadione calcium 300 ppm (T_6). The trees treated with prohexadione calcium at the concentration of 200 ppm exhibited the maximum levels of chlorophyll index (41.416, 39.700), carbohydrates (18.95mg $100g^{-1}$, 26.78 mg $100g^{-1}$), nitrogen content (1.35 per cent, 1.75 per cent), C/N Ratio (14.037, 15.305) at flowering and fruit development stage, respectively. Similarly, the number of days taken for first flowering (46.75), the advancement of flowering was (32 days) earlier than the control, total number of flowers per panicle (1003.25), hermaphrodite flowers (200.50) and percentage of fruit set (0.468), were also higher in the same treatment. In a nutshell, applications of Prohexadione calcium at the concentration of 200 ppm had a positive effect on the early floral induction and fruit set of mango cv. Banganapalli.

Keywords: mango, prohexadione calcium, early flowering, fruit set.

INTRODUCTION

Mango (*Mangifera indica* L.) belongs to the Anacardiaceae family, is indigenous to the Indo-Burma region**.** Mango has been considered as one of the most commercialized fruits of the tropical and sub-tropical countries (Majeedano *et al.,* 2021). It is commonly called as 'King of fruits' in the world market, because of its excellent flavor, attractive fragrance, beautiful shades, delicious taste and medicinal value**.**

In mango, flowering is a vital milestone in fruit production, serving as a key determinant of fruit development and economic yield (Latheef *et al.*, 2022). Excessive vegetative development during flowering leads to poor fruit set and diminished yields in mango cultivar Banganapalli. In South Indian conditions, flower bud differentiation commences during the month of October and the fruit maturation occurs during the month of March to June (Palanichamy *et al.*, 2011). Temperature fluctuation occurs frequently in the month of December, leading to suboptimal and postponed flowering. The postponement of flowering causes inadequate fruit set, resulting in diminished production. Regular flowering is an essential factor for achieving consistent mango yield in subtropical climates. Seasonal flowering is irregular and inconsistent due to environmental condition for floral initiation. Flower induction in mango has close links with hormonal synthesis and its balance in the developing reproductive plant parts. Floral initiation in mango has been shown to be strongly correlated with gibberellins (Upreti *et al.*, 2014). High gibberellic acid (GA) content in plants hinders flowering and promotes vegetative growth,

while low levels of the same compound encourage flowering in mango (Davenport, 2007). Various growth regulating compounds in plants have been evaluated for their effects on inhibiting floral formation in mango across the different countries (Chacko, 1991). Therefore, it is essential to create an effective strategy to stimulate flowering at the optimal time from newly emerged vegetative flush in mango (Lakshmi *et al.*, 2023). One such growth regulating compound is Prohexadione calcium (P-Ca) which regulates the plant growth and flowering by inhibiting gibberellin synthesis. Prohexadione calcium mitigates the vegetative development in fruit plants without compromising the yield and fruit quality. Application of Prohexadione calcium advanced the flowering of the regular bearing mango cultivar by more than 30 days (Abdel Rahim *et al.,* 2011). However, there is limited research on the effects of exogenous application of P-Ca on the dynamic changes of endogenous hormones as well as fruit quality and flavour during mango growth and development. The main objective of this research is to examine the effects of Prohexadione calcium on the early floral induction and flowering physiology of the mango cv. Banganapalli.

MATERIALS AND METHODS

The experiment was conducted in a six year old mango orchard at Pathiri village near Tindivanam, Villupuram District, Tamil Nadu during the year 2024 - 2025, to investigate the impact of Prohexadione Calcium (P-Ca) on early floral induction and flowering physiology characteristics of mango cv. Banganapalli. The trees were planted at the spacing of 5 × 5 m. The experiment was carried out by adopting the Randomized Block Design (RBD) and the treatment were replicated thrice. The trees were sprayed with following treatments *viz.,* Absolute control ($T_{1)}$, Control -Water spray (T_2), Prohexadione calcium 150 ppm (T_3), Prohexadione calcium 200 ppm (T_4), Prohexadione calcium 250 ppm (T_5), Prohexadione calcium 300 ppm (T_6). The application of Prohexadione calcium was done before the signal of flower bud initiation during the month of October, 2024.

A. Sampling and data collection

About 100 terminal shoots, averaging 20 cm in length, were labeled in four directions on the experimental trees. Observations on the effect of Prohexadione calcium on flower induction were conducted at 15 days interval following one month of observation and the number of floral buds generated was recorded to determine the average days necessary for 50% flowering.

B. Total chlorophyll index

Total chlorophyll content in fresh leaves was quantified in SPAD units utilizing a Minolta chlorophyll meter (SPAD 502). Measurements were collected from the uppermost fully expanded leaf (4^{th} or 5^{th} leaf from the apex). SPAD 502 readings were noted during the flowering, fruit development and harvesting stages. Consequently, forty SPAD readings were gathered from fifteen plants to determine the mean SPAD 502 values for each treatment.

C. Total Nitrogen (%)

Microkjeldhal method (Humphries, 1956) was followed for estimating the total nitrogen content in the leaf samples after harvest and the samples were digested with concentrated sulphuric acid and a digestion mixture in a digestion chamber till a light bluish green residue was obtained. The known aliquot was distilled in an alkali medium and the liberated ammonium was absorbed in boric acid mixed indicator solution after complete distillation, disconnected from the receiving flask and then the content was titrated against standard sulfuric acid till the colour changed from green to wine red colour (Piper, 1966).

D. C/N ratio

The carbohydrate (C) and total nitrogen content were determined using the colorimetric method of Somogyi (1952) and the Micro-Kjeldahl method of Piper (1966). The C/N ratio was calculated by dividing the total carbohydrate content by the total nitrogen content.

E. No. of days taken for first flowering

Days taken for first flowering was calculated from the spraying to first flower bud emergences. The observations were recorded individually for each tree and later averaged for each treatment.

F. Number of flowers per panicle

The total count of flowers per panicle was determined by summing the male and hermaphrodite flowers, represented numerically.

G. Number of hermaphrodite flowers per panicle

Five panicles were randomly selected from each tree during full bloom stage and hermaphrodite flowers were counted. Average values for these panicles were taken to represent the number of hermaphrodite flowers per panicle.

H. Fruit set percentage

The duration from panicle initiation to fruit production at the mustard stage was documented. Ten shoots were randomly tagged from the North, South, East and West directions) and the fruit set was recorded. The average duration for fruit set following panicle initiation was calculated and expressed as a percentage.

I. Identification of chemical compounds through GC-MS

The mango leaf samples were taken from T_1 (Control) and T4 (Prohexadione calcium 200 ppm) were collected from 4-5 months old borne on the 4^{th} and 5^{th} nodes from the borne of the shoot (Pathak and Pandey 1976). Then air dried at ambient temperature and ground into a powder for extraction. The powder (5g) was macerated in 80% methanol and permitted to stand for 48 hours at ambient temperature. The solution was filtered using Whatman No.1 filter paper, and the volatile compounds were evaluated with a SHIMADZU QP2010 PLUS Gas Chromatograph Mass Spectrometer. The instrument was fitted with a 30 m × 0.25 mm i.d. HP-5 column, which had a 0.25 mm film thickness and was made of

cross-linked phenyl-methyl siloxane. The oven was initially set to a temperature of 40°C and maintained for a duration of 6 minutes. The temperature was subsequently raised at a rate of 2.5°C per minute until it reached 150°C, and then at a rate of 90°C per minute until it reached 250°C. The temperature of the injection port and ionizing source was maintained at 250°C and 280°C, respectively. The split ratio was 10:1, with a sample volume of 2 µL injected. Following a two minutes delay caused by the solvent, the mass spectrum was obtained from m/z 35 to 300, resulting in a scan rate of 5.27 scans per second. The identification of compounds was performed by comparing the mass spectra and retention duration with those of comparable standards, which were identified at Nanotechnology Research Centre (NRC), SRMIST, Chengalpattu.

RESULTS AND DISCUSSION

A. Physiological parameters

The foliar application of Prohexadione calcium significantly improved mango physiological parameters during the different growth stages. Among the treatments, application of 200 ppm prohexadione-Ca recorded the maximum level of chlorophyll index in different stages, flowering stage (41.416 SPAD) and fruit development stage (39.700 SPAD) compare to absolute control (Table 1). Sabatini *et al.* (2003) observed that Prohexadione calcium enhanced the chlorophyll concentration in the leaves of apple and pear trees and also noted that Prohexadione calcium enhanced net photosynthesis and inferred it positively affected fruit weight and yield. These results are aligned with the research conducted by Singh *et al.* (2020), reported that Prohexadione calcium at 200 ppm significantly increased chlorophyll content and nitrogen assimilation in mango cv. Dashehari under field conditions. Their study showed a similar trend, where higher concentrations above 200 ppm resulted in diminished the beneficial effect of physiological activity. This consistency across the various studies emphasizes the efficiency of Prohexadione calcium and support its role as a promising growth regulator for improving flowering physiology in mango.

The application of 200 ppm Prohexadione calcium recorded the maximum level of carbohydrate content in different stages, *viz.,* 18.95 mg $100g^{-1}$ at the flowering stage and 26.78 mg $100g^{-1}$ at fruit development stage, respectively (Table 1). The mechanism involved by Prohexadione calcium application enhances photosynthesis is associated with the concentration of chlorophyll per unit leaf area and carbohydrate content. Similar results were reported in apple (Prive *et al.,* 2004).

The maximum amount of nitrogen content was recorded in the application of 200 ppm Prohexadione calcium at different growth stages, *viz.,* 1.35 per cent at flowering stages and 1.75 per cent at fruit development stage compare to absolute control (Table 1). Similarly, the higher C/N ratio (14.03 at flowering stage and 15.30 at fruit development stage) was observed in the same treatment. Prohexadione calcium has also been reported as capable of increasing total non-structural carbohydrates (TNC), nitrogen accumulation and stimulating stomatal opening. It is normally associated with large carbon assimilation, which promotes early flowering and fruit set (GUAK *et al.,* 2001).

B. Flowering parameters

The foliar application of Prohexadione calcium significantly influenced the days taken for first flowering in mango. In current study, the application of Prohexadione calcium 200 ppm recorded the early floral induction at (46.75 days), whereas the control showed the delayed flowering (78.75 days). It is also observed that application of 300 ppm of P-Ca delayed the flowering by 71 days (Table 2), indicating that excessive concentrations may suppress floral initiation. The hormonal effect is likely due to the inhibition of gibberellin biosynthesis, promoting reproductive over vegetative growth. These findings are consistent with the results of Banger *et al.* (2021), observed that Prohexadione calcium at optimal concentrations advances flowering in guava by reducing vegetative vigour which enhances floral differentiation.

The maximum number of flowers per panicle (1003.25) and hermaphrodite flowers per panicle (200.50) was observed in the application of 200 ppm Prohexadione calcium while compare with the absolute control (Table 2). This might be due to the presence of higher C/N ratio in the bud which increases the starch accumulation and reduces the vegetative growth thereby resulted in more number of reproductive buds. Similar results were reported by Owens and Stover (1999) in apple and De Oliveira *et al.* (2022) in guava.

Similarly, the higher percentage of fruit set was recorded in the application of 200 ppm Prohexadione calcium (0.46 per cent) compare to absolute control (Table 2). Application of Prohexadione calcium at 200 ppm increased the percentage of flowering and fruit set, due to inhibiting effect of gibberellin and higher C/N ratio (Ramirez *et al.,* 2014). These results are in agreement with Medjdoub *et al*. (2005) reported that Prohexadione calcium enhanced the fruit set of 'Gala' apples.

C. GC-MS Analysis

The results of GC-MS and preliminary photochemical testing indicated that the mango leaves contained numerous bioactive phytoconstituents. In current investigation, the untreated leaves contain kaurene (Table 3) which is a crucial intermediate, acting as the first tetracyclic precursor for gibberellins in untreated sample (Fig. 1). Whereas the compound kaurene is not detected in the treated leaves (Fig. 2). Kaurene is well known intermediate in the gibberellin biosynthetic pathway (Helliwell *et al.,* 1998). Prohexadione calcium is recognized for its capacity to suppress gibberellin (GA) biosynthesis, which substantially promotes early floral inductions and accelerating fruit set (Ziauka and Kuusiene 2010).

Table 1: Effect of Prohexadione calcium on physiological parameters of cv. Banganapalli.

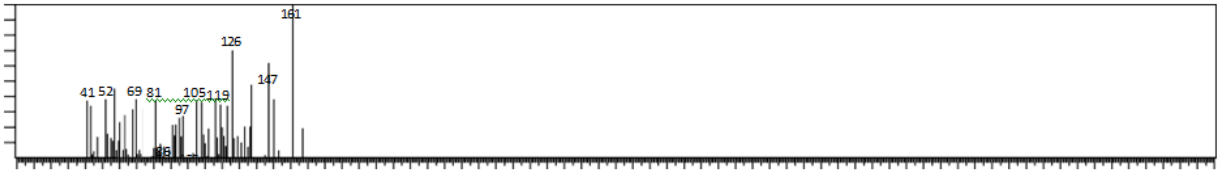
Treatments	Chlorophyll Index (SPAD)		Carbohydrate (mg 100g^{-1})		Nitrogen content (%)		C/N Ratio	
	Flowering Stage	Fruit development stage	Flowering Stage	Fruit development stage	Flowering stage	Fruit development stage	Flowering stage	Fruit development stage
T_1 – Absolute control	27.85	25.95	9.34	12.62	0.86	1.32	10.86	9.52
T_2 – Control (Water spray)	30.36	28.92	10.95	13.61	0.95	1.41	11.52	9.63
T_3 – Prohexadione calcium 150 ppm	40.10	38.40	15.59	18.75	1.26	1.70	12.37	10.98
T_4 - Prohexadione calcium 200 ppm	41.41	39.70	18.95	26.78	1.35	1.75	14.03	15.30
T_5 - Prohexadione calcium 250 ppm	40.59	39.16	16.96	24.34	1.27	1.72	13.35	14.09
T_6 - Prohexadione calcium 300 ppm	37.35	34.89	14.23	16.33	1.17	1.64	12.17	9.95
SE(d)	0.41	0.50	0.28	0.01	0.02	0.01	0.13	0.04
CD (5%)	0.88	1.06	0.61	0.03	0.05	0.01	0.28	0.09

Table 2: Effect of Prohexadione calcium on flowering and fruit set of cv. Banganapalli.

Treatments	No of days taken for 1st flowering	Total number of flowers per panicle	Hermaphrodite flowers per panicle	Fruit set (%)
T_1 – Absolute control	78.75	698.5	157.50	0.28
T_2 – Control (Water spray)	75.25	751.75	160.25	0.29
T_3 – Prohexadione calcium 150 ppm	67	811.25	181.25	0.33
T_4 - Prohexadione calcium 200 ppm	46.75	1003.25	200.50	0.46
T_5 - Prohexadione calcium 250 ppm	55	848.25	195.50	0.42
T_6 - Prohexadione calcium 300 ppm	71	825.50	170.80	0.32
SE(d)	0.12	0.97	0.23	0.02
CD (5%)	0.27	2.08	0.50	0.05

Table 3: GC-MS analysis in control sample of cv.Banganapalli.

Sr. No.	Compound name	Peak area	Retention time	Compound molecular formulae	Biological activity	References
1.	Kaurene	57	13.900	$C_{22}H_{34}O_2$	Intermediate in the gibberellin biosynthetic pathway	Helliwell *et al.* (1998)



Peak:57 Retention Time:13.900, MassPeaks:69 BasePeak:161.15(1827)
Action of gibberellin compound on control leaf sample of cv.Banganapalli

Fig. 1. GC-MS analysis in control sample of mango (*Mangifera indica* L.) cv.Banganapalli.

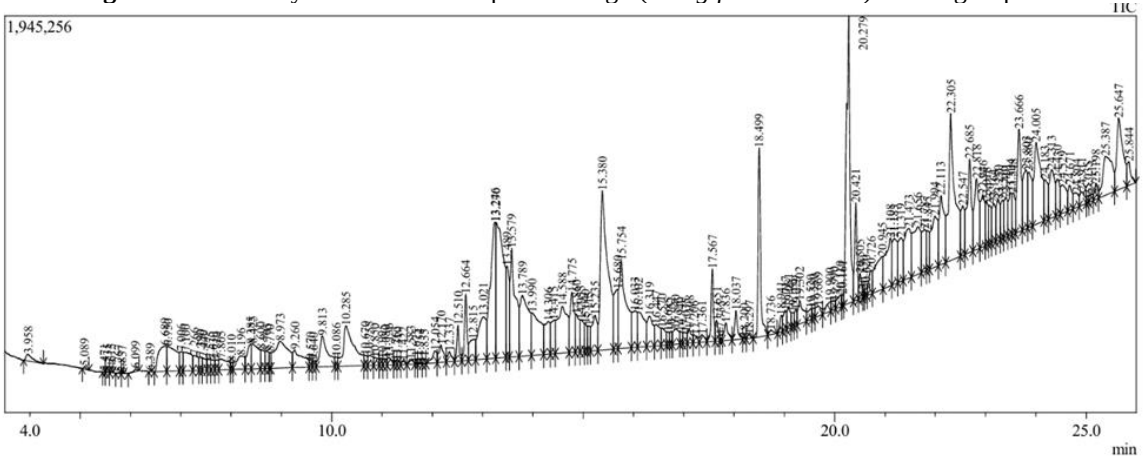


Fig. 2. GC-MS analysis in treated sample of mango (*Mangifera indica* L.) cv.Banganapalli.

Total components of the treated sample of cv. Banganapalli (Gibberellin compound was not identified in the treated leaf sample).

CONCLUSIONS

In a nutshell, applications of prohexadione calcium at the concentration of 200 ppm had a positive effect on the early flower induction and fruit set of mango cv. Banganapalli. It recorded the maximum level of chlorophyll index, carbohydrate, nitrogen content, C/N Ratio at flowering and fruit development stage respectively. Similarly, the number of days taken for first flowering, total number of flowers per panicle, hermaphrodite flowers and percentage of fruit set, were also higher in the same treatment.

FUTURE SCOPE

Prohexadione calcium shows great promise in mango cultivation by promoting early flowering, improving fruit yield, quality and shelf life. Its consistent performance across various climates can be confirmed through extended field trials for wider adoption as a reliable growth regulator.

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Conflict of Interest. None.

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