ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

Determination of Nicotine content in Virginia Tobacco of Ethiopia

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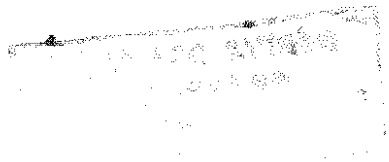
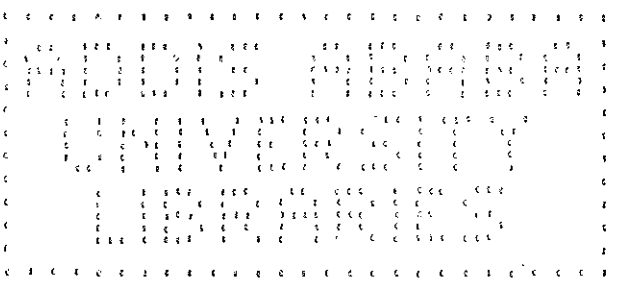


CONTENT

Abstract

Tobacco is a complex chemical matrix, and analysis of one of its components, nicotine, is from the fact that varities are inherently different in their nicotine contents. Varities retain their relative rankings in nicotine content despite differences in weather conditions and locations. In this study, nicotine content of virginia tobacco of Ethiopia has been investigated. 24 different samples were collected from Bilate and Shoa- Robit of Southern and Central parts of the country. Technique that has been applied to the analysis of nicotine in Virginia tobacco was a typical single-beam spectroscopy. Auto data processing by Spectronic Genesys-2 was used to determine the actual nicotine content in Virginia tobacco.



1. INTRODUCTION

Nicotine is named after the tobacco plant Nicotiana tabacum which in turn is named after Jean Nicot, who sent tobacco seeds from Portugal to Paris in 1550 and promoted its medical use. It was first isolated in 1828 by German chemists Posselt and Reimann, its molecular formula (3-(1-methyl-2- Pyrrolidinyl) Pyridine) was established in 1843 by Adolf Pinner and it was first synthesized in 1904. [1]

Presently The Peoples Republic of China, The United States , Brazil and Zimbabwe are the world's largest producers of tobacco. The United States and Brazil are the world's largest exporters of tobacco used primarily in the production of cigarettes. [2]

In evaluating desirability of tobacco on the base of chemical constituents the balance of the constituents may be more revealing than the actual quantity found. For example, tobacco with high ratios of nitrogen to nicotine tend to be light bodied and those with low ratios tend to be heavy bodied, and those with extremely low ratios tend to show paleness of color, slickness of texture and a general lack of desirable character as well as general lack of aroma.

Since nicotine – nitrogen is one of the larger sources of soluble nitrogen in tobacco , the nitrogen – nicotine ratio is closely correleted with the percentage of the total nitrogen in the soluble form . Total nitrogen in the soluble form has been related to subjective estimates of the ability of tobacco to show favourable response to natural aging processes . Tobaccos that have higher nitrogen – nicotine ratio values (above 1.0) are sometimes referred to as ' unbalanced ' . This implies that tobaccos on the other end of the scale with very low nitrogen – nicotine ratio values (below 0.5) should be considered 'unbalanced ', but this is not necessarily true .

These tobaccos frequently may be considered undesirable , but usually because they are too heavy in the body and have associated with this body a high level of nicotine and low level of reducing sugar Low nitrogen – nicotine (0.6-0.7) would be very desirable in medium to light bodied , mature tobaccos possessing a satisfactory sugar to nitrogen balance .

Soluble carbohydrates in cured tobacco are generally considered to have a positive effect on tobacco quality ; whereas , structural carbohydrates have a negative effect . The sugar content of tobacco can be correleted with quality within the range of 12 to 25 percent reducing sugars . Tobacco with very high level of sugar has a smooth texture , dense leaf structure , poor fire holding capacity , and a poor aroma ; which results in an impaired taste quality ; however , if the sugar – nitrogen balance can be maintained , tobacco with the higher sugar concentrations are more desirable . Generally though , if the nitrogenous constituents are high the sugar constituents are low .

The well drained sandy loam and loamy sand soils that are slightly acid and which have a low nitrogen retention capacity, along with the normal climate are favourable for the production of tobacco with desirable flavour and from these conditions help produce leaf with a desirable balance of nicotine and sugars and favourable smoke properties [3]

1.1 Tobacco Species and Composition

Tobacco is a plant, usually grown as an annual, belonging to the Nicotiana genus of the Solanaceae family. Over sixty recognized nicotiana species exist , divided into the subgenera Tabacum, Rustica and Petuniodes. Of these species , however, only two – Nicotiana Tabacum and Nicotiana Rustica are commonly used for consumption. Tobacco is usually a long - day plant, with a maximum height of 25m , though some types, e.g. Oriental, are much smaller. The length and shape of the leaf also depends on the type. Up to 25 leaves may be produced on alternate sides of the stem, until harvest. The vast majority of the world's tobaccos and virtually all which enter world trade are Nicotiana Tabacum. They are classified as

a) *Oriental Tobacco* –mainly grown in the countries of the eastern Mediterraneas (or Orient), hence the name. Relatively small leaves, aromatic qualities. The nicotine content is quite low, being in general below 2% and averaging around 1% or less.

b) *Burley Tobacco* – light to light brown in color with a special aroma. Burley leaf is characterized by high ash content, high nicotine, a low to negligible sugar content and hence a very low proportion of sugar to

nitrogen. . Crops in the field are characterized by a light green, partially chlorotic appearance

c) *Virginia Tobacco*- collective name for certain sorts of tobacco, originating in what is now the states of Virginia[4]. These tobaccos can, depending on the intended use, be Flue- cured, Fire- cured , or Air- cured . Fluc- cured Virginia , grown throughout the world is renowned for its color and pleasant aroma.

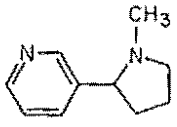
Tobacco is composed of:

1. Inorganic components such as H_2O, K, Na, Ca, Mg, Fe, Trace elements, N,P, S and Si.
2. Organic components such as Carbohydrates, sugar, starch, dextrins pectins, cellulose, lignin and nitrogenous bodies such as aminoacids , purines, bases, proteins and alkaloids that are determined as "***Nicotine***".
3. Organic Acids : phenol derivatives (polyphenols, pigments, resins , essential tobacco oils and paraffins

1.2. Nicotine

Nicotine is an organic compound, an alkaloid found naturally in the night shade family of plants, such as tobacco and tomatoes. It constitutes 0.3 to 5 % of the tobacco plant by dry weight , with biosynthesis taking place in the roots, and accumulates in the leaves. It is a potent nerve poison and is included in many insecticides . In lower concentrations, the substance is a stimulant and is one of the main factors leading to the pleasure and habit - forming qualities of tobacco smoking , Nicotine has limited Carcinogenic effects, inhibiting the body's. ability to destroy potentially cancerous cells; however, nicotine does not promote the development of cancer in healthy cells, In addition to the tobacco plant, nicotine is also found in lower quantities in other members of the Solanaceae (night shade) family, which includes tomato, potato, eggplant (aubergine), and green pepper. Nicotine alkaloids are also found in the leaves of the coca plant. [5] Some characteristic features of nicotine is indicated in Table-1.

Table –1 Typical features of nicotine in their standard state (at 25 ^{0}C, 100kpa)

Chemical name	(S)-3- (1-methyl pyrrolidin-2-yl) pyridine
Chemical formula	$C_{10}H_{14}N_2$
Molecular mass	162.23g/mol.
Density	1.01 g/ml
Melting point	-79^0 C
Boiling point	247^{0}C
Structural formula	

1.2.1 Characteristics of Nicotine

Nicotine is synthesized in the roots of the tobacco plant. The starting materials for its production are relatively simple organic materials photosynthesized in the leaves plus simple form of nitrogen absorbed from the soil. Once formed, nicotine is transported to the leaves and stems where it accumulates. Therefore, a great many climatological and cultural factors; particularly those which would result in a disproportion between the relative amounts of synthesizing roots and storage leaves and the length of time the leaves are in the field , would affect the nicotine content of the leaves [6]

Listed below are some of the factors which will influence the nicotine content of Flue –cured Virginia Tobacco:

* Varities are inherentely different in their nicotine contents. Varities retain their relative rankings in nicotine content despite differences in weather conditions and locations.
* Nicotine accumulation is directly related to the level of nitrogen fertility . Nitrogen is vital for nicotine synthesis and nitrogen delays maturity which increases the opportunity for nicotine accumulation.
* Root damages caused by excess water , high nematode infestation or other root diseases will reduce nicotine content .

* Plant population influences nicotine content . As plants are spaced closer together with increased root competition the nicotine is lowered.
* Available solid moisture plays a major role in regulating the growth and nicotine content of flue- cured tobacco. Low moisture levels lead to restrict leaf area and slow above ground growth rate, both of which will increase nicotine content . On the other hand , high moisture levels tend to increase the rate of . growth and increase the leaf size and possibly lower the available nitrogen level by leaching , all of which will lower nicotine content. Drowned tobacco has a reduced root system which lowers nicotine content .
* Overmaturity results in a slight increase in the nicotine content .
* There is some indication that extending the yellowing period during the curing process will slightly increase the nicotine content
* Stalk position influences nicotine content . The nicotine increase from the primings (lower) to the Tips (upper) stalk positions . (Table-2)

Table –2 Stalk position of Tobacco leaf

Grade	Stalk positions	Nicotine (%)
Primings	1 (lower)	2.12
Lugs	2	2.49
Cutters	3	2.59
Leaf	4	3.17
Tips	5 (upper)	3.27

In general in going from the bottom to the top of the plant, the total nitrogen , alpha amine nitrogen , and petroleum ether extractables increase, the non-volatile acids decrease and the reducing sugars reach a maximum near the middle of the plant. (Fig.1.) [7]

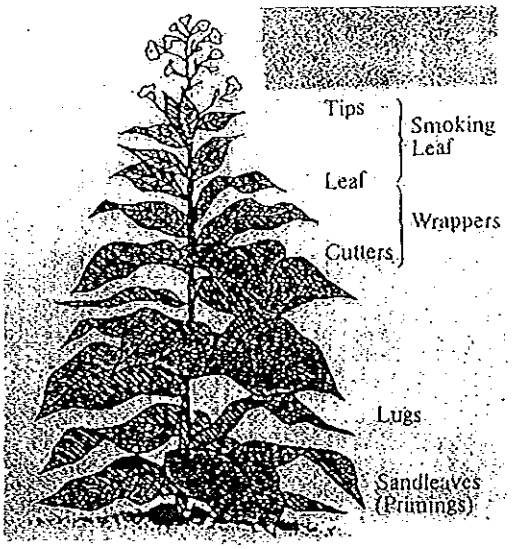


Fig. 1. Stalk position of Tobacco leaf

The range of chemical constituents most commonly and routinely identified in flue- cured Virgina tobacco, are shown in Table- 3. As stated earlier, many factors influence the level and balance of chemical properties of tobacco, but these figures can be considered representative .[8]

Table-3 Range of chemical constitutes in flue-cured Virginia tobacco.

Constituent	Percent (%)
Nicotine	1.5 – 3.5
Nitrogen	1.4- 2.7
Total volatile base	0.3-0.5
Petroleum ether extracts	6.0-8.0
Sugars	8.0-18.0

Nicotine is a hygroscopic oily liquid that is miscible with water in its base form As a nitrogenous base, nicotine forms salts with acids that are usually solid

and water soluble . Nicotine easily penetrates the skin and forms vapors at elevated temperature. [9]

1.2.2 EFFECTS ON THE BOOY

In small doses nicotine has a stimulating effect, increasing activity , alertness and memory . Repeat users report a pleasant relaxing effect. It also increases the heart rate and blood pressure and reduces the appetite. In large doses,it may cause vomiting and nausea, as little as 40mg has been lethal to humans when taken by mouth [10]

1.2.3 THERAPEUTIC USES

The primary therapeutic use of nicotine is in treating nicotine dependence. Controlled levels of nicotine are given to a patient through gums, dermal patches, or nasal sprays in an effort to wean them off of their dependence. Recent studies have indicated that nicotine can be used to help adults suffering from autosomal dominant frontal lobe epilepsy. The same areas that cause seizures in that form of epilepsy are also responsible for processing nicotine in the brain.

Although the amount of nicotine inhaled with tobacco smoke is quite small (most of the substance is destroyed by the heat) it is still sufficient to cause dependence The amount of nicotine absorbed by the body from smoking depends on many factors, including the type of tobacco, whether the smoke is inhaled , and whether a filter is used . For chewing tobacco, or snus which is held in the mouth between the lip and gum, the amount released into the body tends to be much greater than smoked tobacco. [11] .

As nicotine enters the body, it quickly gets distributed through the blood stream and can cross the blood- brain barrier. On average it takes about seven seconds for the substance to reach the brain It acts on the nicotinic acetylcholine receptors. In small concentrations it increases the activity of these receptors, among other things leading to an increased flow of adrenaline, a stimulating hormone . The release of adrenaline causes an increase in heart rate, blood pressure and respiration , as well as higher glucose levels in the blood . Cotinine is a break – down product of nicotine which remains in the blood for up to 48 hours, and so can be used as an indicator of a person's exposure to smoke . In high doses,

nicotine will cause a depolarizing block of the nicotine acetylcholine receptor,which is the reason for its toxicity and its effectiveness as an insecticide

In addition, nicotine increases dopamine levels in the reward circuits of the brain. Studies have shown that smoking tobacco inhibits monoamineoxidase (MAO) an enzyme responsible for breaking down monoaminergic neurotransmitters such as dopamine, in the brain . It is currently believed that nicotine by itself does not inhibt the production of monoamine oxidase (MAO), but that other ingredients in inhaled tobacco smoke are believed to be responsible for this activity . In this way, it generates feelings of pleasure This reaction is simlar to that caused by cocaine and heroin, and is another, reason people kept smoking; to sustain high dopamine levels nicotine and its metabolites are being researched for the treatment of a number of disorders, including ADHD, Parkinson's Disease and Alzheimer's Disease. [12]

1.3 TOBACCO PRODUCTION

1.3.1 CULTIVATION

Tobacco is grown on loamy sand or sandy loam soils or those with an available water-holding capacity of about 0.8 to 1.4 inches of water in the root zone.

Preferred growing conditions for virginia tobacco are:

- *ALITITUDE*- 1050 to 1650 m
- *RAINFALL*- 600 to 800 mm.
- *HUMIDITY*- 70%
- *SOIL* - Light texture, medium sands and sandy loams of which are of low inherent fertility
- Enough *river water* for Irrigation.

The seeding date varies considerably from location to another depending upon the expected transplanting date. It ranges from 60 to 90 days prior to transplanting. The seeding rate will influence the quality of the seedlings. Excessive or inadequate seeding rates results in poor seedlings.[13]

In Ethiopia tobacco seeding has 2 cycles. The first cycle of transplanting seedlings take place in October and extends to the last of November. Harvest begins in march and completes in the mid of April. The second cycle of seedling usually starts in

the mid of May and completes between the last days of July. Harvest of this cycle is usually completed in the last bays of December.

Sufficient fertilizer is needed to grow the seedlings reasonably fast; however, caution must be used to guard against over fertilization. Excess nitrogen promotes excess sucker growth that is difficult to control. Excess nitrogen has been associated with increases in certain insects such as horn worms. Leaf drop and leaf break are encouraged by excess nitrogen.

Experiments have shown that manual topping and hand suckering lead to an increase in root growth. This in turn, increase the plants potential to absorb water and nutrients and to synthesize nicotine. The practice of topping and suckering reduces the drain on the leaves of certain organic and inorganic compounds used for growth by the plant.

Whether or not a plant is topped, and if topped, the time of topping can have a pronounced effect on the cured leaf. The data in Table- 4 indicate that topping improves yield, the alkaloid and sugar content of the cured leaf. The yield increase is much greater for topping plus suckering than for topping and not suckering when compared to the not-topped plant. The same is true for the alkaloid and sugar content of the cured leaf.[14]

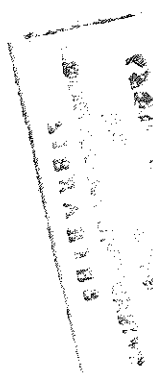


Table 4. Effect of topping and hand-suckering on total alkaloids and reducing sugars.

Treatment	*% Total Alkaloids*	*% Reducing sugars*
Not topped or suckered	1.76	13.30
Topped-not suckered	2.36	17.30
Topped and suckered	2.80	18.20

Tests conducted by J.F Chaplin; Z.T. Ford and R.E Currin, Agricultural experiment station, South carolina

The time of topping with manual sucker control will influence the yield and the quality of a tobacco crop is shown in Table-5a, where manual sucker control was practicd and in Table-5b, maleic hydrazide used for sucker control.

Table-5a Effect of time (Flower Stage) of topping of cured tobacco with manual sucker control

Treatment	% Total Alkaloid	% Reducing Sugar	% Total Nitrogen
Button stage	2.16	24.6	1.75
Early flower stage	1.98	24.3	1.83
Full flower stage	1.85	22.7	1.87
Late flower stage	1.81	20.9	1.87

Table - 5b Effect of time (Flower Stage) of topping of cured tobacco with maleic hydrazide sucker control

Treatment	% Total Alkaloid	% Reducing Sugar	% Total Nitrogen
Button stage	2.59	21.8	2.00
Early flower stage	2.02	22.8	1.90
Full flower stage	1.89	21.8	1.90
Late flower stage	1.95	20.01	1.90

Tests conducted by H.V Marshal, Jr; and Heinz seltmann; N.C State University.

1.3.2 HARVEST

Under normal conditions, tobacco ripens at the rate of 2 to 4 leaves per week, thus the normal harvest rate before mechanization was from 2 to 4 leaves per plant per week for a period of 5 to7 weeks. Under extreme conditions it may be necessary to harvest 7 to 10 leaves per plant in a week to prevent some from being lost, but this is not normal ripening and usually is low quality tobacco. On the other hand, dry weather or excessive quantities

of available nitrogen may reduce the maturity rate enough so that it becomes necessary to skip a week or more of harvesting.

The degree of maturity can have a pronounced influence on the yield and quality of tobacco. The effect of degree of maturity at harvest on nicotine content is shown in Table- 6

Table-6 The degree of maturity on nicotine content

Treatment	*Nicotine %*
Un ripe	2.60
Ripe	2.75
Over ripe	2.95

(Tests conducted by W.G. Woltt; J.A. Wey Brew; and J.M. Corrin, 1981.)

1.3.3 CURING

Proper curing is both a biological and a drying process. In the drying process, there are three stages of moisture removal: yellowing, leaf drying and stem drying stages.

It is desirable to curing to have uniformly ripe tobacco, however, the time and temperature schedule during the yellowing stage can be adjusted; to a limited extent, for slight errors in judgement or ripeness at harvest time. The yellowing is similar to a continuation of the ripening process. Usually, cured leaf that was thoroughly ripe and yellowed over a relatively long period will appear to be more mellow and grainy than tobacco harvested less ripe and yellowed a shorter time.

In the early part of the cure (yellowing stage), the leaf must be kept alive until certain biological processes take place. At the same time, moisture must be removed with out hindering these biological processes.[15]

Another important physical change in the yellowing stage is the loss of water. Under normal conditions, about 20 to 30 percent of the water should be removed from the leaf during yellowing. However, if the leaf is dried too fast the green color may be "set", and certain chemical and biological changes stopped.

The temperature and humidity schedule followed during the yellowing period and the length of time tobacco is yellowed can have a great influence on the yield and quality

of cured tobacco. In fact, the yellowing stage is the most important stage in curing flue-cured tobacco.

Some undesirable changes may take place in curing. Leaf browning or "scalding" frequentely occurs in the critical, leaf drying or color setting stage. This happens when the temperature of the leaf is advanced too rapidly and too high while there is excessive moisture in the leaf. Under those conditions complete browning can occur within a few minutes at a leaf temperature of 130 to 135 ^{0}F. To avoid browning or "Scald" the leaf tissue must be dried to a safe level of around 40 to 50 percent moisture before raising the air temperature above 130^{0}F.

In the "killing out" stage, the objective is to complete the removal of moisture from the stem and leaf so as to preserve the leaf. Once the leaf is dry, major biochemical changes have ceased. The "killing out" temperature should not exceed 160^{0}F. Other wise scorched tobacco, as indicated by red pigment, may result as well as the excessive use of curing fuel.

1.4 METHOD OF ANALYSIS

Technique that has been applied to the analysis of nicotine was UV-visible spectroscopy. As nicotine contains strong UV-Chromophores, it can readlly be detected by UV-detector. The three π-ectrons in the structure of nicotine forming the conjugated system has a profound effect upon the location (λ_{max}) of the band. Thus the absorption spectra of nicotine formed from the transitions involving π-electrons were found at longer wave length in the near-UV region.

1.5 OBJECTIVE OF THE STUDY

- Determine the level of nicotine in flue-cured virginia tobacco of Ethiopia.

2 SAMPLE COLLECTION

In Ethiopia, we have imported tobacco seeds grown in differnt areas of the country, owned by the National Tobacco Enterprise, Ethiopia (NTE, Ethiopia). They are widely grown in Bilate, Awassa and Wollaiyta of Southern Ethiopia. Considerable amounts are also harvested in Shoa- Robit of central Ethiopia. The four plantation areas cover 1,258 hectares of land. Bilate, Awassa, and Wollaiyta cover 250, 200 and 100 hectars

respectively with annual yield of 250,000 , 100,000 and 150,000 kg. Shoa-Robit tobacco plantation covers 708 hectares of land with 850,000kg. of harvest per year. (Data source: NTE, Ethiopia, 2005)

2.1 SAMPLE SITES

The Ripened tobacco leaves were collected from two main farm areas: Bilate of Southern Ethipia and Shoa-Robit of Central Ethiopia. Bilate is some 300 kms, while Shoa-Robit is 215 kms. away from the capital city Addis Ababa

The farm areas and sites of sample collection are illustrated in Table-7 and Table-8

Table-7 Sample sites of Bilate farm land

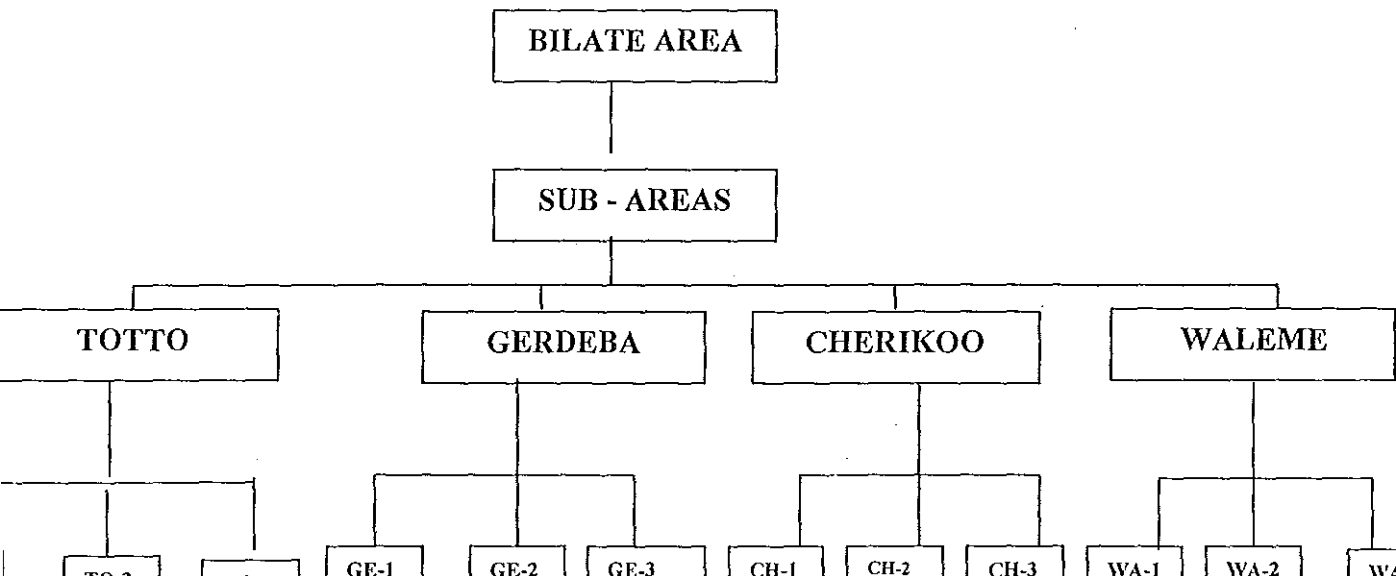
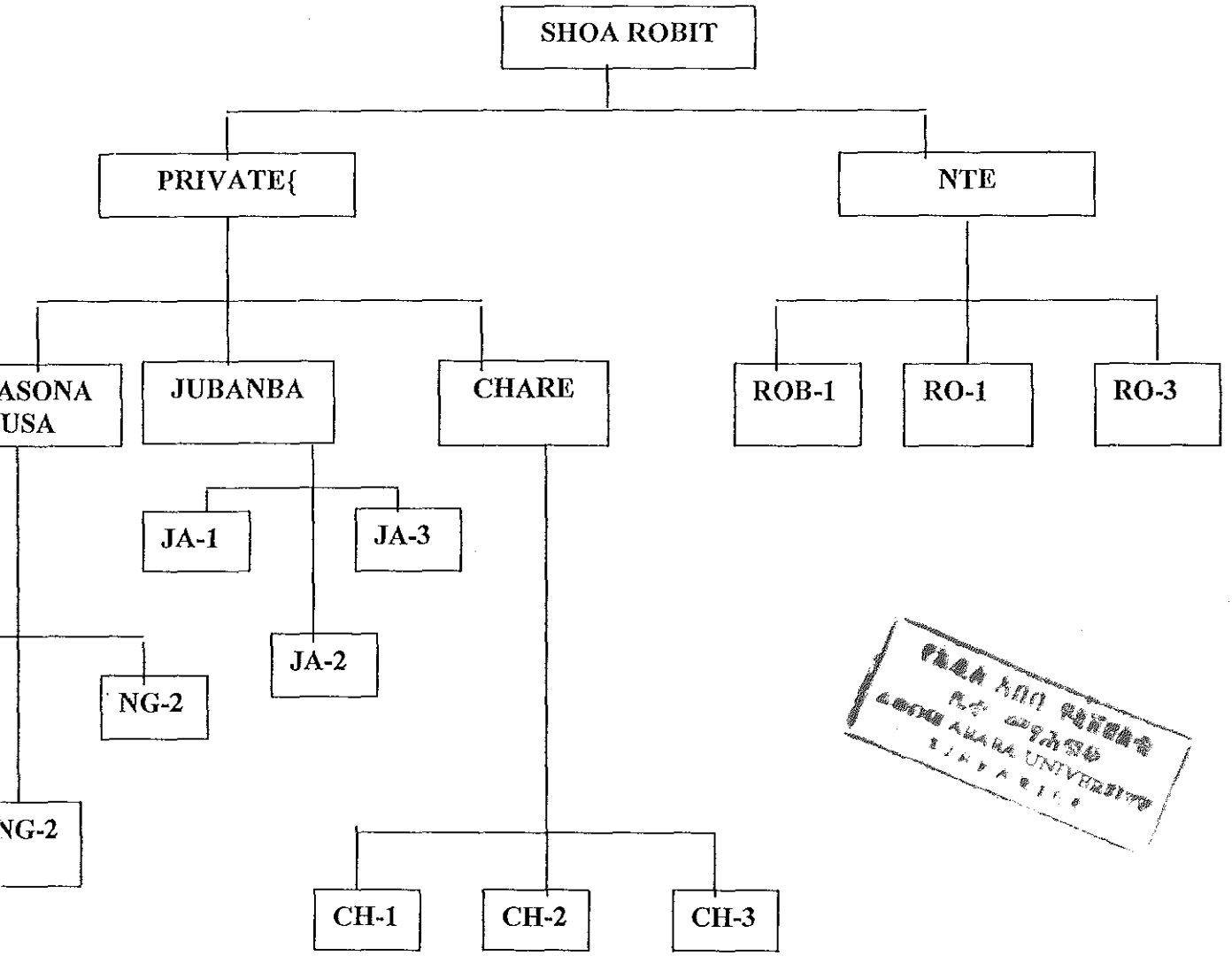


Table- 8 Shoa Robit sample sites.



2.2 CURING ENVIRONMENT

With an understanding of some of the changes that take place in the leaf during the curing operation, it is easier to provide the correct environment for the leaf during each stage of the cure.

The Curer influences the cure by controlling three leaf environmental factors: 1. *Air Temperature* 2. *Air moisture content or relative humidity* and 3. *Air movement.*[16]

2.2.1 Typical curing schedule

The three dry-bulb temperatures selected are well within the safe ranges for each phase of curing : - 100 ^{0}F for yellowing, 130 ^{0}F for leaf drying and 160 ^{0}F for stem drying. The exact wet-bulb reading during the yellowing period must be regulated to fit the needs of the tobacco, but the upper limits shown for leaf and stem drying are conservative. The most critical part of this or any other curing system is proper moisture control during the yellowing period.[17]

Enough moisture must be maintained so that yellowing can be completed before the leaf is dry enough to stop the normal biological changes that must occur during this stage. On the other hand, enough drying must be accomplished so that when yellowing is completed, the leaves will be thoroughly wilted. Under normal conditions a spread of 2 to 3 ^{0}F between the wet and dry-bulb will give the desired rate during the yellowing period. The temperature ranges should be followed in carrying out the simplified curing schedule shown in Fig. 2

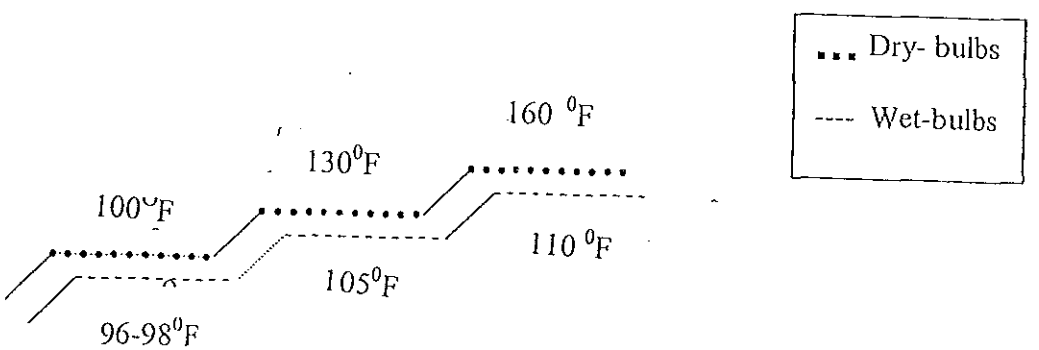


Fig .2 Dry and wet- bulb temperature schedule for average tobacco.

4 RESULTS AND DISCUSSIONS

4.1 ABSORPTION SPECTRA OF NICOTINE

The absorbance of nicotine solution was measured (and plotted) as a function of wave length of the incident light across 200-300 nm. Fig.5. illustrates a plot of UV absorption spectra of a standard solution of different concentrations of nicotine.

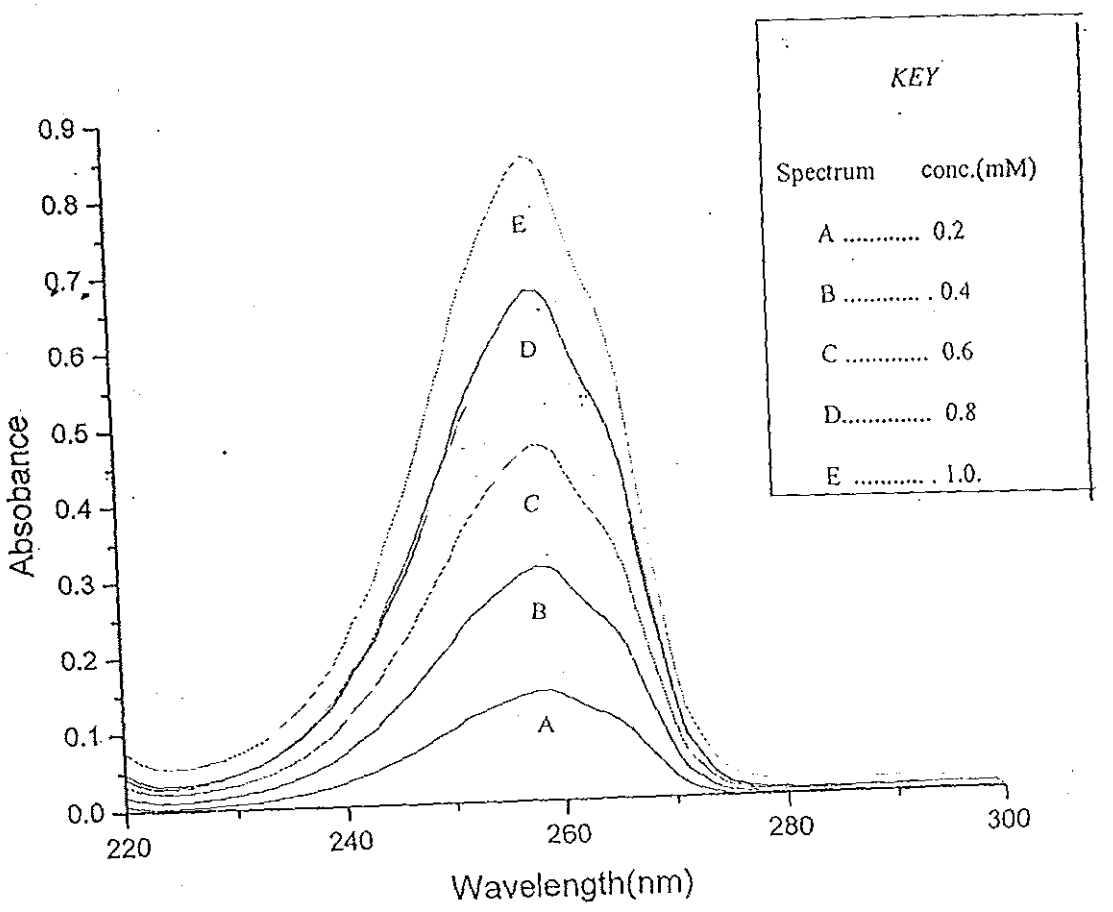


Fig.5 UV- absorption spectra of Nicotine

4.2 Beer's law

The Absorbances of the standard solutions (Table-9) were measured and used to prepare a calibration curve, which in this case is a plot of absorbance versus concentration. The points on the calibration curve should yield a straight line.

Table- 9 Absorbances of standard solutions at 259 nm.

Standard concentration (mM)	*Absorbance*
0.2	0.135
0.4	0.305
0.6	0.469
0.8	0.643
1.0	0.817

Fig.6 Depicts a plot of absorbance against known concentrations of nicotine at 259nm using the data in Table-9.

Linear Regression for the Data in Table-9: $Y = A + B^* X$

.Parameter	Value	Error
A	-0.0368	0.00365
B	0.851	0.00551

R	SD	N	P
0.99994	0.00348	5	< 0.0001

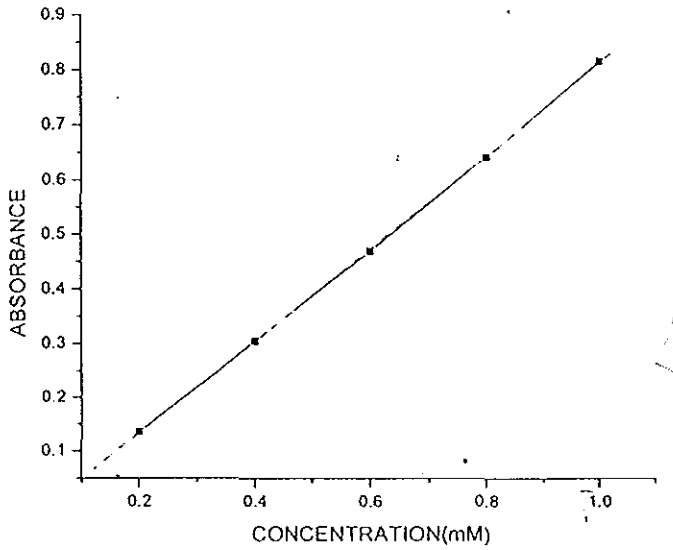


Fig.6. Absorbance versus concentration of standard solutions.

4.3 DETERMINATION OF UNKNOWN SAMPLE CONCENTRATION.

A. calculation of the expected rage of nicotine concentration

- Amount of tobacco used in the analysis -1.0g
- Nicotine distillate collected in a 250 mL volumetric flask
- Amount of nicotine identified in flue-cured virginia tobacco ranges from 1.5 to 3.5%

The above mentioned data helps in calculating the actual mass of nicotine in the extracted nicotine solution and hence the maximum and minimum concentration in conjunction with the commonly determined range of nicotine in flue-cured virginia tobacco.

The data obtained from the standard were used to plot a straight line. The slope and intercept of that line provide a relationship between absorbance and concentration:

$$A = slope\ c + Intercept$$

The absorbance of each of the unknown sample solution, A_U, is then used with the slope and intercept to calculate the concentration of unknown solution, C_U.

$$Cu = \frac{Au\text{-}intercept}{Slope}$$

The absorbance, concentrations and percents of unknown nicotine sample solution in flue-cured virginia tobacco of dfferent sites of Bilate and Shoa Robit farm area are shown in Table- 10 and Table-11 respectively.

Table- 10 Nicotine content of Tobacco collected from Bilate areas

BILATE AREA

SUB-AREAS	CITES	SAMPLE SOLUTION		
		ABSORBANCE	*CONCENTRATION (mM)*	*% NICOTINE*
TOTTO	TO-1	.609	.758	3.074
	TO-2	.607	.756	3.066
	TO-3	.606	.755	3.061
GERDEBA	GE-1	.606	.756	3.066
	GE-2	.608	.757	3.070
	GE-3	.607	.756	3.066
CHERIKOO	CH-1	.609	.758	3.074
	CH-2	.609	.758	3.074
	CH-3	.610	.760	3.082
WALEME	WA-1	.607	.756	3.066
	WA-2	.607	.756	3.066
	WA-3	.608	.757	3.070

Table-11 Nicotine content of Tobacco collected from Shoa Robit area

SHOA-ROBIT AREA							
SAMPLE			PRIVATE				
					SAMPLE		
ABSORBANCE	*CONCENTRATION (mM)*	*% NICOTINE*	SUB-AREAS	SITES	*ABSORBANCE*	*CONC.*	*% NICOTINE*
.593	.740	3.001	**NEFASONA GUSA**	NG-1	.510	.642	2.603
.590	.736	2.984		NG-2	.515	.648	2.628
.595	.742	3.009		NG-3	.518	.651	2.640
			JUBANBA	JA-1	.506	.637	2.583
				JA-2	.500	.630	2.555
				JA-3	.495	.625	2.534
			CHARE	CH-1	.520	.654	2.652
				CH-2	.527	.662	2.684
				CH-3	.521	.655	2.656

The results of samples collected from Bilate area displayed nearly the same nicotine content of tobacco. A variation of negligible difference was obserbed among samples of all sites under Bilte farm land. The conformity of nicotine contents of all the 12 sites were attributed to the uniformity of climatological, biological and cultural practices of the skilled workers.

In Shoa Robit, how ever, there are two types of owner ship of the tobacco plantation: Private owner ship and the NTE ownership. In Private ownership, the land belongs to farmers and the NTE provides them with seedlings, fertilizers and other materials that would facilitate the growth of the tobacco plant. Until harvest, each farmer performs independently in his own land. After harvest, farmers could sell the collected ripened tobacco to the NTE.

The samples collected from different sites of Private ownership showed poor quantity of nicotine. The nicotine content was high for samples under the ownership of the NTE .The reason for such a variation could be accounted mainly for the absence of important practices like topping and suckering which can have a pronounced effect on nicotine content of the leaf.

While comparing samples collected from Bilate with samples of Shoa Robit, Bilate samples showed higher amount of nicotine than that of Shoa Robit. All sample sites of Bilate were found highly nicotine contented. The important factor for this variation is primarily due to soil's water-holding capacity, which is 0.95 inches of water for Shoa Robit and 1.62 inches of water for Bilate in the root zone and the fact that Bilate soils being more loamy sand creates a better favourable condition for growth of tobacco.

Table-12 Reported Nicotine content in Virginia Tobacco.

country	*year imported*	*method of analysis*	*nicotine (%)*	
			tested by the country	*tested by NTE (Ethiopia)*
Italia	1995	----	3.092	3.001
China	1998	----	3.020	2.998
India	1999	----	2.986	2.979
Brazil	2001	Spectronic Genesys-5	3.127	3.122
Zimbabwe	2006	2D-GC	3.180	3.172
Ethiopia	2006	PU8620 UV/VIS/NIR	--------	2.978

5 CONCLUSION

The method applied for the determination of nicotine level in NTE, Ethiopia dry bacco leaf sample displayed conforming results. The quantities are in good agreement th those reported in the literature for local virgnia tobacco and foreign grown virginia bacco, where the standard nicotine content ranges from 1.5-3.5% on dry mass bases. owever, results indicate that the quantity of nicotine is highly dependent upon curing of bacco plant, which is both a biological as well as a drying process. Nicotine cumulation on leaf is also affected by climatological and cultural factors from the time transplanting seedlings up to harvest.

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