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Anatomical study of ethylene induced gum duct formation and scientific gum tapping technique in gum Karaya (*Sterculiaurens* Roxb.)

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Abstract

Gums and resins occupy a prime place among Non-Wood Forest Produce (NWFP/NTFP) and are known to mankind since time immemorial. 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 cannot be re- enter with plant system. Gum Karaya (*Sterculia urens* Roxb.) is a dry deciduous tree belonging to the family *Sterculiaceae* distributed throughout India and Chhattisgarh. The impact of gum enhance ethephon was found significantly superior, regarding the production of biopolymers. 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 be 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 hrs of ethephon treatment in gum Karaya. The histological changes indicated that the gum ducts lysigenously are present in the pith and cortex of the young stem of *Sterculia urens* but absent in the xylem. The commercial tapping of Karaya is done by blazing, peeling, or by making deep cuts at the base of the bole using an axe or a cycle. These methods often lead to the death of the tapped trees. On account of crude tapping methods and over exploitation the population of karaya trees has markedly declined. The harvesting method currently used are traditional and injurious due to which often obtained inferior quality of products. Hence, the study was undertaken in ICAR Network Project to develop the scientific tapping technique for sustainable harvesting in major gum producing tree of Chhattisgarh state to enhance the livelihood of the rural areas as well as to protect the plant and generate the revenue of the government. The 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. The ethephon @ 3.9% in 4 ml in two consecutive doses in 45-60 days intervals at high temperature in month of April to June was found significantly effective for maximum gum production during both the year 2015 and 2016. The physiochemical properties of exudated gum were investigated and gum was found to be mild acidic, least soluble in cold water but absorb water and swell, soluble in hot water but insoluble in organic solvent.

Keywords: *Sterculia urens*, Ethephon, IAA, gum tapping, gum yield, gum duct formation

Introduction

Gum trees are economically important and found in tropical moist and dry deciduous forests, produce a significant quantity of gum, which are widely used as industrial, food and medicinal purposes in India (Bhattacharya, 2012) [6]. 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. The gum exudes from trees and shrubs in tear-like, striated nodules or amorphous lumps. It dries in contact with air and sunlight and forms hard, glass like lumps. Gum production increases at high temperature and limited moisture. (Sao *et al.* 2012) [16] India is a rich center of plant biodiversity having more than 15,000 plant species including about 120 gum yielding plants. India produces annually about 2,81,000 tons of gum (Anonymous, 2013) [2].

Karaya gum is the dried exudate from the tree *Sterculia urens*. It is also known as Thapsi Gum, Gum Kadaya, Kullo, Karei, Kandol, Katilo, Gulu, Katera, Katiera in the trade (Plate.1a). The gum ducts normally occur in the pith and cortex of young stem of *Sterculia urens*. Time course experiments involving mechanical injury to both young and old stems indicate that gum

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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 *et.al.*1983) [18]. Natural gums are present either in the intercellular space (ducts or cavities) of the plant parts or as exudate produced due to injury. The ducts or cavities formed due to injury are called traumatic ducts/cavities. The development of the duct is schizogenous (separation of the duct initials by dissolution of middle lamella), schizolysigenous (separation of the initials followed by lysis of epithelial cells) and lysisigenous (separation of the duct initials followed by lysis of epithelial cells) and lysisigenous (death of the initials). (Nair *et al.*, 1995) [14].

The commercial tapping of Gum karaya is done by blazing, peeling, or by making deep cuts at the base of the bole using an axe or a sickle (Plate.1b).

These methods often lead to the death of the tapped trees. Gum karaya is vital for tribal economy and its trade value is substantial, there is a pressing need to develop a scientific and sustainable tapping method to increase the yield and ensure the survival of the tapped trees. A simple and safe technique of tapping with substantial increase in the yield is developed using ethephon to enhance gum yield and wound healing (Gupta *et al.* 2012) [8]. 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 as ethephon (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. Etkephon 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, 3-IAA) is the most common, naturally-occurring, plant hormone of the auxin class. It is the best known of the auxins, and has been the subject of extensive studies by plant physiologists (Siman and Petrsek, 2011) [22]. An Application of Indole 3-acetic acid (IAA) increase the number of gum ducts in *Sterculia urens* (Setia and Shah, 1977a) [18] However, gum tapping using scientific methods of gum exudation not only increase the life span of the tree but also yields good quality gum of high International value (Gupta *et al.*, 2012) [8].



Plate 1: (a) Gum Karaya tree



Plate 1: (b) Mechanical Tapping method in Gum karaya tree

Materials and Methods

An investigation was carried out at former Central Government Forest Division, Biladi at Tilda block of Raipur (Chhattisgarh) during year 2015 and 2016. The experiment was laid out in Randomized Block Design with three replication and six treatment *i.e.* T₁ control (distilled water), different Etkephon concentration (T₂ 2.34%, T₃ 3.12%, T₄ 3.9%) and IAA (T₅ 400ppm, T₆ 800ppm) for potential gum production in *Sterculia urens* Roxb.

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 cm² area 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) [12]. 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.

Gum tapping method

The chemical gum tapping of selected trees was initiated using different doses of gum enhancer ethephon (2-chloroethyl-phosphonic acid) (trade name Ethrel) having 39%, Indole acetic acid (IAA @400 and 800ppm) in the tree trunk by battery operated drill machine to induce gummosis. The whole treatments were made through a syringe of 10 ml volume. The 4ml gum enhancer was injected twice during the whole period of tapping. First treatment injected in March and second in the month of May.

Two slanted hole of about 6 mm diameter with 1" deep was made on at least one meter tree girth and confined 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 IAA gum enhancer were applied/injected in the hole with the help of syringe and immediately the hole was covered (patched up) by moistened clay. It is observed that the tree starts exuding gum tears after 7-10 days of treatment (Plate.1c).

The exudates gum was picked by hand as large stalactic mass. The distilled water used as control was injected 4 ml (2 ml each hole) in the hole of trees. The rate of gum exudation was measured by application of chemical treatment and collecting the gum at different time intervals in a month. It was collected after one week of the commencement of treatment. It was calculated by weighing the exudated gum and divided it by time. The quantity of gum exudation was measured by

collecting the gum at different time interval in a month and adds them. The yield data per year obtained was compared to

check variation in gum exudates per month on the basis of weight.



Plate 1: (c) Chemical tapping method in gum karaya tree

Physiochemical analysis of exudate gum

The physiochemical analysis of gum samples study was done in Department of Plant Physiology, Agricultural Biochemistry, Medicinal and Aromatic plants, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.) laboratory. Each analysis was repeated three times and values reported in respect of the gum samples are actually the average of three replications.

Determination of pH

The sample powder was thoroughly mixed and 1 g and was dissolved in 100 ml of hot distilled water. The mixture was allowed to stand for 5 min at room temperature before the pH and temperature was recorded using a pre-calibrated pH meter. (Ameh, 2012) [1].

Determination of Solubility

The solubility of the gum was determined in cold and hot distilled water, acetone, and ethanol. 1.0 g sample of the gum was added to 50 mL of each of the above mentioned solvents and left overnight. 25 mL of the clear supernatants were taken in small preweighted evaporating dishes and heated to dryness over a digital thermostatic water bath. The weights of

theresidue with reference to the volume of the solutions were determined using a digital top loading balance and expressed as the percentage solubility of the gums in the solvents. (Eddy *et al.* 2012) [71].

Determination of Protein

Crude protein content of the gum was determined using the Kjeldahl method with the nitrogen content being multiplied by a factor of 6.25. (Rodriguez *et al.* 2004) [15].

Results and Discussion

Gummosis process in stem

The observation was undertaken to elucidate the gum duct formation and histological changes in *Sterculia urens* Roxb by gum tapping associated with gummosis process is presented in Plate.2.

In *Sterculia urens* Roxb, control bark sample show the little presence of gum cavities. The stem has normal gum ducts only in the pith and cortex. Gum ducts or cavities are normally absent in the wood. Wood is characterized by diffuse or occasionally banded parenchyma, broad multiseriate rays and thick walled fibers. Administration of ethephon into the stem induced extensive development of

gum cavities in the secondary xylem. The cavities are developed from the axial parenchyma cells formed after ethephon treatment. Upon ethephon treatment, the ray cells remain intact, but only axial parenchyma cells are formed from the fusiform initials. The cambium soon renewed its normal function and consequently a band of traumatic tissue consisting of only axial and ray parenchyma cells are formed in the outer sapwood. The axial parenchyma cells undergo active transverse divisions and the derivatives enlarge to form vertical files of isodiametric cells, the cavity initials. They are mostly thin walled, and have dense cytoplasm and large nuclei. The cavity initially develops lysigenously from a group of such cells.

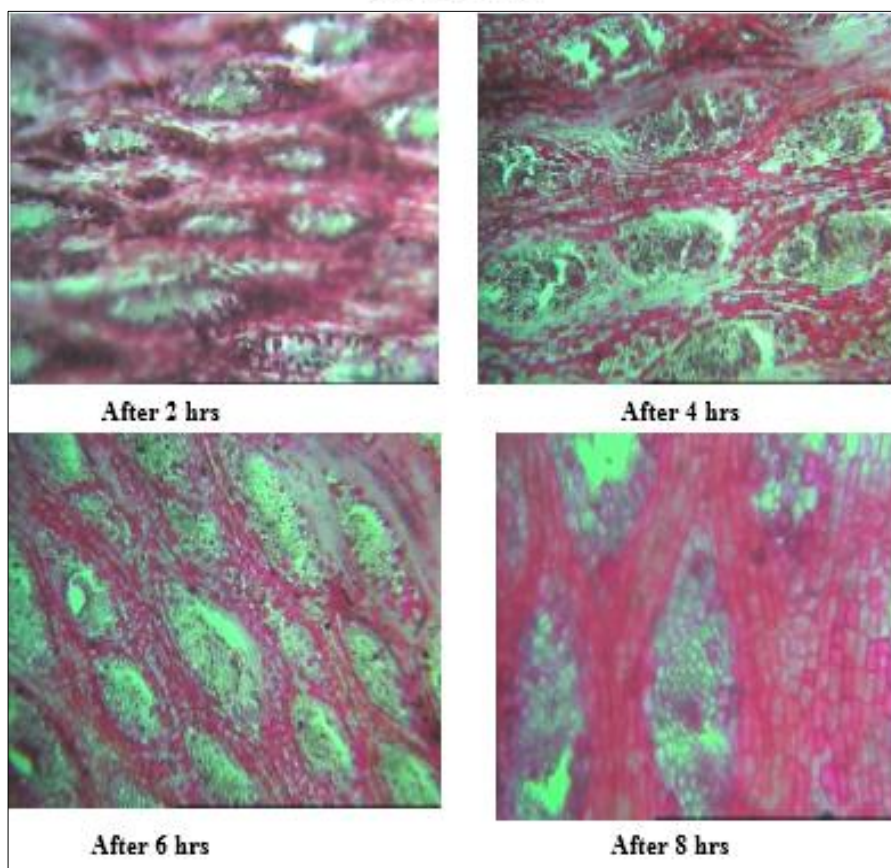
The lysis is triggered by the disintegration of the vertical file of cells proceeded by the darkening of cytoplasm and disappearance of nuclei. Lysis of more cells progresses in vertical and tangential directions forming a cavity. But a definite epithelium is not formed around the cavities. Tangential widening of a cavity is limited by multiseriate rays

which remain mostly intact. Almost all axial parenchyma cells undergo lysis forming a system of tangentially anastomosing cavities around the intact islands of multiseriate rays. The cavity is filled with disintegrating cells and gummy substances. Nevertheless, at places of extensive cavity formation, some multiseriate rays also disintegrate. But always ray cells are the last to be affected. In radial longitudinal sections, the cavities appear as vertically elongated system interrupted by multiseriate rays.

Shah and Setia (1976) ^[20] observed that the lysigenously formed gum ducts are present in the pith and cortex of the young stem of *Sterculia urens* but absent in the xylem. The gum cavities are induced upon ethephon treatment. Vander Molen *et al.* (1977) ^[24] also reported that the cell walls are transformed into gum may be cells of mature xylem or of cells in specialized parenchyma groups which differentiate in the cambium and later disintegrate and form the gum and duct lumen. Similar findings were also reported by Wilde and Edgerton (1975) ^[25] and Setia (1984) ^[19].



Control bark



After 2 hrs

After 4 hrs

After 6 hrs

After 8 hrs

Plate 2: Anatomical section cutting of *Sterculiaurens* Roxb bark (Gummosis process)

Rate of gum exudation (g) in *Sterculia urens* Roxb

The data pertaining to the rate of gum exudation (g) in *Sterculia urens* Roxb during 2015 and 2016 is presented in Table.1 and Fig.1.

The significant variation in rate of gum exudation was observed during March to June. The maximum rate of gum exudation was obtained in month of May in both the experimental years 2015 and 2016 (440.33 and 530.1 g, respectively), followed by April and March. The minimum rate of gum exudation was observed in month of June in both the experimental years.

The rate of gum exudation in *Sterculia urens* Roxb tree was significantly highest in the month of May and followed by April as compared to March and June during the year 2015 and 2016. It might be due to temperature and relative humidity played a significant role for the process of gummosis and gum exudation. Gum yield was positively related with mean temperature as the temperature increased and relative humidity decreased there was increased in gum yield. The gum yield was significantly higher when tapping was carried out during the sharp decrease of the relative humidity (Harmand *et al.*, 2012) [9]. Similar result were also reported by (Bhatt and Mohan Ram, 1990) [4] indicated that 0.8 to 0.9 kg of good quality gum can be obtained per tree by introducing 4 ml of ethephon containing 960 mg of the active substance through a hole in the sapwood in April/May. It is supported by Harsh *et al.* (2013) [10].

Amongst the treatments, ethephon @3.9% (T₄) was found significantly superior (717.05 g, 777.45 g) followed by @3.12% ethephon (T₃) and @2.34% ethephon (T₂). The minimum rate of exudation was observed in @IAA 800 ppm (T₆) (21.43 g, 22.789 g) in both the experimental years. However, control (T₁) where treated with distilled water

produced negligible amount of gum. Gupta *et al.* (2012) [8] reported that the different doses of ethephon injected in the tree trunk of *Sterculia urens* by drilling method and 4 ml ethephon, if injected twice, first in mid-March and second in first week of May per tree, further enhances the gum exudation without any apparent ill effect on the health of the tree.

The pooled analysis of effects of chemical tapping in quantity of gum exudation in *Sterculia urens* Roxb is depicted in Table.2.

The quantity of gum was significantly highest in T₄ @3.9% ethephon (239.02 g and 259.15 g) followed by T₄ @3.12% ethephon (137.51 g and 172.36 g) and T₂ @2.34% ethephon (66.80 g and 87.67 g), whereas in T₅ @400 ppm IAA (15.84 g and 17.30 g) and minimum exudation was obtained in T₆ @800ppm IAA was obtained maximum gum exudation as compared to treated with @800ppm (7.14 g and 7.59 g) concentration of IAA. However, in T₁ (control, distilled water 4ml) no exudation was observed in gum karaya trees. The pooled average data revealed that the effect of chemical tapping in quantity of gum exudation was significantly obtained highest when treated with @3.9% (249.08 g) concentration of ethephon followed by concentration of 3.12% (154.93 g) and @2.34% (77.23 g). The gum enhancer ethephon treated with concentration of @3.9% and @3.12% produced more gum over control. It may be due to the treatment with ethephon has induced formation of gum ducts and cavities in the stem of *Sterculia urens* (Babu and Menon 1989) [3]. An application of IAA increased number of gum ducts in *Sterculia urens* (Setia and Shah, 1977a) [18]. Sharma and Prasad (2013) [21] also supported that the ethephon application leads sapwood and these results in the clogging of vessels of secondary xylem with gummy material.

Table 1: Effect of gum inducing chemical ethephon and IAA on rate of gum exudation (g) in Gum karaya (*Sterculia urens* Roxb) during year 2015 and 2016

Treatments	Temp.(°C) RH (%)	2015				Total	2016				Total
		March	April	May	June		March	April	May	June	
		34.45	38.1	42.15	35.88		35.51	38.8	42.82	37.91	
T ₁ Control (Distilled water)		0	0	0	0	0	0	0	0	0	
T ₂ (2.34% Ethephon)		31.29	41.23	64.28	63.59	200.39	41.48	68.57	95.5	57.47	263.02
T ₃ (3.12% Ethepon)		82.46	125.88	123.87	80.33	412.54	91.29	161.26	175.18	89.9	517.63
T ₄ (3.9% Ethephon)		155.91	198.94	221.89	140.31	717.05	174.09	221.82	229.24	152.3	777.45
T ₅ (400 ppm IAA)		6.21	11.15	23.27	6.89	47.52	8.67	10.4	24.33	8.44	51.81
T ₆ (800 ppm IAA)		3.81	5.81	7.02	4.79	21.43	4.97	6.42	5.85	5.54	22.78
Total		279.68	383.01	440.33	295.8	1398.93	320.5	468.47	530.1	313.65	1632.06

Table 2: Pooled average data of effect of gum inducing chemical ethephon and IAA on quantity of gum exudation (g) in Gum karaya (*Sterculia urens* Roxb)

Treatment	2015	2016	Pooled
T ₁ Control (Distilled water)	0	0	0
T ₂ (2.34% Ethephon)	66.80	87.67	77.23
T ₃ (3.12% Ethepon)	137.51	172.36	154.93
T ₄ (3.9% Ethephon)	239.02	259.15	249.08
T ₅ (400 ppm IAA)	15.84	17.30	16.57
T ₆ (800 ppm IAA)	7.14	7.59	7.36
Factors	C.D.	SE(d)	SE(m)
Year	N/A	6.039	4.27
Treatment	21.832	10.46	7.396
Interaction year x treatment	N/A	14.792	10.46

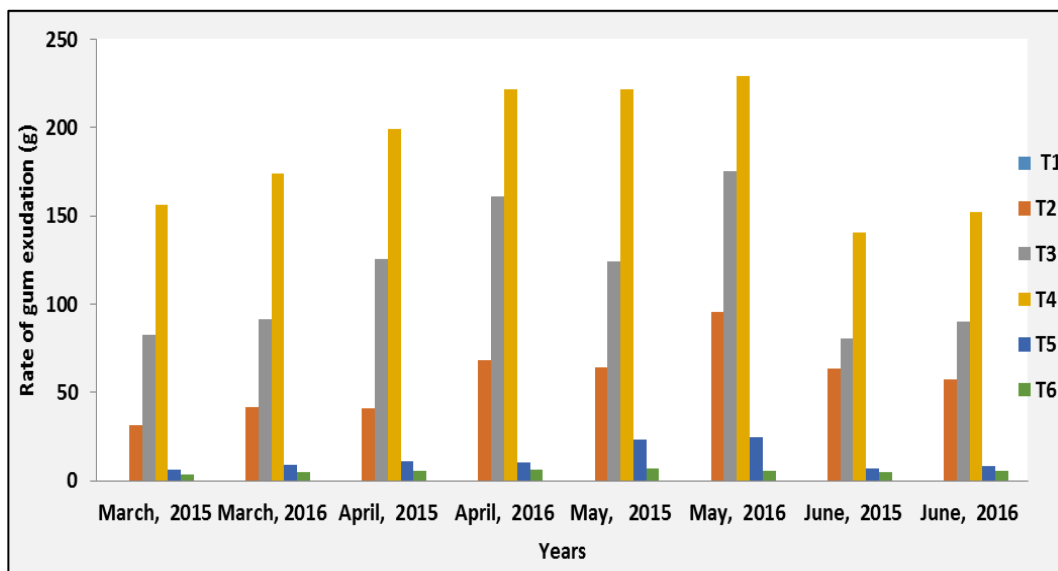


Fig 1: Effect of gum inducing chemical ethephon and IAA on rate of gum exudation (g) in Gum Karaya (*Sterculia urens* Roxb) during year 2015 and 2016

Physiochemical analysis exudate gum of *Sterculia urens* Roxb.

The pH, solubility and protein content were evaluated and results obtained are summarized in Table.3.

The measured pH of gum is 4.8, indicating that the gum is mild acetic. This result was agreed with (Taher, 1998)^[23].

The solubility of karaya gum in cold water and hot water 10.66% and 31.34% respectively, indicating that the solubility of the gum is temperature dependent. The karaya gum least soluble in cold water. The gum particle do not dissolve but absorb water and swell extensively to more than 60 times the original volume, producing a viscous colloidal solution. The swelling behavior of gum karaya is caused by the presence of acetyl groups in its structure (Le Cerf *et al.*, 1990)^[13]. Since solubility is expected to increase with increase in temperature, the solubility of the gum in hot water is higher than the corresponding solubility in cold water. On the other hand, the samples were not soluble in acetone but sparingly soluble in ethanol. These indicate that the studied gum is ionic in character unlike the covalent counterpart that is soluble in organic solvents (Sarah *et al.*, 1998)^[17].

The sparingly solubility of the gum observed in ethanol due to the fact is that it can ionize to produce hydroxyl ion (OH⁻) and the value of its dielectric constant is higher than those of acetone. Consequently ethanol has some polar character over acetone which is characterized by low value of dielectric constant. The solubility was also closer to the result obtained by (Yadav *et al.*, 2015)^[27]. The protein content of karaya gum was found 1%. The most of the gum had a very low protein content and was referred to as an arabinogalactan (Hussein *et al.*, 2006)^[11].

Table 3: Effect of gum inducing chemical ethephon and IAA in Physio-chemical properties of *Sterculia urens* Roxb.

Parameter <i>Sterculia urens</i> Roxb. gum	
pH	4.8
Solubility (2% w/v of solution)	
Cold water	10.66%
Hot water	31.34%
Acetone	0
Ethanol	0.40%
Protein	1%

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. In spite of the competition from synthetic products, natural gum and resins are preferred in certain industries as they are superior. 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.

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