Research Article

Changes in Gum Yield and Anatomy of *Commiphora wightii* (Arnott.) Bhandari in Response to Foliar Application of PGR and Nutrients

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Abstract

A two year field study with foliar application of plant growth regulator (PGRs) and nutrients on *Commiphora wightii* (Arnott.) Bhandari, was carried out to explore the relationship of leaf area, chlorophyll content, the density and circumference of gum ducts, gum ooze and yield. Increase in the leaf area of *C. wightii* treated with PGR and nutrients over the control though was non significant but the total affect due to cumulative increase in the total leaf area of a plant and photosynthesis cannot be ruled out. The leaf chlorophyll content (a, b and total) was significantly highest in Humic acid treated *C. wightii* over rest of the treatments. It was followed by that in micronutrient treated *C. wightii* plants. The mean gum ooze immediately after incision was highest in the micronutrient treated *C. wightii* but was at par with that of Humic acid and more than that in the control plants. The weight of gum w/v was significantly highest in micronutrient treated *C. wightii*, followed by Humic acid. *C. wightii* treated with micronutrients had the highest density of gum ducts in the month of December while it was highest in the March among the *C. wightii* plants treated with Humic acid. Thus, *C. wightii* treated with micronutrients can be tapped three months earlier i.e. December. It has another advantage as the atmospheric temperature remains low for rest two months which may prevent loss of aromatic content of guggul gum. Foliar applications of PGR and nutrients improved leaf area, chlorophyll content, gum duct density, duct circumference and guggul yield of *C. wightii* over the control treatment.

Keywords: Data deficient, Guggul, Gum duct, mirco-nutrients, Natural resins

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Introduction

Gum resin and oleo-resin producing plants are of economical [1, 2] and ecological [2, 3] interests. These products are non wood forest produce, serves as cash crop to millions of poor and tribal communities [1] living either in the forest [4] or in the arid regions [5] of the world. Natural resins and gum producing plants are usually slow growing and generally found in semi-arid [6] and arid regions [7] across the world. Destructive tapping of these plants [8] are threatening them in their natural stand [9] and degrading the ecology. Losses of these valuable plants are equally a threat to the socio-economy [10] of the people depending on it [11, 12] for their household income [13] and livelihoods [2]. In this context, the challenge before the scientific community is to develop technologies to conserve the present stock of natural resin and gums yielding plants [14, 15] as well as increase the productivity [16].

Resins and gums are the secondary metabolites [17, 18] of the plant. The production or yield of resin and gum depend on many factors like soil [19], its nutrient status [20], soil moisture [21, 22], location [23], nutrient applications or availability [22], growth period [24], leafy period [22] and genetic characters [25] of the plant. Unfortunately, resin and gum producing plants are never cared for its nutrient status [26] or nutrient replenishment [27], in its natural stand. It is widely acknowledged that the production of resin and gum depends on the allocation of primary metabolites to the sink or storage by the plants [28].

The present two year field study is an attempt to find a relationship with plant growth, physiology, anatomy and gum production *C. wightii* with foliar application of PGR and nutrients.

Materials and Methods

A two years field trial was carried out during 2014-15 and 2015-16 to study the response of foliar application of plant growth regulator (PGR) and nutrients on 40 *C. wightii* plants in its natural stand. The field experiment was conducted in the Chambal ravines of Morena district, Madhya Pradesh, India. Four spray schedules of the five treatments had

eight *C. wightii* plants each. The treatments were T_1-Humic acid, T_2-Urea, T_3-Micro-nutrient, T_4-PGR and T_5-Control. Leaf area, chlorophyll content and ooze gum collection were the observations recorded from the guggul plants.

Physiological observations

Leaf area (cm^2)

Ten leaves per plant were randomly select for measuring the leaf area by Laser Area Meter (Model LI-300).

Chlorophyll content (SPAD)

The chlorophyll content was measured with the help of Chlorophyll meter (Soil Plant Analysis Devise-502 Plus) on two plants per replication. Ten leaves per plant were randomly selected for measuring the chlorophyll content and expressed in terms of SPAD units.

Chlorophyll 'a' and 'b' (mg/g)

The chlorophyll 'a' and 'b' were estimated in mg/g by the method suggested by Arnon[29] on two plants per replication.

Gum

Observations of gum production of the treated *C wightii* were recorded by making diagonal incision on its tertiary branches by Jawahar Guggul Blazer, followed by immediate collection of the oozing gum for a minute in calibrated vials. The volumes of the guggul gum (ml/incision) collected in vials were recorded immediately in the field. The dry weight of the gum (g/ooze) recorded on the Digital balance in the Project laboratory, Department of Entomology, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur.

Anatomical studies

Tertiary branches of size 3 inches from the treated as well as of control *C. wightii* plants collected in February, May, July, September, November, December 2015 and March 2016 were preserved in Formalin (10%). Transverse sections of these samples were carried out in the College of Forestry, Kerala Agriculture University, Thrissur, Kerala for the density and dimension of gum ducts.

Staining procedure

The specimens after thoroughly washing with distilled water were subjected to transverse section with Digipro. The slides of transverse sections of the specimen samples were stained with saffranin following the procedure suggested by Johansen (1940), later washed thoroughly with distilled water to ensure complete dehydration. This was followed by subsequent dipping in xylone and finally mounted in DPX mountant to prepare the slides (size 75mm X 25mm and thickness of 1mm) and covered by cover slips.

Image analysis

Microscopic examination and quantification of sections were undertaken using an image analyzer (Leica-DM 750).The image analyzer consisted of a microscope, digital camera and desktop computer that provided quick and accurate data. Digital images were analyzed by the software-Lasez Digipro for measurement like density, circumference and width of oleo gum resin ducts.

Results and Discussion

Leaf area

The mean leaf area of *C. wightii* varied in different treatments but there was no significant difference in the leaf area of *C. wightii* among different treatments (**Table 1**). Among various determinants of plants, leaf area plays significant role in influencing light interception, transpiration, photosynthesis and plant productivity [30]. Leaf area and shape being a genetic character of any plant species, larger variation due to treatments over the control cannot be expected. However an increase in leaf area, even if it is non significant, influence the overall increase in the biomass of the plant as well as its physiology. Bhat et al. [31] reported maximum leaf area (129.70 cm^2) and dry matter content (26.51%)

in seedless grapes with 3ppm of N-(2-Chloro-4-pyridyl)-N′-phenylurea (CPPU) and 0.4ppm of Brassinosteriod (BR) combination along with 25 ppm of gibberellic acid (GA). Laxmipathi et al. [32] also found the highest leaf area in cashew sprayed with GA3 @ 50 ppm and ethrel @ 50 ppm.

Table 1 Mean leaf area (cm^2) of *C wightii* under different treatments

Treat	2014-15					2015-16					Pooled				
	T_1-HA	T_2-Urea	T_3-MN	T_4-PGR	T_5-CN	T_1-HA	T_2-Urea	T_3-MN	T_4-PGR	T_5-CN	T_1-HA	T_2-Urea	T_3-MN	T_4-PGR	T_5-CN
R_1	1.38	1.66	1.56	1.85	1.42	1.71	1.52	1.48	1.68	1.49	1.54	1.59	1.52	1.77	1.45
R_2	1.48	1.55	1.09	1.65	1.31	1.66	1.62	1.39	1.54	1.37	1.57	1.58	1.24	1.59	1.34
R_3	1.31	1.46	1.23	1.32	1.47	1.64	1.47	1.41	1.47	1.62	1.48	1.47	1.32	1.39	1.54
R_4	1.63	1.27	1.71	1.71	1.68	1.81	1.55	1.67	1.88	1.44	1.72	1.41	1.69	1.79	1.56
Mean	1.45	1.49	1.40	1.63	1.47	1.71	1.54	1.49	1.64	1.48	1.58	1.51	1.44	1.64	1.48
SEm± : 0.052, CD(5%): NS						SEm± : 0.038, CD(5%): NS					SEm± : 0.039, CD(5%): NS				

The two years pooled data analysis revealed that, the leaf area was the highest (1.64) in T_4-PGR followed by T_1-Humic acid and remaining treatments. There was still no significant difference in the leaf area among different treatments. This increase in leaf area with PGR might be related to the fact that it promotes leaf area through the increase of cell division in higher plant [33-35]. Higher leaf area values recorded with PGR may also be due to increased rate of photosynthesis in the shoot [36, 37] as reported in grape [31]. Role of PGRs in increasing leaf area can be ascribed to their influence on cell division and cell elongation. Thus, a cumulative increase in leaf area of the whole plant may lead to an increase in the overall photosynthesis and production of primary metabolites when compared with the control plants of *C. wightii*. The plants have a tendency to mobilize excess primary metabolites produced to its sink and converted into secondary metabolites as oleo-resin in the present case.

Chlorophyll content (SPAD)

Rapid, non-destructive estimation of total chlorophyll content is a potentially important application for both forest managers and researchers. Estimation of chlorophyll content obtained with portable chlorophyll meters have been reported for a number of agricultural species, including cabbage, cotton, and pea [38], sorghum and pigeonpea [39], muskmelon [40], corn [41] and several fruit tree species [42]. In the present investigation the mean chlorophyll content of the leaves varied among the different treatments during first year analysis but there was no significant difference in the chlorophyll content of leaves among different treatments (**Table 2**). In second year the chlorophyll content though increased in all the treatments yet the difference was non significant. This increase may be due to the cumulative effect of two years of the treatments. In the pooled analysis the mean chlorophyll content was the highest (21.88) in T_1-Humic acid followed by T_2-Urea (19.93), T_3-Micro nutrients (19.21), T_4-PGR (19.20) and T_5-Control (18.50).An increase in the values of chlorophyll readings (SPAD) was reported by Meganid et al. [43] in common bean plants treated with humic acid as compared to untreated plants. Humic acid is known to enhance synthesis of the chlorophyll [44]. High chlorophyll content in the leaves of Green mint (*Mentha spicata*) treated with foliar application of Urea over the control is reported by Hamid et al. [45].

Table 2 Chlorophyll content (SPAD) of *C wightii* under different treatments

Treat	2014-15					2015-16					Pooled				
	T_1-HA	T_2-Urea	T_3-MN	T_4-PGR	T_5-CN	T_1-HA	T_2-Urea	T_3-MN	T_4-PGR	T_5-CN	T_1-HA	T_2-Urea	T_3-MN	T_4-PGR	T_5-CN
R_1	15.90	24.03	17.67	19.57	17.87	31.41	19.84	19.66	22.41	18.91	23.66	21.94	18.66	20.99	18.39
R_2	16.43	19.30	18.27	17.73	16.37	21.47	18.22	18.62	18.90	20.57	18.95	18.76	18.44	18.32	18.47
R_3	29.43	20.03	18.40	21.27	18.10	19.60	23.47	22.39	16.84	16.52	24.52	21.75	20.40	19.05	17.31
R_4	19.93	17.30	18.17	17.43	17.73	20.86	17.22	20.47	19.47	21.91	20.40	17.26	19.32	18.45	19.82
Mean	20.43	20.17	18.13	19.00	17.52	23.34	19.69	20.29	19.41	19.48	21.88	19.93	19.21	19.20	18.50
SEm± : 1.11, CD(5%): NS						SEm± : 1.070, CD(5%): NS					SEm± : 0.519,CD(5%): NS				

Chlorophyll 'a' and 'b'

Chlorophyll is found in the chloroplasts of green plants and is the molecule that performs photosynthesis. The mean chlorophyll 'a' and 'b' in the leaves of *C. wightii* varied significantly among the different treatments. In pooled data analysis the mean value of chlorophyll 'a' was highest (0.68mg/g) in T_1-Humic acid followed by T_2-Urea (0.51mg/g)

while it was lowest (0.40mg/g) in T_5-Control (**Table 3**). The mean value of chlorophyll 'b' (**Table 4**) was highest (0.42mg/g) in T_1-Humic acid followed by T_4-PGR (0.35mg/g). The mean total chlorophyll (a+b) content of the leaves varied significantly among the different treatments (**Table 5**). It was the highest (1.10mg/g) in T_1-Humic acid and significantly superior over the other treatments. The lowest (0.69mg/g) mean total chlorophyll content was recorded in T_5-Control.

Table 3 Mean Chlorophyll 'a' content (mg/g) of *C wightii* under different treatments

Treat	2014-15					2015-16					Pooled				
	T_1-HA	T_2-Urea	T_3-MN	T_4-PGR	T_5-CN	T_1-HA	T_2-Urea	T_3-MN	T_4-PGR	T_5-CN	T_1-HA	T_2-Urea	T_3-MN	T_4-PGR	T_5-CN
R_1	0.66	0.50	0.42	0.53	0.39	0.69	0.47	0.39	0.58	0.41	0.68	0.49	0.40	0.56	0.40
R_2	0.65	0.50	0.43	0.54	0.40	0.76	0.61	0.37	0.43	0.42	0.71	0.56	0.40	0.48	0.41
R_3	0.67	0.51	0.42	0.55	0.39	0.61	0.51	0.45	0.47	0.34	0.64	0.51	0.43	0.51	0.36
R_4	0.66	0.50	0.45	0.53	0.41	0.74	0.48	0.45	0.58	0.45	0.70	0.49	0.45	0.55	0.43
Mean	0.66	0.51	0.43	0.54	0.40	0.70	0.52	0.42	0.52	0.41	0.68	0.51	0.42	0.53	0.40
SEm± : 0.004,CD(5%): 0.011						SEm± : 0.020, CD(5%): 0.063					SEm± : 0.010, CD(5%): 0.030				

HA-Humic acid, MN-Micro-nutrient, PGR-Plant growth regulator, CN-Control

Table 4 Mean Chlorophyll 'b' content (mg/g) of *C wightii*under different treatments

Treat	2014-15					2015-16					Pooled				
	T_1-HA	T_2-Urea	T_3-MN	T_4-PGR	T_5-CN	T_1-HA	T_2-Urea	T_3-MN	T_4-PGR	T_5-CN	T_1-HA	T_2-Urea	T_3-MN	T_4-PGR	T_5-CN
R_1	0.42	0.32	0.28	0.34	0.28	0.46	0.29	0.26	0.36	0.37	0.44	0.30	0.27	0.35	0.33
R_2	0.44	0.30	0.25	0.35	0.26	0.42	0.33	0.33	0.39	0.25	0.43	0.32	0.29	0.37	0.26
R_3	0.41	0.29	0.28	0.32	0.29	0.38	0.42	0.29	0.38	0.34	0.39	0.36	0.29	0.35	0.31
R_4	0.44	0.31	0.25	0.35	0.27	0.41	0.37	0.31	0.29	0.24	0.42	0.34	0.28	0.32	0.26
Mean	0.43	0.31	0.27	0.34	0.28	0.42	0.35	0.30	0.36	0.30	0.42	0.33	0.28	0.35	0.29
SEm± : 0.005,CD(5%): 0.016						SEm± : 0.017,CD(5%): 0.052					SEm± : 0.009,CD(5%): 0.027				

Table 5 Mean Total Chlorophyll (a+b) content (mg/g) of *C wightii* under different treatments

Treat	2014-15					2015-16					Pooled				
	T_1-HA	T_2-Urea	T_3-MN	T_4-PGR	T_5-CN	T_1-HA	T_2-Urea	T_3-MN	T_4-PGR	T_5-CN	T_1-HA	T_2-Urea	T_3-MN	T_4-PGR	T_5-CN
R_1	1.09	0.82	0.70	0.87	0.67	1.15	0.76	0.65	0.94	0.78	1.12	0.79	0.67	0.91	0.73
R_2	1.09	0.80	0.68	0.89	0.66	1.18	0.94	0.70	0.82	0.67	1.14	0.87	0.69	0.85	0.67
R_3	1.08	0.80	0.70	0.87	0.68	0.99	0.93	0.74	0.85	0.68	1.03	0.87	0.72	0.86	0.68
R_4	1.09	0.82	0.70	0.88	0.68	1.15	0.85	0.76	0.87	0.69	1.12	0.83	0.73	0.87	0.69
Mean	1.09	0.81	0.69	0.88	0.67	1.12	0.87	0.71	0.87	0.71	1.10	0.84	0.70	0.87	0.69
SEm± : 0.003,CD(5%): 0.009						SEm± : 0.024, CD(5%): 0.075					SEm± : 0.012, CD(5%): 0.037				

HA-Humic acid, MN-Micro-nutrient, PGR-Plant growth regulator, CN-Control

Application of humic acid enhances chlorophyll [46, 47]. We also observed a significant effect of humic acid in the present experiment and in agreement with Chen et al. [48] who also reported improved chlorophyll 'a' and 'b' in creeping bentgrass (*Agrostis stolonifera*). However, Liu et al. [49] could not find the effect of humic acid in increasing chlorophyll content in *A. stolonifera*, but could enhance net photosynthesis. Some researchers reported no difference in chlorophyll content of the turf with any humic acid treatment [50].

The increased chlorophyll content may be associated with the supply of essential nutrients to the plants. Since chlorophyll synthesis in the plants is directly related to the availability of the physiologically active Fe, N, P and S micronutrients in plants. Hence the availability of these nutrients to plants helps in the formation of chlorophyll in the leaves. Increased chlorophyll 'a', 'b' and total chlorophyll content in green leaves of rice with foliar application of organic solution been observed by Tejada and Gonzalez [51]. Similar results of humic acid on chlorophyll 'a', 'b' and total chlorophyll content of plants have been reported earlier in different studies [46, 47, 52].

The point here to be noted is that significant increase in chlorophyll content in leaves of treated *C. wightii*. A leaf with more chlorophyll means more production of primary and later secondary metabolites in *C. wightii*, over those with no treatments and secondary metabolites influence gum yield in the plants.

Gum yield by volume (ml/minute)

The volume (ml) of guggul gum collected immediately after incision for a minute in calibrated vials varied significantly among different treatments. The two years pooled data analysis of the ooze data revealed significant difference in the yield of gum due to different treatments (**Table 6**) over the control. It was highest (1.09) in T_3-Micro nutrient but at par with remaining treatments. The lowest ooze was recorded in T_5-Control (0.75). However, Samanta et al. [22] found no significant effect on the guggul yield with two doses of nitrogen (17 and 34 mg kg^{-1} soil) in two split doses, but soil moisture below 20 percent significantly affected the gum yield. Basal application of nutrients and irrigation of *C. wightii* in arid region is practically not viable option, while foliar application can be done. Both Micronutrient and Humic acid application increased chlorophyll content of *C. wightii*, which is reflected in the higher gum yield.

Gum yield (wt/vol)

The pooled analysis of the data revealed that the highest (1.53) mean weight of guggul gum was observed in T_3-Micro nutrient, which was significantly superior over T_1-Humic acid (1.24), T_4-PGR (1.04), T_2-Urea (1.01) and T_5-Control (0.95). There was a significant difference in the yield of gum due to different treatments over the Control (**Table 7**).

The oozing gum after incision has moisture content. The speed flow of oozing gum depends on its moisture content or viscosity. More viscous means less moisture leads to slower flow rates. However more viscous also means more dry weight per volume [53].

Table 6 Gum yield by volume (ml/incision) of *C wightii* under different treatments

Treat	2014-15					2015-16					Pooled				
	T_1-HA	T_2-Urea	T_3-MN	T_4-PGR	T_5-CN	T_1-HA	T_2-Urea	T_3-MN	T_4-PGR	T_5-CN	T_1-HA	T_2-Urea	T_3-MN	T_4-PGR	T_5-CN
R_1	0.25	0.20	0.60	0.25	0.20	1.60	1.20	1.90	1.30	1.10	0.93	0.70	1.25	0.78	0.65
R_2	0.20	0.25	0.50	0.25	0.25	1.80	1.60	1.60	1.50	1.30	1.00	0.93	1.05	0.88	0.78
R_3	0.25	0.20	0.50	0.30	0.25	2.00	1.40	1.80	1.10	1.50	1.13	0.80	1.15	0.70	0.88
R_4	0.20	0.20	0.25	0.30	0.20	1.60	1.60	1.60	1.40	1.20	0.90	0.90	0.93	0.85	0.70
Mean	0.23	0.21	0.46	0.28	0.23	1.75	1.45	1.73	1.33	1.28	0.99	0.83	1.09	0.80	0.75
SEm± : 0.049,CD(5%): 0.152						SEm± : 0.069,CD(5%): 0.187					SEm± : 0.036, CD(5%): 0.113				

Table 7 Gum yield by weight (g/ooze) of *C wightii* under different treatments

Treat	2014-15					2015-16					Pooled				
	T_1-HA	T_2-Urea	T_3-MN	T_4-PGR	T_5-CN	T_1-HA	T_2-Urea	T_3-MN	T_4-PGR	T_5-CN	T_1-HA	T_2-Urea	T_3-MN	T_4-PGR	T_5-CN
R_1	0.31	0.14	0.87	0.23	0.21	2.18	1.75	2.64	1.87	1.92	1.24	0.95	1.75	1.05	1.07
R_2	0.10	0.17	0.58	0.21	0.33	2.42	1.82	2.39	1.90	1.58	1.26	1.00	1.48	1.06	0.96
R_3	0.19	0.11	0.33	0.39	0.15	2.33	1.90	2.57	1.92	1.84	1.26	1.00	1.45	1.15	0.99
R_4	0.10	0.03	0.19	0.48	0.11	2.26	2.13	2.66	1.30	1.48	1.18	1.08	1.42	0.89	0.79
Mean	0.18	0.11	0.49	0.33	0.20	2.30	1.90	2.57	1.75	1.71	1.24	1.01	1.53	1.04	0.95
SEm± : 0.077,CD(5%): 0.239						SEm± : 0.068,CD(5%): 0.211					SEm± : 0.032, CD(5%): 0.098				
HA-Humic acid, MN-Micro-nutrient, PGR-Plant growth regulator, CN-Control															

The most important resin canal trait in the past studies reveals that resin yield for Norway spruce and pine trees are depend upon the diameter of resin canals [54]. The gum resin already stored in these ducts ooze out immediately by injury [55]. As resin ducts are oriented parallel to the longitudinal axis of stem [56], horizontal cut resulted in higher gum as it cuts higher number of resin ducts as compared with the cuts of other orientations. Also, larger cut size increases the number of exposed ducts and resulted in higher gum yield. Fertilizer and irrigation also favours guggul gum production. Hence, gum yield also depends on nutrition and site soil factors.

In various past research findings, plant growth regulators; have been reported to increase exudation of gum or resin in *Anogiessus latifolia*, *Acacia senegal*, *C. wightii*, *Sterculia urens* and *Mangifera indica* (www.fao.org). Previously it is proved that among some other factors, applied chemicals [57], and genetics of trees [58] may affect the resin production.

Anatomical studies

Resin secretory structures of *C. wightii* are predominantly found in the bark. There is no consistency in the mode of development, distribution and structure of ducts even in the different tissues of the same organ of a plant body [59]. Axial and radial resin canals form a three-dimensional network in the intact zone. In this relatively small zone, which accounts for less than half of the thickness of the inner bark, the network of resin canals is mostly intact and so is most likely to be functional for short- and long-distance resin transport. Radial resin canals connect the canal network of the bark to the wood. This hints to the possibility of radial transport of resin between wood and bark. Previous studies showed similar results for *B. serrata* from India [60] and interconnected canals are also reported for Pinaceae [61-63] and Araliaceae [64].

Density of gum ducts (no./mm^2)

The density of gum ducts (no./mm^2) was highest during March in all the treatments except that of micro nutrients, where it was highest in December (37.29). In the month of March density of gum ducts per mm^2 was highest (46.68) in PGR followed by Humic acid (36.31), Urea (33.77) and Control (28.37) (**Table 8**, **Figure 1**). Thus in comparison to the Control, the density of gum ducts was 39.22 percent more in PGR followed by Humic acid (21.87%), Urea (16.00%) and Micro nutrient (5.43%). Increase in the density of gum ducts in comparison to the Control may be due to increasing the yield of biomass of plant. The highest density (37.29) of gum ducts in *C. wightii* treated with micronutrients was in December. It does mean that, the tapping of *C. wightii* treated with micronutrient can be done in December instead of March i.e. three months earlier, than the traditional tapping schedule of *C. wightii* in Chambal ravines of Madhya Pradesh. On the other hand the density of gum ducts in *C. wightii* treated with PGR (46.68), Humic acid (36.31) and Urea (33.74) was highest in March. This is an indication that *C. wightii* treated with the above are ready for tapping in March.

Table 8 Density of gum ducts (no./mm^2) in *C. wightii* under different treatments

Date of sampling	Density of gum ducts (mm^2) in different treatments					SEm±	CD(5%)
	T_1-Humicacid	T_2-Urea	T_3-Micro nutrient	T_4-PGR	T_5- Control		
21.02.15	21.89	10.47	17.03	17.84	10.61	1.00	3.12
29.05.15	11.16	14.47	12.00	11.05	12.30	1.11	3.45
17.07.15	16.21	20.00	16.63	12.12	10.58	1.54	4.80
18.09.15	21.42	20.12	19.47	15.83	17.98	1.16	3.60
19.11.15	22.76	19.08	25.57	17.61	14.78	1.34	4.16
23.12.15	22.12	19.94	37.29	24.27	23.85	3.90	12.12
08.03.16	36.31	33.77	26.91	46.68	28.37	2.66	8.25

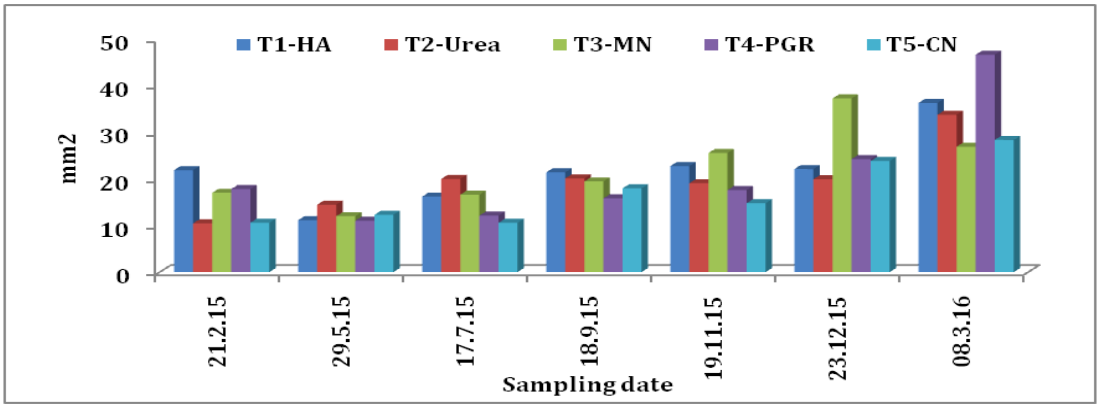

Figure 1 Periodic variation in the density of gum ducts in *C. wightii* under different treatments

With increasing distance from the cambium, the density of axial resin canals decreases strongly. This decrease is caused by dilatation occurring due to increasing tangential strain as trees grow in circumference [64, 65]. The dilatation is realized by the production of new parenchyma cells that are formed by phloem parenchyma cells which regain meristematic status [64, 66].

In a study, Arbellay et al. [67] found increase in axial resin duct density in *P. menziesii* and *Larix occidentalis* due to the formation of tangential rows of traumatic resin ducts, especially in the first and second year after injury though the radial density did not change significantly. Moreira et al. [68] reported that density of constitutive resin canals in

the cortex and the total canal system was ~1.5-fold higher in *Pinus pinaster* plants under limited P availability than in fully fertilized plants. Availability of P did not significantly influence the inducibility of resin canal traits. Negative genetic correlations between plant growth and the density of constitutive canals in the xylem and total canal system, but only under conditions of limited nutrition was found. These results demonstrated that differentiation of constitutive anatomical-based defense was affected by P limitation.

Circumference of gum duct (in mμ)

The mean circumference of gum ducts in *C. wightii* differed significantly among the treatments. The mean circumference of gum duct was highest in the month of December, 2015. It was highest (6461.69mμ) in Micro-nutrient followed by Urea (6123.34 mμ), PGR (5793.93 mμ) in Humic acid (5135.32 mμ) and in the Control (4097.69 mμ).The circumference of gum duct in treated plants was increased in comparison to non treated plant of *C. wightii*. The highest percent of increase over the control was recorded in Micro nutrient (36.58%) followed by Urea (33.08%), PGR (29.28%) and Humic acid (20.21%) in the month of December (**Table 9**, **Figure 2**).

During March the values of mean circumference of the ducts was highest (5173.69 mμ) in PGR followed by the Control (4796.57mμ), Humic acid (4338.36mμ), Urea (4017.57mμ) and Micro nutrient (3852.53mμ). No such studies have been conducted on any gum/resin producing tree species regarding effect of foliar application of growth regulators and nutrients on circumference of gum ducts.

Table 9 Mean circumference of gum duct (in mμ) in *C wightii* under different treatments

Date of sampling	Mean circumference of gum duct (mμ) in different treatments					SEm±	CD(5%)
	T_1-Humic acid	T_2-Urea	T_3-Micro nutrient	T_4-PGR	T_5- Control		
21.02.15	4410.78	4150.66	3038.35	3309.10	4054.69	109.38	339.74
29.05.15	5640.60	3656.71	5011.68	5023.80	3984.93	514.57	1598.27
17.07.15	5369.85	4650.16	4102.33	3999.82	3676.62	202.72	629.64
18.09.15	3693.17	3983.99	3824.59	3775.25	3765.69	220.23	684.03
19.11.15	5293.84	4600.21	5036.64	4299.85	5367.99	389.74	1210.54
23.12.15	5135.32	6123.34	6461.69	5793.93	4097.69	548.51	1703.68
08.03.16	4338.36	4017.57	3852.53	5173.69	4796.57	321.54	998.70

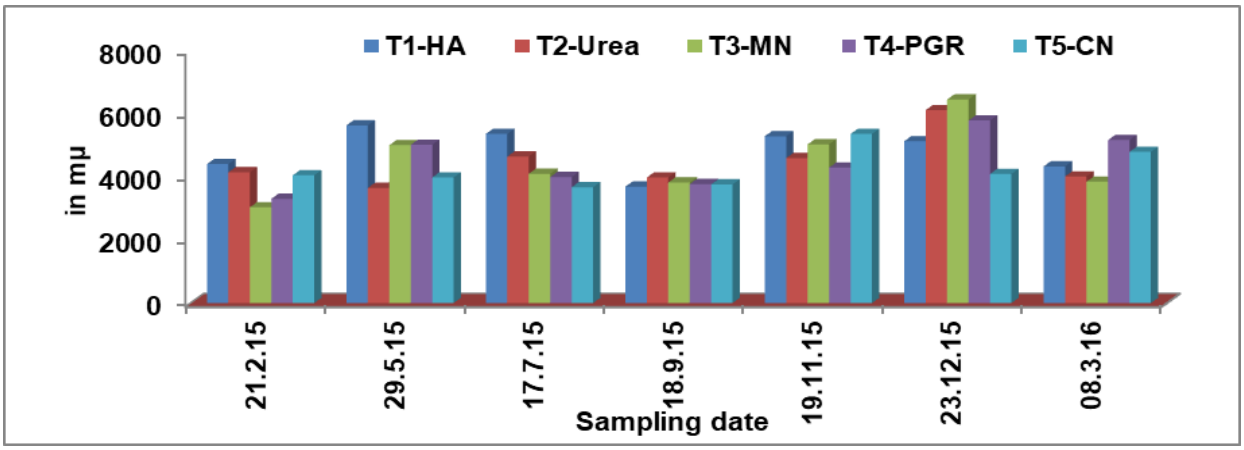

Figure 2 Periodic variation in the circumference of gum ducts in *C wightii* under different treatments

Width of gum ducts (in mμ)

The mean width of gum ducts of *C. wightii* differed significantly among the treatments. The mean width of gum duct was maximum in the month of March 2016. It was maximum (62.22mμ) in Humic acid followed by PGR (61.34mμ), Urea (60.69mμ), Micro nutrient (58.19mμ) and Control (52.78) in the same month. Thus in comparison to the Control, the width of gum ducts 15.17 percent more in Humic acid followed by PGR (13.96%), Urea (13.03%) and Micro nutrients (9.30). The gum duct width of treated plants increased as compared to non treated plant of *C wightii*(**Table 10**, **Figure 3**). In a similar study, foliar potassium application had only a slight effect on needle morphology of Scots pine. The sclerenchyma cell walls were thinner, the xylem area was larger, and the resin ducts were smaller in needles of *Pinus sylvestris* with a low K concentration than in needles with a high or intermediate K

concentration [69]. The decrease in size and number of resin ducts with decreasing foliar K concentration may indicate that the terpene based defensive mechanism is compromised in trees suffering from K deficiency [70].

Table 10 Mean width of gum ducts (in mμ) in *C wightii* under different treatments

Date of sampling	Mean width of gum ducts (mμ) in different treatments					SEm±	CD(5%)
	T_1-Humic acid	T_2-Urea	T_3-Micro nutrient	T_4-PGR	T_5- Control		
21.02.15	47.03	38.00	53.75	51.63	45.31	1.12	3.47
29.05.15	39.50	27.94	33.81	35.31	34.06	1.13	3.51
17.07.15	58.78	52.13	56.75	57.06	43.81	3.42	10.64
18.09.15	53.03	41.56	36.06	52.38	42.38	3.77	11.71
19.11.15	27.84	26.26	28.44	32.00	31.63	1.34	4.15
23.12.15	46.75	56.88	41.75	55.81	44.31	2.06	6.39
08.03.16	62.22	60.69	58.19	61.34	52.78	3.32	10.32

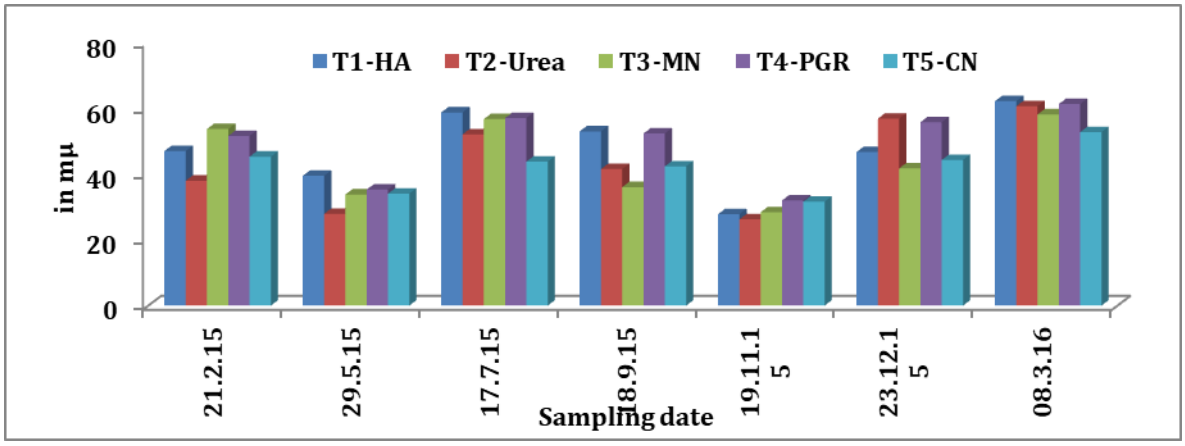

Figure 3 Periodic variation in the width of gum ducts in *C wightii* under different treatments

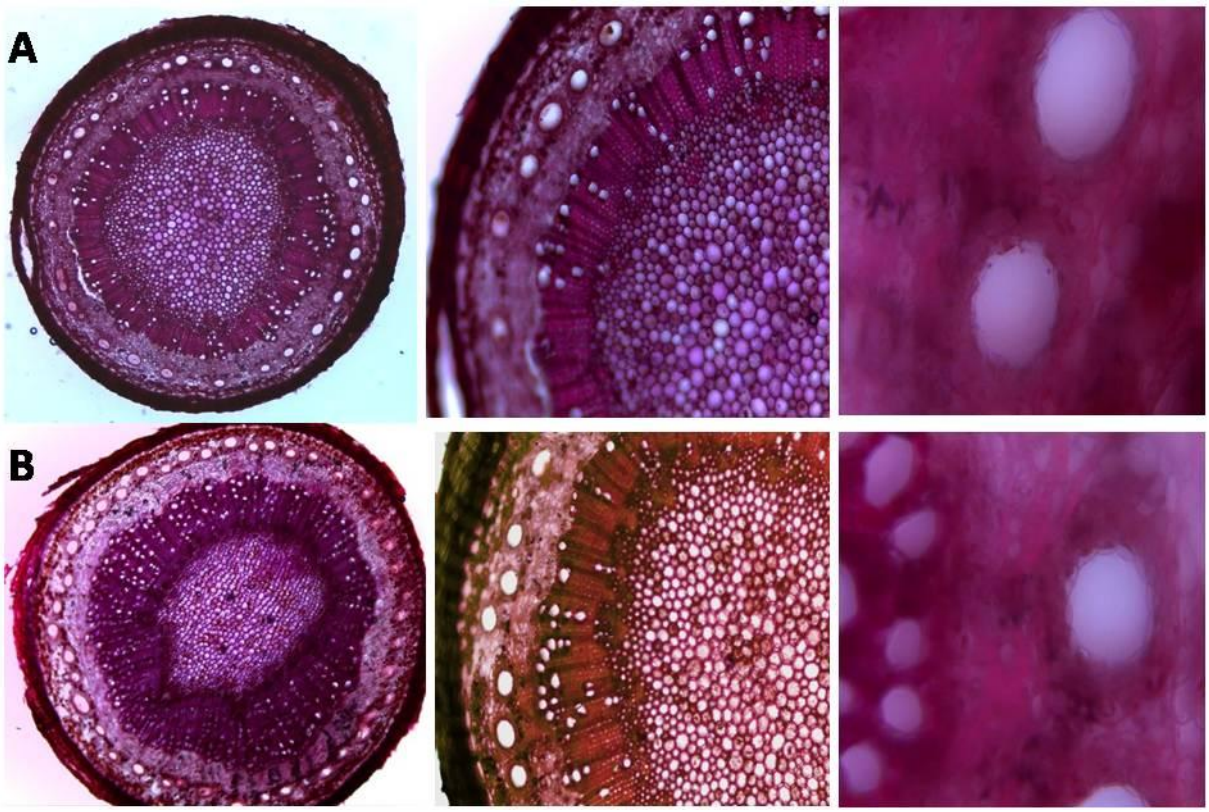

Figure 4 Section cuttings of the *C. wightii* branches with the treatment of A. T_1- Humic acid and B. T_2- Urea

Many researchers have worked on gummosis numerous plants attribute gum formation, to cell wall decomposition. The cell walls which according to them are transformed into gum may be of cells of mature xylem [71, 72], or of cells of specialized parenchymatous groups of cells that differentiate in the cambium, and disintegrate and form the gum and the duct lumen [73].

The gum ducts are usually elliptical, stretched horizontally when filled with gum before its release. Thus, the short dimension (width) during gum filled stage appears lesser as there is increase in circumference. However, after the release of gum (i.e. tapping), the gum duct shrinks horizontally, which may have lead to increase in its width as observed in March. Thus low width in November and December may be an indication of gum filled ducts. In December the width of gum duct was lowest in micro nutrient treatment which means the gum ducts were more stretched and had highest gum content. In a study conducted by Setia et al. [56] round to oval shaped gum-resin ducts were seen distributed irregularly in the phloem of *C. wightii*. They were found to be oriented parallel to the longitudinal axis and anastomose in the tangential planes. Each duct is lined by a single layer tangentially-flattened cell.The previous anatomical studies showed that gum resin present in the resin ducts is distributed irregularly in the phloem, whereas specialized cells present at the border of these ducts synthesizes and secrete the gum resin into the duct lumen [56, 74].

Conclusions

The information obtained in the present study is important to understand the effect of foliar sprays PGR and nutrients on resin yield and will help to formulate recommendations for developing a more sustainable tapping regime. This may the first study that describes the effect of PGR and micronutrients on resin secretory structures in the bark of *C. wightii*. The two year study reveals that foliar application of PGR and nutrients increases gum yield in *C. wightii*. Based on the anatomical changes, there is an indication that foliar application of micronutrients can advance the tapping period from March to December, i.e. three months ahead of the traditional tapping period of *C. wightii* in Chambal ravines of Madhya Pradesh.

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