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Effect of flowering regulating chemicals and girdling on winter flushing, yield and fruit quality in Litchi cv. China

Pragya Kumari[1], Sanjay Kumar Singh[2*] and Sanjay Vyas[1]

[1] *Govt. Holkar [Autonomous] Science College, Indore 452 001, Madhya Pradesh, India*
[2] *ICAR-National Research Centre on Litchi, Muzaffarpur 842 002, Bihar, India*

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Abstract
A study was conducted on effect of various bio-regulators and girdling on winter flushing, flowering intensity, fruit yield and quality of litchi cv. China. The experimental results revealed that winter flushing was inhibited by girdling (removal of bark at 3mm width in 75 % branches] and application of paclobutrazol (PBZ). The number of floral panicles per tree was highest due to spray of KNO_3 and prohexadione-Ca (207.50 and 206.17 respectively), while least in control trees (109.16). The fruit retention, average fruit weight was insignificant amongst the treatments. Spray of KNO_3 during September end, led to highest yield in terms of number of fruits per tree (2,427) and in weight (55.42 kg per tree). The control trees had only 35 fruit per tree (i.e., 0.80 kg per tree). KNO_3 also led to highest pulp percent (65.35 %) of the fruits. Spray of salicylic acid caused highest fruit ascorbic acid (29.26mg/100g pulp) and highest total sugar (22.4 %) of the fruit. Besides lowest yield, the least pulp content (50.61 %), total anthocyanin content (6.15 mg per 100 g pulp), total sugar (11.80 %) and reducing sugar (9.03 %) were observed in control trees. The highest TSS (22.60° Brix) and TSS/acidity ratio (66.97) of the fruits were recorded in paclobutrazol applied trees while least titratable acidity was found after spray of prohexadione-calcium. Thus, spray of KNO_3 (2.0 %) was able to record highest yield and pulp content in litchi cv. China while salicylic acid @ 2000 ppm application was found very effective for improving fruit quality attributing characters. Girdling and spray of prohexadione-Ca in litchi tree can also be an effective intervention for yield enhancement

Key words: Bio-regulators, Fruit drop, KNO_3, prohexadione-Ca, Salicylic acid.

Introduction

Litchi (*Litchi chinensis* Sonn.), is an important subtropical evergreen fruit crop belonging to the family Sapindaceae (or soapberry family) and sub-family Nepheleae, which has about 150 genera and more than 2000 species. India is the second largest producer of litchi in the world after China with an annual growth of 4.30 per cent in area and 7.20 per cent in production in the country. India produces 7.16 lakh tonns litchi from approximately 1.0 lakh ha area every year (ICAR-NRC on Litchi, 2020). Bihar as such accounts for 32 per cent area and 42 per cent production in litchi in the country (Sahni et al., 2020). Low production and poor edible flesh yield of litchi in all cultivars of economic importance constitute the chief reasons for its restricted cultivation. The information on use of growth regulators and their influence on shoot behaviour, flowering, panicle induction, fruiting and their inter-relation is lacking particularly in juvenile litchi orchard.

Potassium compounds like KNO_3 and KH_2PO_4 are found to be promising with regard to flower induction and improving bearing potential of litchi. Foliar application of potassium nitrate induces flowering and improves fruit quality in litchi. Shortage of potassium can affect many metabolic processes like rate of photosynthesis and

*Author for Correspondences: Phone : 9546891510, Email: sanjay.singh3@icar.gov.in

translocation (Marschner, 2003). Paclobutrazol has been reported to bring regularity of bearing in litchi cv. China and its alternative prohexadione-calcium (Pro-Ca) can be used instead of paclobutrazol (PBZ), as the residue of PBZ in fruits of litchi has been reported by many researchers. Pro-Ca is an inhibitor of gibberellin (GA) synthesis, and due to low toxicity and limited persistence, gets metabolized or decomposed within 6 to 7 weeks of application and so it can be used to control the vegetative growth in litchi. When applied during the early stages of vegetative growth, the Pro-Ca reduces the levels of GA_1 (highly active), and leads to accumulation of its precursor, GA_{20} (inactive) in plant tissues (Evans et al., 1999). Polyamines like spermidine (*Spd*) predominate during flower bud differentiation. When *Spd* reaches a certain level, it can promote the initiation of a flowering gene, thereby triggering the synthesis of a special protein and finally the formation of the flower primordium. *Spd* application promotes flowering during floral induction by increasing *MdGA2ox2* (gibberellin 2-oxidase) through GA_3 reduction and increasing *MdNCED1* and *MdNCED3* (9-cis-epoxycarotenoid dioxygenase) through ABA enrichment during 60 to 80 days after full bloom (Qin et al., 2019). Girdling is a known practice to promote flowering, fruit set, retention, size, colour, and sugar content (Smith et al., 2005). When these applications are combined, the response will be greater. The uses of various bio-regulators were reported by several workers as an alternative approach to minimize fruit drop, enhance yield and ensure regular flowering. Salicylic acid induces flowering in trees by acting as a chelating agent. It functioned as endogenous growth regulator for flowering and florigenic effects (Pieterse and Muller, 1977).

Litchi suffers from alternate bearing and yields are often irregular. Productivity in off-years is unacceptably low. In this paper, we explored the effect of various management practices on the soil temperature, soil moisture, and flushing in litchi and their inter-relations in a sub tropical region. Further, the effect of foliar application of bio-regulators and practice of girdling on winter flushing, yield and fruit quality in litchi cv. China was also investigated.

Material and methods

The experiment was conducted during 2019-2020 at experimental farm of ICAR-National Research Centre on Litchi, Mushahari, Muzaffarpur, Bihar, India. The experimental site is situated at 26° 5' 87'' N latitude 85° 26' 64'' E longitudes at an elevation of 210 m above the mean sea level and experiences a sub-tropical climate. The climate is typical representative of warmer/tropical fringe of subtropical climate. Average annual rainfall is about 1180 mm out of which about 90% is received during June to October in south west monsoon. Mean monthly temperature ranges from 15 to 36°C, with minimum during January and maximum during May. Daily maximum temperature reaches up to 43.5°C whereas daily minimum temperature goes below 4°C. The experimental soil was alluvial with sandy loam texture, and calcareous having pH of 7.5 - 8.0.

Twelve year old uniform sized trees of litchi cv. China spaced at 8 x 8 m were selected for this experiment. The details of treatment were: foliar spray of KNO_3 (2.0 %), foliar spray of prohexadione calcium (0.5 g a.i. /L); paclobutrazol (2.5 g a. i. per m canopy diameter) application through trunk soil line pore method; girdling with 3 mm intensity practiced in 75 % branches; foliar spray of salicylic acid (2000 ppm); foliar spray of KH_2PO_4 @ 0.5 %; foliar spray of spermidine @ 0.01 millimolar and untreated trees (control) i.e., without any spray or girdling. All the treatments were imposed during last week of September (after 100 days of harvesting). The chosen design was randomized block design (RBD) considering one plant as a unit. The eight treatments were imposed in triplicate. For spray scheduling, the proper weighing of different chemicals was done and stock solution of each chemical was prepared by dissolving them in small quantity of water. Final volume was made to 5 litre by adding water i.e. enough for spraying one tree. All the chemicals except paclobutrazol (PBZ) were sprayed in the morning hours with a rocker sprayer (after adding Active-20 as a surfactant) by end of September. The PBZ solution was poured around the tree trunk in a shallow circular trench (about 10 cm deep). Girdling was done in the middle of September. For girdling, a strip of bark of 3 mm width was removed in a circular fashion around the selected primary branch with the help of pruning saw. The phloem portion was removed carefully without damaging the xylem.

The soil moisture and temperature were measured using STP-2 (Soil Temperature Probe) of EGM-5 Portable CO_2 Gas Analyzer (Amesbury, MA 01913 USA) and expressed in °C with accuracy of ± 0.3 °C at 25 °C. To monitor the flush emergence pattern and floral panicles per tree, 10 shoots in each direction were tagged (i.e., 40 shoots per tree) and number of winter flushes was counted on whole tree while number of floral panicle per tagged shoot were counted over numbers of shoots tagged after harvesting. The fruits were harvested at fully matured and ripened stage since they do not ripen after harvest. Harvesting was done manually by picking the fruits. Total number of fruits was counted per tree after harvest. Fruit yield was also recorded in kg/tree. The fruit retention was calculated using the formula.

Fruit retention (%) =

$$\frac{\text{No of fruit retained per panicle}}{\text{No of fruit set initially per panicle}} \times 100$$

The per cent fruit drop was worked out by subtracting per cent fruit retention from 100 fruits set per panicle and average was worked out. Data on fruit weight and size were recorded at maturity (determined by change of peel colour to deep red, flatness of tubercles and smoothness of the epicarp). Fruit weight, pulp weight, and stone weight were recorded using an electronic balance and expressed in grams. The weight of seed, pulp and pericarp were obtained from three fruits and per cent of weight of seed, pulp and pericarp of total fruit weight was calculated.

Total soluble solids (TSS) was estimated by a hand refractometer (Atago 3T Abbe) and expressed in °Brix value which was corrected at 20 °C with the help of temperature correction chart (Ranganna, 1986). Titratable acidity (expressed as a percentage of malic acid) was determined by titrating a known quantity of blended (homogenized) pulp, diluted with distilled water against standard sodium hydroxide (0.1 N) solution using phenolphthalein as indicator, and results were expressed in percentage. TSS: acid ratio was calculated by dividing the TSS value by acidity.

For determining the ascorbic acid, aril (10 g) was ground with 3 per cent metaphosphoric acid (HPO_3) solution and titrated against the dye (2, 6-dichlorophenol-indophenol) till end point of pink colour was reached. The titre value was recorded and calculated (mg per 100 g of pulp) according to the method described by Ranganna (1986). The total sugar of litchi fruits was estimated by the Lane and Eynon method. In this method, invert sugar reduces the copper in Fehling's solution to red insoluble cuprous oxide. The sugar content was estimated by determining the volume of the unknown sugar solution required to completely reduce a measured volume of Fehling's solution (Jayaraman, 1981).The reducing sugar of litchi pulp was determined by dinitrosalicylic acid method suggested by Ranganna (1986). Fifteen litchi fruits (five in each of the three replicates), were used to prepare samples for total anthocyanin estimation. Five grams of pericarp from five litchi fruits were finely sliced, extracted and well stirred with 50 ml of 1% weight HCl for 2 h at a pH of 1.0 (Fuleki and Francis, 1968). The extract was centrifuged at 4500 rpm for 20 min and filtered through Whatman No. 1 filter paper. The absorbance reading of the anthocyanins was then measured photometrically at 515 nm.

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

Results and Discussion

Winter flushing pattern and panicle emergence

Based on soil moisture and temperature during the (critical) winter season, it was observed that the soil temperature did not vary with treatments (in 2020). The data presented in Table 1 indicated that when soil moisture was higher (i.e., 12-14 %), spray of KH_2PO_4 (0.5 %) caused inhibition of winter flushes (13.66) followed by inhibitory role of girdling (46.00) of litchi trees. There was no significant effect of treatments on soil temperature as such in the year 2020. However, untreated trees recorded highest soil moisture content (15.30 %) which would have conducive for emergence of undesirable winter flushes. Further, spermidine (0.01mM) and KH_2PO_4 (0.5 %) led to highest number of panicles per tree (352) i.e. 50 % more number of panicles in comparison to control trees during the year 2020. Kaur-Sawhney et al. (1988) also observed that the exogenous spermidine (*Spd)* application caused more than 20% of floral buds to develop in tobacco thin-layer tissue cultures, whereas all of the buds were vegetative when the cultures were lacking in *Spd*, indicating the direct role of *Spd* in floral differentiation. Although, in the first year of experiment, the spray of KNO_3 (256.66) and girdling (211.66) were better to induce floral panicles, in second year (111.67), other bearing regulating chemicals were better to induce panicles. However, the pooled data showed insignificant results on number of floral panicles per tree due to treatments, but it was found that KNO_3 (256.66, 158.33) ensured more number of panicles per tree for sustainable yield followed by spray of Pro-Ca (170.66, 241.67) (Table 1).

Girdling in 75 per cent of branches with 3 mm intensity proved better over other treatment to bring 211.66 numbers of panicles per tree in the year 2019 (Table 1). Ramburn (2001) also found girdling of branches of 3 to 4 cm diameter with hardened flush in May, or foliar application of 0.5 g paclobutrazol + 0.4 g of ethephon per litre promoted flowering in unproductive litchi cv. Tai So in Mauritius. The use of plant growth regulators or retardant such as paclobutrazol, ethephon and SADH (succinic acid - 2, 2-dimethyl hydrazide) and cincturing have been reported to improve flowering and restrict vegetative flushing in litchi, although the results can often be inconsistent. It is reported that the paclobutrazol and KH_2PO_4 spray increase the number of pure panicle up to four times (Satish Chand et al., 2017).

Effect on yield and fruit quality

Bearing regulating chemicals and girdling distinctly influenced the fruit yield and quality attributes in litchi cv. China (Table 2). The highest number of

Table 1. Flushing pattern and panicle emergence affecting by soil moisture and temperature in litchi cv. China

Treatments	*Soil Moisture (%)		*Soil Temperature (^{0}C)		*No. of flushes/ tree		No. of panicles/tree		Pooled
	2019	2020	2019	2020	2019	2020	2019	2020	Average
KNO_3 (2.0 %)	8.73	14.36	13.36	18.03	20.00	120.00	256.66	158.33	207.50
Prohexadione-Ca (0.5 g per L)	5.16	12.60	13.70	18.03	180.00	248.33	170.66	241.67	206.17
Paclobutrazol (2.50 g)	2.80	13.73	13.53	18.13	70.00	142.00	7.33	208.33	107.83
Girdling	4.56	10.96	13.36	17.90	147.66	46.00	211.66	111.67	161.66
Salicylic acid (2000 ppm)	4.36	9.16	13.53	18.23	23.000	86.66	62.33	263.33	162.83
KH_2PO_4 (0.5%)	5.10	12.30	13.63	18.03	389.33	13.66	7.66	321.67	164.66
Spermidine @ 0.01mM	2.50	8.33	13.56	17.96	140.00	52.00	8.33	352.00	180.16
Control	4.76	15.30	13.73	18.06	132.66	68.33	3.33	215.00	109.16
$CD_{0.05}$	2.58	4.16	0.22	NS	158.61	114.34	4.11	NS	NS
SE(m)	0.845	1.36	0.072	0.081	51.792	37.33	43.23	57.86	46.31

*observation taken during 15th December-15th January and year written as following year fruits per tree (2,427) was recorded with KNO_3 followed by spray of prohexadione-Ca (2,006) and girdling (1,448). Highest yield due to foliar application of KNO_3 could be attributed to enhanced ethylene biosynthesis which ultimately promoted floral induction and resulted in highest yield (Mishra et al., 2011). KNO_3 is reported to cause increased carbohydrate reserves and better fruit set (Beevers and Hageman, 1969). Prohexadione-Ca showed increased fruit set as it reduced fruit abscission. The untreated trees bore only 35 numbers of fruits per tree. The C/N (carbohydrate/nitrogen) ratio in leaf and shoot increased sharply from September to January due to the application of chemicals and cincturing, which might have been another reason for higher yields (Mandal et al., 2014) in this investigation.

None of the treatments had any significant effect on fruit retention percentage in litchi cv. China. Girdling caused 26.45 per cent fruit retention followed by KNO_3 application (25.66%) and spray of prohexadione-Ca (25.48 %). Girdling improved leaf N content which consequently improved C: N ratio and carbohydrate content, hence it reduced fruit abscission and increased fruit retention (Shao et al., 1998). Hung and Nghi (2006) observed that in litchi cv. Binhkhe, PBZ applied @ 20 g a. i./tree in late August-early September at mature bud stage resulted in the inhibition of winter bud emergence, reduced inflorescence size but increased female flowers, fruit set and yield by 61.5-85.2 % than the control. Yeshitela et al. (2004) also recorded yield increase up to two to three fold in comparison to control trees in mango cv. Tommy Atkins due to soil drenching of paclobutrazol. One of the possible reasons for increased yield might be due to *de novo* biosynthesis of auxin and other growth regulating or promoting chemicals at initial stage due to the additional stimulus produced by external application of all these chemicals.

Application of different bearing regulating chemicals and girdling had no significant effect on average fruit weight in litchi cv. China. The application of salicylic acid @ 2000 ppm led to highest fruit weight (23.73 g) followed by PBZ (23.66 g). Salicylic acid might have directed the flow of metabolites to the developing fruits which consequently improved fruit yield and seed weight. The control trees had 20-30 % smaller (19.70 g) fruits than the best treatment. Garad et al. (2013) also reported that foliar application of KH_2PO_4 was beneficial for increasing fruit size and weight of mango fruits. The byproduct of fruit, i.e. peel, seed and pulp, of different treatments are represented in Table 2 and it was observed that KNO_3@ 2.0 % recorded highest pulp recovery (up to 65.35 %) followed by trees sprayed with spermidine (62.10 %) which had least seed percentage (15.43 %). The control trees had only 50.61 % pulp recovery with more (18.45 %) seed content in the fruits (Table 2).

Physico-chemical properties of fruits

There was significant effect of bearing regulating chemicals and girdling on fruit quality parameters in litchi cv. China. Paclobutrazol brought sweetness in the fruits as TSS up to 22.60 ^{0}B with highest

Table 2. Effect of different bearing regulating factors on yield and fruit quality attributes in litchi cv. China.

Treatments	No. of fruits/ tree	Fruit retention (%)	Fruit Yield (kg/ tree)	Average fruit weight (g)	Peel (%)	Pulp (%)	Seed (%)
KNO_3 (2.0 %)	2,427.67	25.66	55.42	22.04	15.89	65.35	16.55
Prohexadione-Ca (0.5 g per L)	2,006.67	25.48	41.53	21.48	21.82	54.96	18.52
Paclobutrazol (2.50 g)	40.67	24.35	0.93	23.66	19.37	54.42	18.47
Girdling	1,448.67	26.45	31.76	22.65	14.85	52.13	19.60
Salicylic acid (2000 ppm)	584.67	22.84	13.23	23.73	20.63	51.55	20.04
KH_2PO_4 (0.5%)	70.00	22.28	1.49	21.70	16.83	57.60	20.53
Spermidine @ 0.01mM	67.67	20.53	1.50	23.07	14.75	62.10	15.43
Control	35.00	16.67	0.80	19.70	18.41	50.61	18.45
$CD_{0.05}$	898.30	NS	18.86	NS	2.07	1.61	2.17
SE(m)	293.31	4.90	6.16	0.76	0.68	2.28	0.71

Table 3. Effect of different bearing regulating factors on physico-chemical attributes in litchi cv. China.

Treatment	TSS (°Brix)	Titratable acidity (%)	TSS/Acidity ratio	Ascorbic acid (mg/100g pulp)	Total sugar (%)	Reducing sugar(%)	Anthocyanin (mg/100)
KNO_3 (2.0 %)	21.53	0.33	66.76	28.15	18.20	10.77	19.90
Prohexadione Calcium (0.5 g per L)	17.47	0.29	61.44	22.07	16.84	8.73	8.77
Paclobutrazol (2.50 g)	22.60	0.34	66.97	25.19	19.59	11.30	25.67
Girdling	18.53	0.38	49.46	22.59	17.55	9.27	23.67
Salicylic acid (2000 ppm)	21.47	0.39	54.72	29.26	22.41	10.73	24.32
KH_2PO_4 (0.5%)	20.20	0.39	51.80	24.82	12.57	10.10	20.43
Spermidine @ 0.01mM	21.37	0.40	53.12	22.59	19.98	10.68	14.50
Control	18.07	0.35	52.07	24.07	11.80	9.04	6.15
$CD_{0.05}$	1.20	0.05	9.95	3.94	5.34	0.60	8.98
SE(m)	0.39	0.02	3.25	1.29	1.74	0.20	2.93

reducing sugar content (11.30 %) was recorded. Vijayalakshmi and Srinivasan (2000) also found improvement in fruit quality in terms of TSS, reducing and non reducing sugars, ascorbic acid and carotenoids in 'Alphonso' mango due to paclobutrazol application. Prohexadione-Ca@ 0.5g/L and control trees had TSS ranging from 17.47 to 18.07 ^{0}Brix only. However, the titratable acidity was lowest (0.29 %) in trees sprayed with pro-Ca while spermidine caused highest titratable acidity (0.40 %). The vitamin C content of fruits up to 29.26 mg per 100 g pulp was recorded in trees sprayed with salicylic acid @2000 ppm followed by spraying of KNO_3 (28.15 mg per 100 g pulp). In congruence of this result, Ball (1997) also observed that constituent levels of vitamin C, pH, T.S.S., and titratable acidity of pepper fruits treated with salicylic acid concentrations were higher than those of control fruit. In this investigation, the least value (22.07 mg per 100 g pulp) of ascorbic acid was recorded with prohexadione-Ca. It was observed that the highest total sugar of the fruits (22.41 %) was recorded with spray of salicylic acid while control trees had lowest fruit total sugar contents (11.80 %). Reducing sugar was lowest (8.73 %) with prohexadione-Ca which was on par with the control. The fruit quality parameters like anthocyanin content was highest due to PBZ i.e., 25.67 mg per 100 g pulp (Table 3), which was on par with six other treatments. Once again the lowest anthocyanin content was observed in untreated trees (6.15 mg per 100 g pulp). Other research workers also reported increased antioxidant ability and ascorbic acid content in other fruits due to salicylic acid.

Thus, KNO_3 @ 2% resulted in better yield, i.e., 55.42 kg per tree, highest pulp percentage, while salicylic acid provided better sized fruits (23.73 g) which was on par with KNO_3. SA enhanced ascorbic acid content (29.26 mg per 100g pulp) and total sugar (22.40 %) content of the fruits. Girdling was better for fruit retention (26.45 %) and paclobutrazol @ 2.5g ensured higher fruit reducing sugar (11.3 %) and fruit anthocyanin contents (25.65%) in litchi cv. China. Thus it may be concluded from the research study that overall, KNO_3 application was better in terms of yield, fruit and quality characters in litchi cv. China if applied by end of September month, and thus potassium nitrate could replace the need for vegetative dormancy period, and induced higher flowering rates as compared to application of other plant growth regulators.

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