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Gum tapping technique and anatomical study of ethylene induced gum duct formation in Dhawda (*Anogeissuslatifolia)*

Vidya Bhushan Kuruwanshi, Pratibha Katiyar and Shabnam Khan

Abstract
Gums are important natural biopolymers demand from biological system under stress situation *i.e.* disease injury to bark etc. being used as a principal components in food, pharmaceutical industries and play a key role in social and livelihood of tribal communities. Gums are metabolic by-products of plant tissues either in normal course or often as a result of disease or injury to the bark or wood of certain plants and it can not be re-entered with plant system. Dhawda *(Anogeissuslatifolia)* also known as Gum ghatti is the dried exudates tree belonging to family *Combretaceae*. The commercial tapping of Dhawda is done by blazing, peeling, or by making deep cuts at the base of the bole using an axe. These method soften lead to the death of the tapped trees. On account of crude tapping methods and over exploitation the population of Dhawda trees has markedly declined. The harvesting method currently used is traditional and injurious due to which often obtained inferior quality of products. Hence, the study was undertaken in ICAR Network Project for research work at village Khargadih, district Raipur (Chhattisgarh) to develop the scientific tapping techniquefor sustainable harvesting in major gum producing tree of Chhattisgarh state during year 2015. The various chemical methods are used for tapping purpose. Chemical tapping method using ethephon and IAA injected by battery operated drill machine. However, temperature and relative humidity also play significant role in gum exudation. Theethephon @ 2.34% in 4 ml in two consecutive doses in 45-60 days intervals at high temperature in month of April toJune was found significantly effective for maximum gum production. The process of gummosis related to biotic and abiotic stress of tree plant and plays significant role in production of biopolymers (gum/resin). Therefore, the physiological tool was used to impose artificial stress *via* various concentration of ethephon (slow releasing substance of ethylene) as gum enhancer in stem of dhawda to find out the mechanism of gummosis and compared with mechanical tapping method. The biological (anatomical), studies were done *via* taking the sample of soft (sapwood) after injecting the gum enhancer ethephon at different time intervals. It can observed that the application of ethephon enhance the process of gummosis, due to formation of gum duct. The gum duct formation was observed in histological analysis of bark section within 2-4 hrs of ethephon treatment in dhawda. The histological changes indicated that the gum ducts arises schizogenously by the formation of intercellar spaces among a group of axial parenchyma cells in dhawda.

Keywords: *Anogeissuslatifolia*, Ethephon, IAA, Gum tapping, Gum duct formation

Introduction
Chhattisgarh is a pioneer state of India and has vast variety of minor forest produce to favourable agro climatic conditions resulting in good forest area *i.e.* 43.6 % of total. Gums are primarily categorized as Grade-I of Karaya (*Sterculiaurens*) and Grade-II of Dhawda (*Anogeissuslatifolia*), Babool (*Acacia indica*) and Khair (*Acacia catechu*) in Chhattisgarh state.

Dhawda (*Anogeissuslatifolia)* or Gum ghattior Indian gum is a complex non-starch polysaccharide **(Plate.1 a)**. The tree grows extensively all over the country, more commonly in the dry deciduous forests in Western Ghat and dry plateaus of Vindhyachal, Satoura and Western Ghat range of mountains extending in Madhya Pradesh, Chhattisgarh Bihar and Orissa. Trees have a greyish bark and leaves that turn red in dry season. The exudate tears are normally less than one cm in diameter and often occur in large vermiform masses. The gum has a glassy fracture and the colour of the exudate varies from nearly light brown to dark brown. Generally, color varies in relation to the age of the exudate (Al-Assaf *et al.*, 2008). It has been widely employed in food, pharmaceuticals, paper and other industries due to its excellent emulsification and thickening property (Deshmukh *et al.*, 2012). Time course experiments involving mechanical injury to both young and old stems indicate that gum ducts are also formed in the xylem within 30-40 minutes. These ducts, called as traumatic ducts, are formed as a result of breakdown of xylem cells. A traumatic duct shows an irregular lumen

without any distinct epithelial cells. Histochemical test reveals that the nature of the gum produced in these ducts is similar to that in the normal ducts. (Setia and Shah,1983).

Traditionally trees are tapped by blazing, stripping of the bark or making deep cuts in the base of the tree with axe. Trees are tapped to increase gum yield by making incisions in the bark or treating with stress hormone ethylene or ethylene-releasing compounds such aethephon (2-chloroethylphosphonic acid). The idea to use ethephon as gum inducer came from the thought that if ethylene is supplied artificially to the tree *via* the application of ethephon, the developmental response to stress could be accelerated, and, consequently, more gum exudates could be obtained. Ethephon can mimic the effect of water stress, as it releases the stress hormone ethylene in plant tissues. The gum yield increase with increase in concentration of ethephon. Indole-3-acetic acid (IAA) is the most common, naturally-occurring, plant hormone of the auxin class. The development of induced vertical ducts in response to auxin exogenous application does not initiate immediately after applying this phytohormone but only approximately a month, similar to the time gap observed between the beginning of vascular activity and the formation of duct in natural conditions (Fahn 1979). In addition, auxin promotes the expression of ACC synthase gene which encodes for a key enzyme regulation ethylene production (Chae and Kieber, 1999).

Materials and Methods

An investigation was carried outat village Khargadih, Tilda block of Raipur (Chhattisgarh) during 2015. The experiment was laid out in Randomized Block Design with three replication and six treatment *i.e.*T_1control (distilled water), different Ethephon concentration (T_2 0.78%, T_3 1.56%, T_4 2.34%) and IAA (T_5 400 ppm T_6 800 ppm) for potential gum production in Dhawda *(Anogeissuslatifolia)*.

Gum tapping method

Twoslanted hole of about 5mm diameter with 1" deep is made at one feet above the collar of the tree with the help of battery operated drill machine. After that,4 ml (2 ml each hole) dose of ethephon and IAAgum inducerinjected in the hole with help of syringe and immediately the hole iscovered (patched up) by moistenedclay. It is observed that the tree starts exudating gum tears after 8-10 days of treatment **(Plate.1)**. The exudates gum was picked by hand as large stalactic mass. The quantity of gum exudation was measured by collecting the gum at different time interval in a month and adds them, which was divided by number of treated trees. The yield data obtained was compared to check monthly variation in gum exudation per trees.

Gummosis process in stem

The traumatic duct formation and studied the histological changes during their development tapping or injury. The gum enhancer treated tree bark was cut about 2.5 cm long pieces and removed square wood block about 3 cm^2area with hammer and chisel. The formalin acetic acid alcohol solution (FAA) solution was made of 90 ml of 70% ethyl alcohol, 5 ml glacial acetic acid and 5 ml acetaldehyde for 100ml solution. The treated bark were cut at 2, 4, 6 and 8 hrs time interval and fixed in (FAA) and embedded paraffin wax using conventional methods (Jensen, 1962). The histological/anatomical test of bark sample has been done in College of Forestry, Y.S. Parmar University of Horticulture and Forestry, Solan (Himachal Pradesh), India.

Results and Discussion

Effect of gum inducing chemical ethephon and IAA in quantity of gum exudation (g)

The data pertaining to themonth wise effect of gum inducing chemical ethephon and IAA in quantity of gum exudation (g) of *Anogeissuslatifolia* shown in **Table.1 and Fig.1.**

The significant maximum rate of exudation was achieved in the month of May followed by April, June and March during the year 2015. It might be due to the variation in temperature and relative humidity that was found to be significantly effective on tapping and consequently gum yield. Giri*et al.* (2008) reported that the trees of *Anogeissuslatifolia* for tapping gums naturally ooze out mostly in summers. However, to increase the yield of gum sometimes people make incision in the tree bark. It is mainly harvested in March to mid-June. Similar findings also reported by Kramer and Kozlowski (1979).The quantity of gum was found significantly maximum in T_4(ethephon @2.34 %) (662.25g) followed by T_3 (ethephon @1.56 %) (394.16g) and T_2 (ethephon @0.78 %) (197.66g). The minimum exudation was observed in T_6 (IAA@800 ppm) (42.87 g). However in control T_1 (distilled water) were no exudation at all. This indicated that the gradual increases in ethephon concentration also increase the process of gummosis and ultimately increased the quantity of exudation in Dhawda. Bhatt (1987) reported that the gummosis is enhanced by ethephon application and 466 fold increases in gum yield was recorded in plants treated with 1600mg of active ethephon substance during April- May when plants becomes leafless.

Histological study of *Anogeissuslatifolia* bark for gummosis process

The observation was undertaken to elucidate the gum duct formation and histological changes in *Anogeissuslatifolia* by gum tapping associated with gummosis process is presented in **Plate.2** and summerized below:

The control bark sample of *Anogeissuslatifolia* showed the presence of gum cavities in the bark presumably as a response to wounding. However, the treated samples show the formation of tangential bands of gum cavities in the axial parenchyma of the sapwood. The elongated cavities are oriented parallel to the longitudinal axis of the tree trunk and anastomose tangentially. Multiseriate rays are observed admist the anastomosing cavity system, although no cavities are recorded in the multiserate rays. The cells suspended in close vicinity to the multiserate rays indicate that some of the peripheral cells become detached and move into the lumen of the gum cavity. In the sapwood, a gum cavity arises schizogenously by the formation of intercellar spaces among a group of axial parenchyma cells. However, the occurance of isolated cells suspended within the gummy material of the cavity and their enlargement emphasize the lysigenous mode of gum cavity. Xylem vessels in the vicinity of the traumatic cavities are usually plugged by a gummy material.

Abeles(1973) reported that *Anogeissuslatifolia* has no natural pre-formed gum producing tissue system in the wood but the gum cavities are induced schizo-lysigenously in the axial parenchyma of sapwood upon ethephon treatment. Ethylene may cause gum pocket or cavity or cyst formation in plants. The loss of middle-lamellar cohesiveness and the breakdown of the primary cell walls in the phloem tissue in and around gum pockets. Ghosh and Purkayastha (1959) also observed that the induced exudation is associated with the development of traumatic gum cavities in the secondary xylem. The traumatic development of gum ducts or cavities in the

secondary xylem due to mechanical injury or ethephon treatment is also reported in *Anogeissuslatifolia.*The cell walls are transformed into gum may be of cells of mature xylem orof cells in specializedparenchyma groups which differentiate in the cambium and later disintegrate and form the gum and duct lumen (Vander Molen *et al.*, 1977).

Dhawda *(Anogeissuslatifolia)* tree

1. Making hole with battery operated drill

2. Injected gum inducer ethephon machine

3. Covering the hole by moistened clay

4. Exudated gum tears

Plate 1: Chemical gum tapping method in Dhawda *(Anogeissuslatifolia)* tree

Table 1: Effect of gum inducing chemical ethephon and IAA in quantity of gum exudation (g) *Anogeissuslatifolia* during year 2015

Treatments		March	April	May	June	Total
	Temp.(^{0}C)	**34.45**	**38.1**	**42.15**	**35.88**	**(g/tree)**
	RH (%)	**48.25**	**38.4**	**39.75**	**56.12**	
T_1		0	0	0	0	0
T_2		28.74	44.25	70.79	53.88	197.66
T_3		67.96	121.95	129.32	74.93	394.16
T_4		135.54	186.42	202.7	137.59	662.25
T_5		13.02	46.96	41.24	30.88	132.1
T_6		6.6	10.11	19.42	6.74	42.87
CD at 0.05% level						32.05
SEm±						25.11
CV (%)						11.56

Note: T_1 control (distilled water), Ethephon concentration (T_2 0.78%, T_3 1.56%, T_4 2.34%) and IAA (T_5 400ppm, T_6 800ppm).

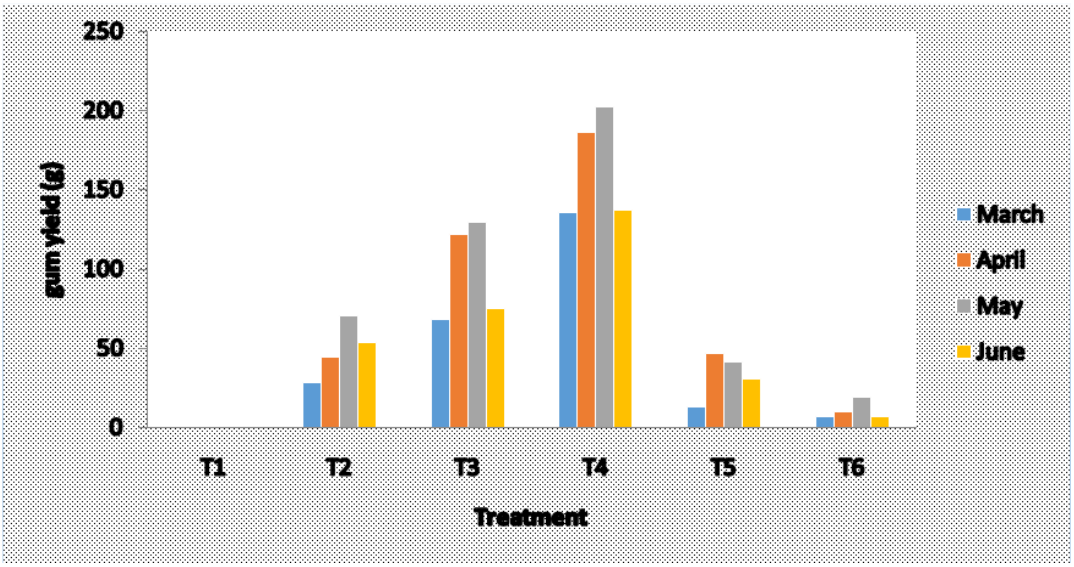
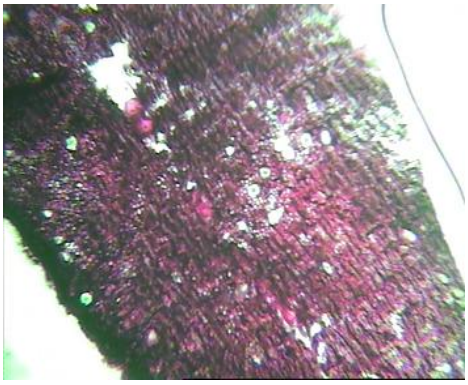
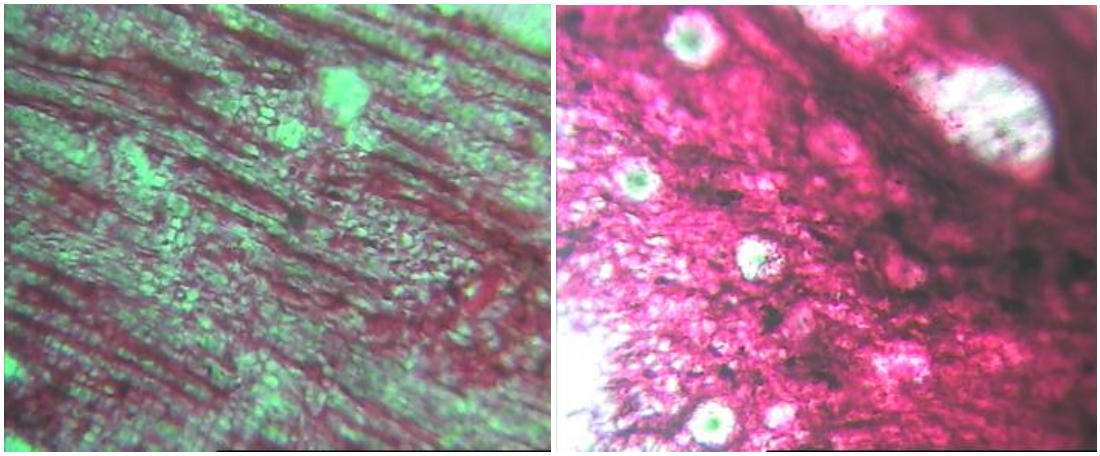


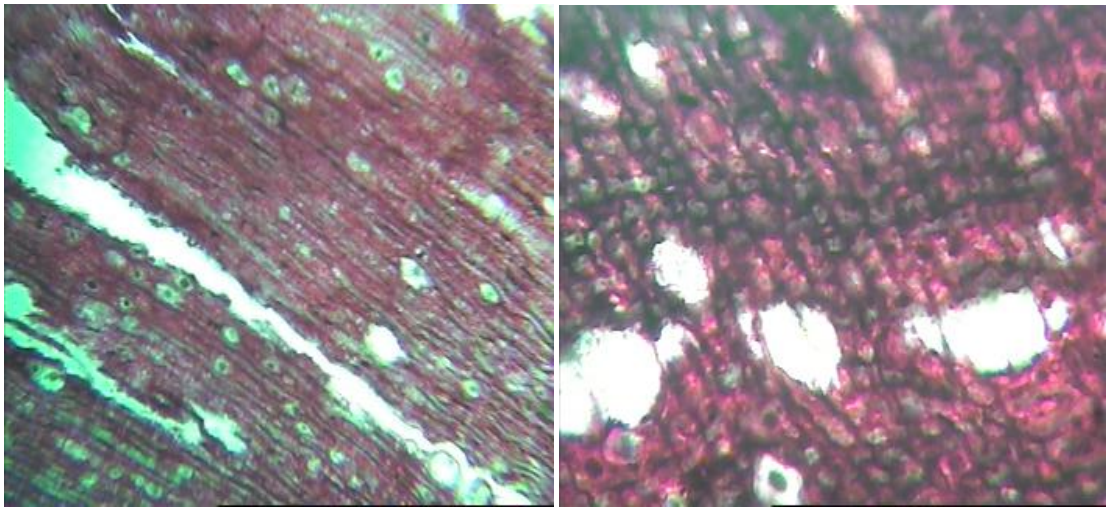
Fig 1: Monthwise quantity of gum exudation (g)*Anogeissuslatifolia* in year 2015



Control



2 hrs



6 hrs

Plate 2: Histological study of *Anogeissuslatifolia*bark for gummosis

Conclusion

The future of natural gum industry is uncertain and therefore, a thorough economic study of the national and international trade is necessary. Synthetic products are preferred by the industry because of the uncertain supply and cost of natural gums. However, unstable oil prices, decreased production and high costs of the synthetic material create a promising future for natural gums and resins. The tapping methods used are brutal and injurious to the plants, often leading to their death. The technology available is old and the innovations are essential for sustainable yield and quality control. A concerted effort by researches and agencies such as research institution, Universities and non-governmental agencies is urgently needed to improve all aspects of the industry such as tapping, collection, processing, grading, classification and marketing. R and D are completely lacking in the area of utilization of natural gums and resins. The industry completely depends on traditional and certain ad hoc investigations by individuals. Research into genetic improvement and selection of species for production of gums and resins should be initiated which may lead to establishment of plantation of these species. Gum and resin industry can provide employment and a steady additional income to rural people and thereby stop their migration into the towns and cities.

References

1. Abeles FB. Ethylene in Plant Biology. Academic Press, New York. 1973, 134
2. Al-Assaf S, Amar V, Phillips GO. Characterization of gum ghatti and comparison with gum arabic. In: Gum and Stabilizers for the Food Industries 14, Eds Williams, P.A. and Phillips, G.O. Royal Society of Chemistry, 2008; 316:280-290.
3. Bhatt JR. Gum tapping in *Anogeissuslatifolia* (Combrataceae) using ethephon. Current Science. 1987; 56(18):71
4. Chae HS, Kieber JJ. Trends Plant Science. 1999; 10:291.
5. Deshmukh, Anand S, Setty, Mallikarjuna C, Badiger, Arvind M, Muralikrishna KS. Gum ghatti: A promising polysaccharide for pharmaceutical applications. Carbohydrate Polymer. 2012; 87:980-986.
6. Fahn A. Secretory Tissue in Plants. Academic Press, London, 1979.
7. Ghosh SS, Purkayastha SK. An anatomical study of wood and bark of *Anogeissuslatifolia* tree with special reference to gum exudation. Indian Forester. 1959; 88:92-99.
8. Giri SK, Prasad N, Pandey SK, Prasad M, Baboo B. Natural Resins and Gums of Commercial Importance at a Glance.Indian institute of Natural Resins and Gums, Ranchi, Jharkhand, 2008, 38.
9. Jensen WA. Botanical Histochemistry. W.H. Freeman and Company, San Francisco, London, 1962.
10. Setia RC, Shah JJ. Effect of Morphactin, IAA and Kinetin on gum canals in *Sterculiaurens* Roxb. Indian Journal of Experimental Biology. 1977; 15:297-301.
11. Shah JJ, Setia RC. Histological and histochemical changes during the development of gum canals in *Sterculiaurens.*Phytomorphology, 1983; 26:151.
12. Simon S, Petrasek J. Why plants need more than one type of auxin. *Plant Science.* 2011; 180(3):454-460.
13. Vander Molen GE, Beckman CH, Rodehorst E. Vascular gelation: A general response phenomenon following infection. - Physiol. Plant Pathol. 1977; 11:95-100.