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Effects of pruning and chemicals on growth behaviour of acid lime (*Citrus aurantifolia* Swingle) cv. Kuliana lime

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Abstract
Acid lime being a tropical fruit crop, its flushing and flowering is dependent on the variation of climatic factors. Generally, the timing and rate of vegetative and reproductive growths are regulated by autonomous or environmental factors. Unmanaged growth leads to various insect and pest attacks, reduction in quality and yield etc. Growth regulators as well as chemicals are widely used for controlling the plant growth and enhancing the yield and quality. Present experiment was carried out to find the effects of pruning and chemicals on growth behaviour of acid lime (*Citrus aurantifolia* Swingle) cv. Kuliana lime during 2017-18 at Horticultural Research Station, Department of Fruit Science and Horticulture Technology, O.U.A.T., Bhubaneswar. Results showed that, the interaction effect of urea (5%) in pruned plants i.e. treatment P_1C_1, was the best in all most all the foliar growth parameters viz. earlier sprouting initiation (24 days after pruning), number of new shoots (24.57), length of the shoots (21.33cm), numbers of leaves per shoot (18.66), leaf area (27.47cm²), chlorophyll content (0.65, 1.36 and 1.29 mg/g fresh weight of chlorophyll-a, chlorophyll-b and total chlorophyll respectively), leaf dry weight (2.79mg). Maximum (26.31 LUX) light interception was recorded in treatment P_1C_3. The duration taken for sprouting was found maximum (55.25 days) in P_2C_1. Therefore pruning and application of urea 5 per cent is the best way for optimizing the vegetative growth in Kuliana Lime. Further research must be carried out to know the best combination of pruning and chemicals on the yield attributing traits.

Keywords: Acid Lime (*Citrus aurantifolia* Swingle), pruning, chemicals, urea, GA_3

Introduction
Citrus species is grown through-out the world in tropical and subtropical climate, where there is suitable soil and sufficient moisture available to sustain the trees. The finest quality table citrus fruits are grown in non humid irrigated subtropical areas. India ranks fifth among major lime and lemon producing countries in the world. It is grown in more than 100 countries under tropical, subtropical and Mediterranean climatic conditions and considered to be one of the most remunerative fruit crops that have a lasting niche in the International trade and world finance.

Acid lime (*Citrus aurantifolia* Swingle) belongs to the family Rutaceae. It is native of India and South Eastern China (Hume, 1957) [9]. In India, citrus fruits are cultivated in an area of 985 thousand hectare with a production of 11,419 thousand million tonnes (NHB, 2016) [21]. Among citrus species lime occupies 248 thousand hectare in area and 2,364 thousand metric tonnes in production. It is largely cultivated in Andhra Pradesh, Maharashtra, Tamil Nadu, Gujarat, Rajasthan and Bihar. Odisha accounts for 2.4% and ranked 9th to the national production of citrus fruits. Mayurbhanj, Keonjhar, Koraput, Ganjam, Gajapati, Dhenkanal are the major lime growing districts in Odisha (Ministry of Agriculture, Horticulture Statistics at a Glance, 2015) [18]. Kuliana lime is a local elite cultivar grown in Mayurbhanj district of Odisha and popular for its size, aroma and juice content (Mishra *et al*., 2018) [19].

Generally, Citrus has naturally sympodial growth habit, forming a large bush (18 - 20 feet tall), if left un-pruned. Pruning, the judicious removal of any vegetative part, is an important cultural operation for the fruiting trees. Pruning prolong the bearing age of the tree. An un-pruned tree becomes very large, inhibiting light penetration. As a result, leaf sprout is decreased and photosynthetic activity remains low. Light becomes a limiting factor in crowded groves and pruning improves light access. Sunlight not only influences the flowering and fruit

set but also enhances fruit quality and colour development of fruit. The main reason for pruning fruit trees is to foster a high quality yield (Lewis and McCarty, 1973) [17]. Pruning along with application of desired growth regulators are an essential in citrus species for heavy flowering and yield. Thus this study aim to know the best combination of pruning and chemical (growth regulator) for promoting desire result in the vegetative growth parameters, which is the indication of final yield and quality.

Materials and Methods

An experiment was carried out at Horticultural Research Station, Department of Fruit Science and Horticulture Technology, O.U.A.T., Bhubaneswar during September 2017-18 on five year old Kuliana lime, planted at spacing of 4×4m. The research station is situated 60 km away from Bay of Bengal having an altitude of 25.9 m above Mean Sea Level. It is located at 20˚.15' N latitude and 85˚.52' E longitude. The climate here is warm, humid with distinct summer, rainy and winter seasons.

The present experiment was conducted in Factorial Randomized Block Design (F-RBD) with 2 factors {first factor includes pruning (P_1) and without pruning (P_2). The second factor includes urea 5% (C_1) 1 month after pruning, GA_3 20ppm (C_2) and 50ppm (C_3) 2 months after pruning, NAA 100ppm (C_4) after complete flowering and initiation of fruiting and water spray (C_5) 1 month after pruning} and 10 treatments (P_1C_1, P_1C_2, P_1C_3, P_1C_4, P_1C_5, P_2C_1, P_2C_2, P_2C_3, P_2C_4 and P_2C_5) each replicated thrice. Pruning was done on 15th October, 2017 under Bhubaneswar condition.

The date of new shoots initiation was recorded when small light green sprouts appear on the selected tagged branch of plant in all the four directions. The days taken were recorded from initiation of sprout to cessation of sprout of vegetative growth. New shoots in each tagged branch were counted in all the four directions as number of new shoots per branch. Ten selected shoot from each tagged branch was measured with meter tape and expressed in centimetre and millimetre respectively as Length and diameter of the shoot, while the fully grown leaves were counted and expressed in number of leaves per shoot. Ten representative leaves per plant were randomly selected and area of leaves was measured with the help of the formula given by Shrestha and Balakrishnan (1985) [22] and expressed in cm². Canopy spread of each tree in North-South and East-West direction was measured using measuring tape in cm. The relative growth of canopy of the plant during fruiting stage is measured. The leaf dry weight (after harvesting of fruits) was recorded in mg (Dubey and Yadav, 2004) [6]. Light interception percentage of light is calculated by using Digital Luxmeter (TES 1332) in the open field. Plant canopy temperature is measured with Infrared Thermometer (Equinox DT8530). Chlorophyll a, b and total chlorophyll was measured by spectrophotometer in acetone method keeping 80% acetone as blank. The chlorophyll content of the third leaf from the top was measured at 52 and 93DAP (days after pruning) on three random plants using chlorophyll meter (Hanstech model CL01). The SPAD values were recorded as the average value of chlorophyll at lower, middle and upper portion of the leaf of sample plant. Carotenoid content in the leaves (third leaf) were determined by using the method stated by method of Jonsan (1998) [12]. Total flavonoid content was determined according to Eberhardt *et al*. (2000) [7].

Result and Discussion

Vegetative growth is the ultimate parameter for final yield and quality of crop. Inappropriate growth leads to various undesirable conditions viz. poor yield and quality, insect and disease incidence etc. Hence proper care should be taken to control the vegetative growth by timely cultural operations.

Effect of pruning and chemical spray on date and duration of sprouting of vegetative shoot

The interaction effect of pruning with foliar spray of chemicals was significantly differ among treatments while the pruned plants having application of urea 5% (P_1C_1) was found to be earlier in sprout initiation (24 days) and days (32.33) taken for sprouting. The remaining treatments have shown non-significant effect. The duration taken for sprouting was found to be maximum (55.25 days) in un-pruned + 5% urea (P_2C_1) (Table-1). Highest level of pruning gave the best result as it caused better movement of air and light in to the inner part and thereby resulted in greater photosynthesis. This increased photosynthesis activity of the plants leads to higher accumulation of the photosynthates, which were utilized by developing shoots. Combinations of chemicals such as urea increased the availability of nutrients and provide nitrogen resulted in better food reserve and enhanced number of sprouts with earlier sprouting. These results are in line with that of Nath and Baruah (1999); Boughalleb *et al*. (2011) and Kundu *et al*. (2011) [20, 3, 15].

Effect of pruning and chemical spray on number of new shoots per branch

Highest number of shoots (24.57) with urea 5% and pruning (P_1C_1) was obtained where as un- pruned plant (P_2C_1) possessed 20.83 numbers of shoots (Table-1). Higher level of pruning increases number of laterals which might be due to simply removal of hormonal influence and resource sink of the apical meristem. Hence it plays a large role in shifting basal meristem determination to ward new shoots and floral initiation. Urea increases the nitrogen uptake, better food reserve and thereby enhances overall growth of the plant. These results are in agreement with Guimond *et al*. (1998); Nath and Baruah (1999); Boughalleb *et al*. (2011) and Kovaleski *et al*. (2015) [8, 20, 3, 14].

Table 1: Effect of pruning and chemical spray on Days taken for initiation of sprouting from pruning, duration of sprouting, number of new shoots per branch and length of shoot in Kuliana Lime

Chemical spray	Days taken for initiation of sprouting from pruning			Duration of sprouting (Days)			Number of new shoots per branch			Length of shoot (cm)		
	P_1	P_2	Mean	P_1	P_2	Mean	P_1	P_2	Mean	P_1	P_2	Mean
C_1	24.00	32.33	28.16	55.25	33.00	44.12	24.57	20.83	22.75	21.33	17.81	19.57
C_2	36.08	45.02	40.01	26.37	16.43	21.40	17.67	15.67	16.67	16.24	15.06	15.65
C_3	37.33	44.67	41.00	26.17	18.60	22.38	19.67	16.85	18.26	16.25	15.72	15.98
C_4	36.33	44.83	40.58	28.22	17.84	23.03	19.30	17.65	18.47	16.92	15.63	16.27
C_5	37.00	46.67	41.83	24.93	18.50	21.71	19.60	16.33	17.66	16.17	13.54	14.85
Mean	35.26	42.76	39.01	32.18	20.93	26.56	20.66	17.46	18.76	17.38	15.55	16.46

	SE(m)±	C.D (5%)	SE(m)±	C.D (5%)	SE(m)±	C.D (5%)	SE(m)±	C.D (5%)
P	0.44	1.33	0.28	0.84	0.46	1.38	0.51	1.52
C	0.70	2.10	0.44	1.33	0.73	2.18	0.32	0.96
P × C	0.99	2.96	0.63	1.89	1.04	3.09	0.72	2.14

Effect of pruning and chemical spray on length of the shoot, number of leaves and leaf area

Spraying of urea and pruning had shown significant effect and maximum shoot length (Table-1) shown in P_1C_1 treatment(21.33cm), second best was observed in P_2C_1 (17.81cm). Maximum number of leaves was found in the plant treated with urea 5% (C_1) that is 17.04cm. The pruned plant applied with urea (P_1C_1) showed to be highest number of leaves (18.66) whereas 13.12 numbers of leaves were found in control (Table-2). Likewise highest mean leaf area (26.01 cm^2) was obtained from P_1C_1 where as in control it was 21.25 cm^2 (Table-2). The increase in vegetative growth of the plant by urea attributed to the association of nitrogen in the synthesis of protoplasm and in the primary manufacture of amino acids and increased auxin activities. As a result, meristematic activities increase which in turn increase the vegetative growth. Pruning leads to better light penetration and build a better microclimate for plant growth and development, which helps in maintaining sink and source ratio by producing more vegetative bud when combined with urea. Similar results have also been reported by Choudhary *et al*. (2013); Jagtap *et al*. (2013) and Jain *et al*. (2014) [4, 10, 11] in citrus.

Table 2: Effect of pruning and chemical spray on number of leaves in new shoot, leaf area and leaf dry weight of Kuliana Lime

Chemical spray	Number of Leaves per Shoot			Leaf area (cm^2)			Dry weight of leaf (mg)		
	P_1	P_2	Mean	P_1	P_2	Mean	P_1	P_2	Mean
C_1	18.66	15.42	17.04	27.47	24.55	26.01	2.79	1.47	2.13
C_2	14.88	13.60	14.14	23.55	21.75	22.65	1.54	1.35	1.44
C_3	15.01	13.55	14.28	22.40	23.17	22.78	1.55	1.18	1.36
C_4	15.11	13.85	14.48	24.86	22.27	23.56	2.33	1.40	1.86
C_5	14.85	13.12	13.98	22.68	19.83	21.25	2.38	1.11	1.74
Mean	15.66	13.90	14.78	24.19	22.31	23.25	2.11	1.30	1.71
	SE(m) ±		CD (5%)	SE(m) ±		CD (5%)	SE(m) ±		CD (5%)
P	0.25		0.75	0.63		1.88	2.79		1.47
C	0.39		1.18	0.40		1.19	1.54		1.35
P×C	0.56		1.67	0.89		2.65	1.55		1.18

Effect of pruning and chemical spray on leaf dry weight

Pruning and foliar spray of chemicals has shown significant difference on leaf dry weight (Table-2) and recorded maximum (2.79mg) in treatment pruning + urea 5% (P_1C_1) and minimum (1.11mg) in treatment P_2C_5 (Control). The superiority of urea may be based upon its properties. It is the consequence of foliar absorption of same compound which leads to more green matter accumulation and reproduced as high dry weight. The similar finding was obtained by Nath and Baruah (1999) [20].

Effect of pruning and chemical spray on chlorophyll content

Pruning (P_1) has shown superior and significant results with maximum chlorophyll content in leaf (34.09 SPAD value) at 52 day after pruning and (27.63 SPAD value) at 93 day after pruning, least was recorded in un-pruned (P_2) with (24.73) and (21.99) at 52 and 93days after pruning respectively. Combined with Urea 5%, it has shown best result that is (41.59) at 52 days after pruning (Table-3). It might be due to the fact that pruning led to enhance root growth there by caused increased absorption of nutrients elements and transfer them to the leaves. N enhanced chlorophyll synthesis, because chlorophyll embedded in the thylakoid membrane and its synthesis enhanced incresemement the number of grana and thylakoid per granum. Urea stimulates nutrient uptake especially nitrogen which has role in the assimilation of numerous amino acids that are subsequently incorporated in proteins and nucleic acid, which provides framework for chloroplast, mitochondria and other structures in which most of the biochemical reactions occurs and resulted into increase in chlorophyll content of leaves, photosynthetic efficiency and translocation of metabolites from the source to sink. Similar results also found by Bondada and Syvertsen (2003); AL-Hamdawi (2009); Kazi *et al*. (2012); Yadav *et al*. (2012) and Dhaliwal *et al*. (2013) [2, 1, 13, 23, 5].

Table 3: Effect of Pruning and foliar spray of chemicals on chlorophyll content of Kuliana Lime

Chemical Spray	Chlorophyll content of Leaf					
	52 DAP			93 DAP		
	P1	P_2	Mean	P_1	P_2	Mean
C_1	41.59	26.65	34.12	30.91	23.98	27.44
C_2	28.52	24.27	26.39	28.86	23.60	26.23
C_3	33.05	26.70	29.87	23.33	21.25	22.29
C_4	37.01	23.85	30.43	28.34	20.29	24.31
C_5	30.30	22.20	26.25	26.74	20.87	23.80
Mean	34.09	24.73	29.41	27.63	21.99	24.81
	SE(m)±		C.D (5%)	SE(m)±		C.D (5%)
P	0.41		1.25	0.40		1.20
C	0.66		1.97	0.64		1.90
P×C	0.93		2.78	0.905		2.69

Effect of pruning and foliar spray of chemicals on canopy spread

The maximum (244 cm and 214.80 cm) canopy spread was recorded in un-pruned (P_2) in north - south (N-S) and in east - west (E-W) direction respectively. Minimum (224 cm and 205.20cm) was observed in treatment pruned (P_1) in north - south and in east - west direction. Chemicals had shown significant effect on canopy spread. Highest (256 cm and 236.50 cm) canopy spread in north - south (N-S) and in east-west (E-W) direction observed in treatment urea 5% (C_1). Remaining treatments were found statistically at par with control (C_5). Interaction effect of pruning and urea 5% found not significant. Maximum (273cm and 242 cm) canopy spread observed in north - south (N-S) and east-west (E-W) direction in treatment without pruning + urea 5% (P_2C_1) and minimum (214 cm and 173 cm) canopy spread observed in north-south (N-S) and east-west direction in treatment P_2C_5 (Table-4).

Table 4: Effect of pruning and foliar spray of chemicals on canopy spread, canopy temperature and light interception in Kuliana Lime

Chemical Spray	Canopy Spread (cm)						Canopy Temperature (˚c)			Light interception (LUX)		
	North-South			East-West								
	P_1	P_2	Mean	P_1	P_2	Mean	P_1	P_2	Mean	P_1	P_2	Mean
C_1	239	273	256	231	242	236.50	-0.93	0.26	-0.33	25.46	17.32	21.39
C_2	207	243	225	197	215	206.00	-0.09	0.20	0.05	23.51	16.45	19.98
C_3	238	243	240	215	214	214.50	-0.47	0.15	-0.16	26.31	16.68	21.49
C_4	225	221	223	210	225	217.50	-0.44	0.39	-0.25	24.62	15.92	20.27
C_5	214	240	227	173	173	173.00	-0.28	0.49	0.10	22.57	15.07	18.82
Mean	224	244	234	205.20	213.8	209.50	-0.44	0.39	0.02	24.49	16.28	20.39
	SE(m)±		C.D (5%)	SE(m)±		C.D (5%)	SE(m)±		C.D (5%)	SE(m)±		C.D (5%)
P	2.79		8.31	1.58		4.71	0.47		1.41	0.44		1.32
C	4.42		13.14	2.50		7.44	0.75		2.23	0.70		2.08
P×C	4.62		18.58	3.54		10.53	1.06		3.16	0.99		2.95

Highest canopy spread (N-S and E-W) found in P_2C_1 i.e. un-pruned tree showed better canopy spread with urea 5% application. Combination of urea gave the best result as fertilizer increased the availability of nutrients by increasing the absorption and mobilization of nutrients which was supplied by organic and inorganic fertilizers resulted better food reserve which enhanced plant height (Nath and Baruah, 1999; Boughalleb *et al*., 2011; Kundu *et al*., 2011 and Lal and Dayal, 2014) [20, 3,15, 16].

Effect of Pruning and Chemical Spray on Canopy Temperature

Negative values in the treatment show the more transpiration rate and sufficient water is available to plants and positive value indicate the less transpiration rate and plant is under water stress. The least which is the best was recorded as treatment C_1 (-1.32˚C) and the highest in treatment C_5 (0.89˚C). Pruning (P_1) has significant effect and recorded the least (-1.32˚C), which was considered as the best for canopy temperature with good transpiration and maintained plant water status. Un-pruned has the positive value (1.18˚C) with canopy temperature which indicates less transpiration. Interaction effect of pruning and urea treatment (P_1C_1) was found significantly different from the rest of the treatments imposed (Table-4). The findings are in line with Nath and Baruah (1999) [20].

Effect of pruning and chemical spray on light interception

Maximum (24.49 LUX) light interception recorded in pruned treatment (P_1) and minimum (16.28 LUX) in treatment P_2 (Table-4). Maximum (21.49 LUX) light interception observed in treatment C_3 and second best (21.39 LUX) recorded as treatment C_1. The remaining treatments (C_1, C_2, C_4 and C_5) were statistically at par. Pruning (P_1) has significant role in light interception and was recorded maximum (24.49 LUX) than in unpruned (P_2) treatments. Among the Interaction of pruning and chemical spray, best result was recorded in treatment P_1C_1. Pruning helps the plant to open up for more light penetration which helped to circulate air, light humidity inside plant canopy and ultimately lead to more light interception (Nath and Baruah, 1999) [20].

Effect of pruning and foliar spray of chemicals on Chlorophyll a, b and total Chlorophyll

Maximum (0.49, 0.76 and 0.82 mg/g fresh weight) chlorophyll a, b, total chlorophyll content in leaf recorded in P_1 and the minimum (0.34, 0.37 and 0.53 mg/g fresh weight) chlorophyll a, b and total in leaf recorded P_2 (Table-5). Chemical spray has superior effect over the control. Maximum (0.54, 0.86 and 0.81 mg/g fresh weight) chlorophyll a, b and total, content in leaf was recorded in treatment C_1 and the remaining other treatments were statistically at par. Interaction of pruning and chemicals had shown significant difference over unpruned + chemicals. Maximum (0.65, 1.36 and 1.29 mg/g fresh weight) observed in treatment (P_1C_1), however the remaining treatments were statistically at par. Least (0.29, 0.40 and 0.49mg/g fresh weight) chlorophyll a, b and total in leaf were found in treatment P_2C_5.

Effect of pruning and chemical spray on carotenoid content in leaf

Chemical spray and pruning had significant effect on carotenoid content in leaf (Table-5). Maximum (27.81 mg/g fresh weight) and minimum (22.47mg/g fresh weight) carotenoid content was recorded in pruned (P_1) and in un-pruned (P_2) treatments, respectively. Coming to chemicals, maximum (27.44 mg/g fresh weight) carotenoid content was observed in treatment NAA 100ppm (C_4) and the remaining treatments (C_1, C_2, C_3 and C_5) were found to be statistically at par. Interaction of pruning and NAA spray had shown significant effect. Among the interaction effect, maximum (30.91 mg/g fresh weight) carotenoid content in leaf was recorded in treatment P_1C_4 and minimum (24.99 mg/g fresh weight) observed in treatment P_2C_5.

Effect of pruning and chemical spray on flavonoid content in leaf

The maximum (117.93mg/100g dry weight) and minimum (111.74 mg/100g dry weight) flavonoid content was recorded in P_1 and P_2 treatments, respectively. Among the chemicals, maximum (118.16 mg/100g dry weight) flavonoid content was observed in treatment C_4 and the remaining treatments (C_1, C_2, C_3 and C_5) were found to be statistically at par. Interaction of pruning and NAA spray had shown significant effect on flavonoid content in leaf. Maximum (121.88 mg/100g dry weight) flavonoid content in leaf was recorded in treatment pruning + NAA 100ppm (P_1C_4) and minimum (104.60 mg/100gdry weight) observed in treatment un-pruned control (P_2C_5) (Table-5).

Conclusion

All the vegetative characters like early initiation of sprouting (28.16 days) and longer duration of sprouting (44.12 days), higher numbers of new shoot per branch (22.75), highest length of the shoot (19.57cm), number of leaves on emerging shoot (17.04) leaf area (26.01 cm^2), maximum chlorophyll content (34.12and 27.44 mg/g fresh weight at 52 and 93 DAP) highest canopy spread, canopy temperature as well as higher

leaf dry weight was found on the plant applied with urea 5 per cent as compared to other treatments.

As regards the pruning, it showed superiority over without pruning in point of view like days taken to sprouting and duration (35.26 and 32.18 days), higher numbers of new shoot per branch 20.66, highest length of the shoot (17.58cm), number of leaves on emerging shoot (15.66), leaf area (24.19 cm^2), maximum chlorophyll content, carotenoid and flavonoid content in leaf but lower canopy spread with adequate and good enough light interception as well as higher leaf dry weight.

All the parameters viz. sprouting initiation and duration, number of new shoots, length of the shoots, numbers of emerging leaves, leaf area, chlorophyll content, leaf dry weight, light interception were found significantly superior in pruned plants applied with Urea 5 percent.

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