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Effect of pruning and paclobutrazol application of acid lime (*Citrus aurantifolia* Swingle) cv. Balaji under Sub-Himalayan Terai region of West Bengal

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Abstract
Acid lime is considered as most important fruit crop. Acid lime is also known as Kagzi lime or Neebu. It is considered to be native of Malayan peninsula. It belongs to the family Rutaceae, with chromosome number (2n=18). An experiment was conducted at the farmer's field at Gopalpur, Cooch Behar during the year 2018 to 2020. The trial was laid out using 2 factor RBD design with three replications. Acid lime cv. Balaji has pruned at 25cm, 50cm, 75cm and soil drenching with paclobutrazol @ 2.5ml and 5ml/ meter canopy spread of the trees on three times at 50 days interval beginning immediately after pruning (June) to September and were compared with control. The result revealed that the morphological and reproductive characters significantly affected by Medium pruning (50cm from apex) and paclobutrazol @ 5.0 ml/meter canopy of the tree application and their combinations. It was concluded that the treatment T_9 medium pruning (50cm) of tree and paclobutrazol application @ 5 ml/tree in acid lime were vital for regulating tree height, Canopy volume, stem girth and increased total number of flowers per shoot, fruit setting percentage and fruit retention percentage.

Keywords: Acid lime, pruning, paclobutrazol, vegetative growth, flowering, fruiting

Introduction
Acid lime is a profusely branched thorny shrubs or small tree. The leaves are small with narrowly winged petioles. The flowers are small, pure white and are borne in clusters. The fruits are more or less round or oval, smooth having thin rind (papery) attached tightly. The immature fruits are dark green in colour which changes to light yellow when ripe. The colour of the pulp is light greenish-yellow; taste is acidic, aromatic; cells fine and shiny. The numbers of segments are 9-11. The numbers of seeds per fruit are 9-10.

Citrus has naturally sympodial growth habit, farming a large bush if left un-pruned. Pruning is an important tree management practice to regulate vegetative growth and flowering in many fruit crops. The beneficial effects of pruning are related with management of canopy architecture, alteration of biochemical system and early flowering (Singh *et al.* 2009) [25]. The main object of pruning the bearing trees is to maintain the framework and to secure the higher yield and better grade fruits in each year. Singh *et al.* (2004) [26] observed that citrus trees, which were begun to decline in vigour, yield and size of fruit, need pruning to help the restore their condition. Pruning has been practiced for ages in controlling tree size because it has much less stimulating effect on shoot re-growth. The pruning is done to restrict excessive vegetative growth and to maintain a balance between leaf/fruit ratio, fruit size, fruit colour and other quality attributes. Excessive tree vigour can reduce flower bud formation, fruit set and result in reduced fruit quality. Pruning by removing the vigorous growing shoots increase the light intensity in cropping zone and colour intensity. Pruning also reduces the growth due to reduction in photosynthetic capacity and ultimately carbohydrate reserve by reducing the leaf area index and the spread of the canopy.

Pruning and application of paclobutrazol are simple and effective strategies recommended in many fruit crops for controlling the tree vigour and promoting flowering and enhanced production efficiency (Rani *et al.* 2018). In the recent years, paclobutrazol, a growth retardant has been used with considerable success to induce early flowering for off-season production in

several fruit crops such as apple (Trump 1987) [33], peach (Martin *et al.* 1987) [18], mango (Karki 2000) [15] and citrus (Snowball *et al.* 1994) [27].

Materials and methods

Treatments details

The experiment was conducted during 2018 to 2020 at the farmer's field in Gopalpur, Coochbehar, West Bengal, India. Acid lime cv. Balaji has pruned at 25cm (light pruning), 50cm (medium pruning), 75cm (heavy pruning) and soil drenching with paclobutrazol @ 2.5ml and 5ml/ meter canopy spread of the trees on three times at 50 days interval beginning immediately after pruning (June) to September and were compared with control (No pruning and Only water).T_1 (P_0 D_0) No pruning + No Drenching (Control), T_2 (P_0 D_1) No pruning + 2.5 ml/meter canopy of the tree, T_3 (P_0 D_2) No pruning + 5.0 ml/meter canopy of the tree, T_4 (P_1 D_0) Light pruning (25cm from apex) + No Drenching (Control), T_5 (P_1 D_1) Light pruning (25cm from apex) + 2.5 ml/meter canopy of the tree, T_6 (P_1 D_2) Light pruning (25cm from apex) + 5.0 ml/meter canopy of the tree, T_7 (P_2 D_0) Medium pruning (50cm from apex) + No Drenching, T_8 (P_2 D_1) Medium pruning (50cm from apex) + 2.5 ml/meter canopy of the tree, T_9 (P_2 D_2) Medium pruning (50cm from apex) + 5.0 ml/meter canopy of the tree), T_{10} (P_3 D_0) Heavy pruning (75cm from apex) + No Drenching, T_{11} (P_3 D_1) Heavy pruning (75cm from apex) + 2.5 ml/meter canopy of the tree), T_{12} (P_3 D_2) Heavy pruning (75cm from apex) + 5.0 ml/meter canopy of the tree.

Observation and method of estimation

Plant height (m)

The height of the plants was measured (from ground level to the terminal shoot) with the help of measuring device at the time of treatment application and at harvest and calculation of increase in plant height during the experimental period with the help of following formula:

Increase in plant height (m) = $(P_2 - P_1)$

Where:- P_2 = Plant height at harvest, P_1 = Plant height at the time of treatment application

Canopy volume (m^3)

Canopy volume of a plant was measured by using height and spread of this particular plant. The formula is: *canopy volume* = $4/3\ \pi\ a^2\ b$, where a = ½ of plant height and b = average of east - west and north - south spread (West wood *et al.* 1983) [36]. Percentage increase in canopy volume was measured by: $[(Final\ volume - Initial\ volume) / Initial\ volume] \times 100$.

Stem/trunk girth (cm)

Stem/trunk girth (cm) was measured at the base 20 cm above the trunk) of the tree with help of digital caliper in two directions which are at right angles to each other and thereafter averages of the two measurements were computed in centimeter.

Total number of flowers per plant

It was recorded from six tagged plants for each treatment. For this purpose six plants were randomly selected for each treatment and total numbers of flowers from six plants were counted.

Fruit setting percentage

Four branches were randomly selected and tagged on the plant and the number of flowers counted separately on each branch after that number of fruits counted. The fruit setting percent was calculated with following formula:

Fruit setting (%) = (Number of set fruits/ Number of flowers) x100

Fruit retention percentage (at harvest)

Initial number of fruit per shoot were counted and the number of fruit retained till harvested were recorded and these data were used for calculating the percentage of fruit retention at harvest by using following formula.

$$\% \text{ of fruit retention at harvest} = \frac{\text{Number of fruits per shoot at harvest}}{\text{Initial number of fruit per shoot}} \text{ X } 100$$

Result and Discussion

Plant height

The data regarding effect of pruning and paclobutrazol on plant height of acid lime for the year 2018-2019 and 2019-20 are mentioned in Table-1. Different treatments including control (no pruning and water application) were statistically analysed by two factor randomized block design. Perusal of the data indicated significant differences among plant height under different treatments in both years.

During both years significantly increase in plant height was found. All the treatment indicated significant variation over T_1 (*i.e.* control). During both years, The increase in plant height was found significantly maximum (0.560 m) (0.550 m) under treatment T_1 (*i.e.* control) followed by T_2 (0.550 m) (0.530 m) in which trees were treated with no pruning + 2.5ml/meter canopy of the tree and minimum (0.310 m) (0.310 m) under treatment T_9 in which trees were pruned with 50cm from apex (Medium pruning) and treated with paclobutrazol @ 5ml/ meter canopy, respectively.

The pooled mean data of the both years revealed that, maximum increase in plant height (0.560 m) were recorded with treatment T_1 in control trees and minimum (0.310 m) under treatment T_9 in which trees were treated with medium pruning (50cm from apex) and paclobutrazol @ 5.0 ml/meter canopy of the tree application.

The reduction in plant height with the use of paclobutrazol might be due to the inhibitory effect of paclobutrazol on gibberellins biosynthesis pathway at the sub-apical meristem, which ultimately reduced cell elongation, rate of cell division and decreased the shoot growth (Swathi *et al.* 2018).

Inhibition in gibberellins activity following check in the conversion of ent-kaurene to ent-kaurenoic acid in the gibberellins biosynthetic pathway has been attributed as the possible primary mechanism by which paclobutrazol restricts the vegetative growth. Similar results were reported by Quinlan and Webster (1982) [21] in plums and cherries, Teferi *et al.* (2010) [31] in Tommy Atkins mango, Tandel and Patel (2011) [30] in mango, Rani *et al.* (2018) [22].

The above results confer with the findings of Balamohan and Gopu (2014) [5] in Alphanso who reported that the light pruning of current seasons' growth is advantageous for tree vigour regulation without influencing the flowering. Such growth reduction responses of pruning might be result of decline in photosynthetic area, delay in leaf development and changes photosynthetic production and their translocation. Similarly, the plant growth response of pruning and paclobutrazol was observed in the study are in line with the findings of (Srilatha *et al.* 2015) [28] and (Sarkar and Rahim 2012) [24] in mango and in Lemon (Jain *et al.* 2000) [13] could be consequences of modifications in photosynthesis besides

reduction of gibberellins. The results indicated that the medium pruning (50cm from apex) and paclobutrazol (5.0 ml/meter canopy of the tree) reduced the tree vigour.

Canopy volume

The data on effect of pruning and paclobutrazol on canopy volume of Acid lime for the year 2018-2019 and 2019-20 are mentioned in Table-1.

During both years and pooled mean significant increase in canopy volume was recorded. In first year (2018-19), second year (2019-20), pooled mean, maximum increase canopy volume (0.400 m^3), (0.390 m^3), (0.400 m^3) was found under treatment T_1 with control followed by T_2 (0.390 m^3) (0.370 m^3) (0.380 m^3) and minimum (0.170 m^3) (0.180 m^3) (0.180 m^3) in T_9 which trees were treated with Medium pruning (50cm) from apex and paclobutrazol @ 5.0 ml/meter canopy of the tree application, respectively.

The above results are similar with the findings (Vu and Velenosky, 1988) [34] in citrus they reported that application of paclobutrazol results reduced canopy size (42 per cent). The above results also similar to the findings of Balamohan and Gopu (2014) [5] in Alphanso that the light pruning of current season's growth is advantageous for tree vigour regulation without influencing the flowering. Such growth reduction responses of pruning might be result of decline in photosynthetic area, delay in leaf development and changes photosynthetic production and their translocation.

The reduced canopy spread might be due to reduced vegetative growth through reduction in cell elongation and internodes extension ultimately retarding plant growth by via inhibition of gibberellins biosynthesis in the presence of paclobutrazol which ultimately reduce the canopy spread. Similar results were reported by Swathi *et al.* (2018) [29] in jamun and Teferi *et al.* (2010) [31] in Tommy Atkins mango.

Stem/trunk girth

The data on effect of pruning and paclobutrazol on stem girth of Acid lime for the year 2018-2019 and 2019-20 are mentioned in Table-1.

During both years and pooled mean significant increase in stem girth was recorded. In first year (2018-19), second year (2019-20), pooled mean, maximum increase stem girth (0.710 cm), (0.700 cm), (0.710 cm) was found under treatment T_1 with control followed by T_2 (0.660 cm) (0.640 cm) (0.650 cm) and minimum (0.390 cm) (0.370 cm) (0.380 cm) in T_9 which trees were treated with medium pruning (50cm from apex) and paclobutrazol @ 5.0 ml/meter canopy, respectively.

Similarly, the stem girth was increased by application of paclobutrazol in Lisbon lemon reported by Geroge *et al.* (1993) [9]. Similarly, the plant growth response of pruning and paclobutrazol was observed line with the findings of (Rani *et al.* 2018) [22] in lemon, (Srilatha *et al.* 2015) [28] and (Sarkar and Rahim, 2012) [24] in mango could be consequences of modifications in photosynthesis besides reduction of gibberellins. They were the combined treatments of pruning and paclobutrazol of tree vigour as compared to pruning of paclobutrazol treatments alone. From the result of (Srilatha *et al.* 2015) [28] it was apparent that the morphological attributes trunk girth was significantly reduced by pruning alone in cvs. Raspuri and Dashehari and by paclobutrazol in all the three cultivars Raspuri, Dashehari and Amrapali.

Number of flowers per shoot

The data presented on number of flowers per shoot as influenced by various levels of pruning and paclobutrazol was collected and presented in Table-2. The number of flowers per shoot has non-significantly differed between the treatments.

During both years, medium pruning (50 cm from apex) and drenching of Paclobutrazol @ 5 ml per meter (T_9) produced non-significantly highest number of flowers per shoot (16.86) (16.66) followed by treatment-8 *i.e* medium pruning (50cm from apex) and Paclobutrazol @ 2.5 ml per meter (16.10) (16.10). Whereas, the lowest number of flowers (9.33) (9.10) per shoot was recorded in control treatment (T_1).

The pooled mean data of the both years revealed that, maximum highest number of flowers per shoot (16.76) were recorded with treatment T_9 in which trees were treated with medium pruning (50cm from apex) and paclobutrazol @ 5.0 ml/meter canopy and minimum (9.22) under treatment T_1 in control trees.

Highest number of flowers per shoot was recorded with application of Paclobutrazol which could be due to the fact that Paclobutrazol inhibit the bio synthesis of GA_3 and inter nodal elongation. This reduces the availability of GA_3 thus, resulting in the production of more reproductive shoots as reflected in the results. Paclobutrazol might have acted as anti gibberellic compound and arrested the vegetative bud development, nucleic acid synthesis and protein metabolism (Rani *et al.* 2018) [22].

Tripathi and Dhakal (2005) [32] and Devi *et al.* (2011) [7] who reported the effect of Paclobutrazol on off-season flower induction and increase more number of flowers per shoot in acid lime. Several other workers have also observed that Paclobutrazol when applied to soil significantly increased the number of flowers per shoot in citrus (Dhakal and Guzman 1992) [8]. Paclobutrazol inhibits GA_3 biosynthesis by blocking especially the steps in the oxidation of ent-kaurene to ent-kaurenoic acid. It mainly inhibits the function of kaurene synthetase (Sterrett 1985). Therefore, flower induction in woody fruits including acid lime may be attributed to the reduced level of gibberellins below critical level for flowering.

The reason might be due to suppression of vegetative growth by PBZ which could have led to enhancement of total phenol content of terminal buds and altered the xylem to phloem ratio of the stem, which in turn altered the assimilate partitioning more towards reproductive shoots. Similar results were reported by Swathi *et al.* (2018) [29] in jamun, Anusuya and Selvarajan (2014) [2] in mango and Jasmine *et al.* (2011) [14] in mango.

Fruit set percentage

The data presented on fruit set percentage as influenced by various levels of pruning and paclobutrazol was collected and presented in Table-2. The fruit set percentage has significantly differed between the treatments.

During first year (2018), medium pruning (50 cm from apex) and drenching of Paclobutrazol @ 5 ml per meter (T_9) produced significantly highest fruit set percentage (62.56) followed by treatment-8 *i.e* medium pruning (50cm from apex) and Paclobutrazol @ 2.5 ml per meter (57.10). Whereas, the lowest fruit set percentage (46.82) was recorded in control treatment (T_1).

During second year (2019), medium pruning (50 cm from apex) and drenching of Paclobutrazol @ 5 ml per meter (T_9) produced non-significantly highest fruit set percentage (62.00) followed by treatment-8 *i.e* medium pruning (50cm from apex) and Paclobutrazol @ 2.5 ml per meter (56.42). Whereas, the lowest fruit set percentage (45.90) was recorded in control treatment (T_1).

The pooled mean data of the both years revealed that, maximum highest fruit set percentage (62.28) were recorded with treatment T_9 in which trees were treated with medium pruning (50cm from apex) and paclobutrazol @ 5.0 ml/meter canopy and minimum (46.36) under treatment T_1 in control trees.

The increased fruit set per cent due to medium pruning might be due to removal of excess shoots and movements of assimilates to fever growing points as well as the increased fruit set in pruned trees might be due to more nutrient and adequate moisture to the fruits in the remaining shoots of the pruned trees. These findings are in arguments with the finding of Nanthakumar and Balakrishnan (1986) [19] in ber, Arora and Yamdagni (1985) [3] in sweet lime, Ingle *et al.* (2001) [12] in acid lime and Khan and Syamal (2004) [16] in Kagzi lime, Patil *et al.* (2018) [20] in acid lime.

The increase in fruit set with soil applied paclobutrazol might be due to its effect on shifting of assimilates, mineral element and soluble proteins in leaves, stem and root (Wang *et al.* 1985) [35]. These were in close conformity with the results obtained by Tripathi and Dhakal (2005) [32] in acid lime, Arunadevi *et al.* (2019) [4] in acid lime var. PKM-1 and Ghadage *et al.* (2015) [10] in acid lime.

This might be due to significantly higher fruit set in the paclobutrazol treated plants which had a favourable impact on culminating in to higher fruit number per plant. In this context, Kurian *et al.* (2001) [17] reported that paclobutrazol appeared to favourably alter the source sink relationship of mango to support fruit growth with a reduction in vegetative growth. Similar results were obtained with by Samini (2014) [23] in peach cutivars "Kardi" revealed that application of paclobutrazol increased final fruit set. Swathi *et al.* (2018) [29] in jamun cv. Chintamani revealed that application of paclobutrazol increased fruit set.

Fruit retention percentage (at harvest)

The data presented on fruit retention percentage as influenced by various levels of pruning and paclobutrazol was collected and presented in Table-2. The fruit retention percentage has significantly differed between the treatments.

During both years (2018, 2019), medium pruning (50 cm from apex) and drenching of Paclobutrazol @ 5 ml per meter (T_9) produced significantly highest fruit retention percentage (55.37) (55.30) followed by treatment-8 *i.e* medium pruning (50cm from apex) and Paclobutrazol @ 2.5 ml per meter (51.60) (51.46). Whereas, the lowest fruit retention percentage (29.23) (29.20) was recorded in control treatment (T_1), respectively.

The pooled mean data of the both years revealed that, maximum highest fruit retention percentage (55.34) were recorded with treatment T_9 in which trees were treated with medium pruning (50cm from apex) and paclobutrazol @ 5.0 ml/meter canopy and minimum (29.22) under treatment T_1 in control trees.

It might be due to pruning which created more area accelerating the photosynthetic activity by better penetration of sunlight, which has brought an increase in starch assimilation resulting in an increase fruit retention percentage. These results are in accordance with the findings of Ahmad *et al.* (2006) [1] and Ingle *et al.* (2001) [12].

This might be due to significantly higher fruit retention in the paclobutrazol treated plants which had a favourable impact on culminating in to lower fruit drop. The role of paclobutrazol in inhibiting formation of abscission layer was well known. Auxin content in fruits during 2-3 weeks after pollination was low and the ability of fruits to mobilize food material was poor due to low auxin level which results in fruit drop. As the fruit develops, the amount of auxin rises rapidly which was helpful in mobilization of food material (Chacko *et al.* 1972) [6]. At this stage the competition among developing fruits starts and the fruits which compete less successfully were forced to drop. The result of the present study was in accordance with the findings of Gulab sanodiya (2015) [11] in acid lime, Rani *et al.* (2018) [22] in lemon.

Table 1: Effect of pruning and paclobutrazol on morphological parameters of acid lime cv Balaji

Treatments combinations	Plant height (m)			Canopy volume (m^3)			Stem/trunk girth (cm)		
	YI	YII	**Pooled**	**YI**	YII	**Pooled**	**YI**	YII	**Pooled**
T_1 (P_0 D_0)	0.560	0.550	0.560	0.400	0.390	0.400	0.710	0.700	0.710
T_2 (P_0 D_1)	0.550	0.530	0.540	0.390	0.370	0.380	0.660	0.640	0.650
T_3 (P_0 D_2)	0.510	0.520	0.520	0.380	0.370	0.380	0.640	0.620	0.630
T_4 (P_1 D_0)	0.490	0.500	0.500	0.360	0.350	0.350	0.630	0.600	0.620
T_5 (P_1 D_1)	0.390	0.430	0.410	0.270	0.270	0.270	0.430	0.420	0.430
T_6 (P_1 D_2)	0.370	0.370	0.370	0.260	0.250	0.260	0.420	0.400	0.410
T_7 (P_2 D_0)	0.480	0.490	0.490	0.350	0.340	0.350	0.510	0.490	0.500
T_8 (P_2 D_1)	0.340	0.350	0.340	0.190	0.200	0.200	0.420	0.410	0.420
T_9 (P_2 D_2)	0.310	0.310	0.310	0.170	0.180	0.180	0.390	0.370	0.380
T_{10} (P_3 D_0)	0.460	0.480	0.470	0.340	0.320	0.330	0.470	0.460	0.470
T_{11} (P_3 D_1)	0.430	0.500	0.470	0.310	0.300	0.310	0.440	0.420	0.430
T_{12} (P_3 D_2)	0.400	0.450	0.430	0.300	0.290	0.300	0.450	0.430	0.440
S. Em (±)	0.007	0.017	0.0039	0.014	0.008	0.0072	0.018	0.021	0.0091
C.D. (5%)	0.020	0.050	0.0109	0.042	0.024	0.0204	0.052	0.062	0.0258

YI=2018-2019 and YII=2019-2020

Table 2: Effect of pruning and paclobutrazol on reproductive parameters of acid lime cv Balaji

Treatments combinations	Total number of flowers/shoot			Fruit setting percentage			Fruit retention percentage (at harvest)		
	YI	YII	Pooled	YI	YII	Pooled	YI	YII	Pooled
T_1 (P_0 D_0)	9.33	9.10	9.22	46.82	45.90	46.36	29.23	29.20	29.22
T_2 (P_0 D_1)	10.65	10.55	10.60	47.20	46.34	46.77	31.89	31.76	31.82
T_3 (P_0 D_2)	11.60	11.46	11.53	48.50	48.15	48.33	33.32	33.22	33.27
T_4 (P_1 D_0)	12.65	12.22	12.44	50.01	49.23	49.62	36.50	36.30	36.40
T_5 (P_1 D_1)	15.26	15.05	15.16	54.97	54.20	54.59	47.38	47.18	47.28
T_6 (P_1 D_2)	15.90	15.62	15.76	56.29	55.81	56.05	49.60	49.51	49.56
T_7 (P_2 D_0)	13.60	13.00	13.30	52.06	51.67	51.87	38.67	38.43	38.55
T_8 (P_2 D_1)	16.10	16.10	16.10	57.10	56.42	56.76	51.60	51.46	51.53
T_9 (P_2 D_2)	16.86	16.66	16.76	62.56	62.00	62.28	55.37	55.30	55.34
T_{10} (P_3 D_0)	13.78	13.52	13.65	53.05	52.85	52.95	39.60	39.04	39.32
T_{11} (P_3 D_1)	14.22	14.10	14.16	53.56	53.10	53.33	39.92	39.85	39.89
T_{12} (P_3 D_2)	14.94	14.84	14.89	54.54	53.85	54.19	46.92	46.67	46.80
S. Em (±)	0.488	0.413	0.2479	0.954	1.445	0.5011	1.704	1.488	0.8666
C.D. (5%)	NS	NS	0.7022	2.797	NS	1.4195	4.998	4.363	2.4552

YI=2018-2019 and YII=2019-2020

Conclusion

From the study, it was concluded that the medium pruning (50cm from apex) of tree and paclobutrazol application @ 5 ml/tree in acid lime cv. Balaji were vital for regulating plant height, canopy volume, stem girth and increased total number of flowers per shoot, fruit setting percentage and fruit retention percentage in acid lime.

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