

NICOTINE CONTENTS IN HONEY FROM TOBACCO AND NON-TOBACCO GROWING AREAS IN KIGOMA REGION, TANZANIA

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ABSTRACT

Nicotine content in honey is currently the major issue of concern to honey quality in Tanzania. This study was carried out to determine nicotine content in honey from tobacco and non-tobacco growing areas in Kigoma Region, Tanzania. Specifically, the study determined nicotine contents in honey and bee fodder samples. Fresh honey samples were collected from beehives within tobacco and non-tobacco growing areas and from vendors for laboratory tests. Results showed that nicotine content in the fresh honey which was collected from beehives located within tobacco growing areas is significantly higher than that collected from non-tobacco growing areas (0.46µg/g vs. 0.26µg/g, $p < 0.05$). The honey samples from vendors in town and villages had nicotine contents of 0.41µg/g and 2.98µg/g, respectively. It was observed that most of the bee fodder tree species in Miombo woodland had traces of nicotine. Among the most important bee fodder tree species with the highest amount of nicotine were *Brachystegia spiciformis* (20.966µg/g), and *Julbernardia globiflora* (13.168µg/g). Meanwhile *Vernonia colata*, *Combretum collinum* and *Combretum mole* had the lowest nicotine contents of 0.836µg/g, 0.006µg/g, and 0.004µg/g, respectively. Generally, honey samples which were collected from all sources contained different amounts of nicotine. However, these contents were perceived to be tolerable for human health. The sampled bee forage tree species were also observed to have nicotine, which is associated with nicotine in the honey. The study recommends for the establishment of the national standards indicating the allowable amount of nicotine in honey for human consumption. Further research needs to be done in other areas and vegetation types in order to address this concern country-wise.

Keywords: Nicotine Contents, Honey, Tobacco Growing Areas, Non-Tobacco Growing Areas, Kigoma

INTRODUCTION

Tanzania is among the Sub-Saharan countries with the largest forest ecosystem which are covered with Miombo woodlands in more than two thirds of the total forested land (Mbuya *et al.*, 1994; URT, 1998). Miombo woodlands are normally dominated by trees of the closely related genera such as *Brachystegia*, *Julbernardia* and *Isobertinia* (family Fabaceae, subfamily Caesalpinioideae). According to the Tanzania National Forestry Resources Monitoring and Assessment (NAFORMA) report (MNRT, 2015), the woodlands including the Miombo cover about 44,726,246 hectares out of 48.1 million hectares. Kigoma Region is among the regions in the country that has Miombo woodlands with the highest potential for honey production (Mpuya, 2003; Mwakatobe *et al.*, 2016). An increase in honey production in Kigoma Region is a result of the Beekeeping Improvement Project that was implemented between August 2007 and July 2010 by the Ministry of Natural Resources and Tourism (MNRT) with the support from the Belgian Technical Cooperation (BTC), which was implemented in Uvinza, Kibondo, and Kigoma districts (BTC, 2011).

Honey is a natural sweet substance produced by honey bees from the nectar of blossomed flowers (Saba *et al.*, 2013; Waykar and Alqadhi *et al.*, 2016). The composition of honey depends on the plant species visited by the honeybees and the environmental processing and storage conditions (Sanz *et al.*, 2004; Bertoncel *et al.*, 2007; Guler *et al.*, 2007; Zerrouk *et al.*, 2011). It is an important non-timber forest product with a very significant contribution to cash incomes for the rural communities particularly in the Miombo woodland areas. Beekeeping sector in Tanzania generates about US\$ 19 million per annum, employs about 2 million people, helps in bio-diversity, and increases agricultural production through pollination (BTC, 2012). At the household level, the sector contributes up to about 33% of the household income source in the Miombo woodlands of Tanzania (Monela *et al.*, 2000) as opposed to other sources such as agricultural crops and other forest products. Naturally besides honey, there are beeswax, propolis, royal jelly, and honeycomb as the by-products of beekeeping (BTC, 2012). Honey is used in four main ways; (i) direct consumption, (ii) as an ingredient in various products, (iii) for industrial use, and (iv) as a raw material for meals and drinks made from honey. Due to high value of beekeeping products in the international market, it is important to recognise beekeeping as an important provider of income to beekeeping households especially in developing economies.

Bee fodder plants which are available around the apiary are among the factors that influence the quality of the honey. Flowering plants are the sources of nectar and pollen for the honey production. For honey bees to survive, prosper, and be productive, their colonies must have a supply of both nectar and pollen in adequate quantities (FAO, 2015). Therefore, flowering plants are crucial for a colony's life cycle and survival (Castle, 2013). However, not all plants species are equally good for beekeeping, some supply both nectar and pollen abundantly when in bloom, and are often called honey plants; others yield pollen but little or no nectar, and are called pollen plants. A good beekeeping area is the one with nectar and pollen plants growing abundantly and with a relatively long blooming season (Nicolson, 2011; FAO, 2015; Stevenson, *et al.*, 2016). Moreover, depending on the type of flowering plant species, there are different types of honey. It has been established further that the quality of honey also depends on the nicotine content which is a naturally occurring alkaloid found in the plant kingdom especially of the Solanaceae family, predominantly tobacco (Asiyah *et al.*, 2011; Swaileh and Abdulkhalig, 2013; Rand *et al.*, 2015) and on the quality of the soil from which the flowering plant grows. In most cases, the best quality honey is that which is collected from nectar in areas free of chemicals, that is, ecologically pure areas.

Currently, major issue of concern to honey quality in Tanzania is the presence of nicotine. There has been a claim among users of honey that honey harvested within tobacco growing areas has nicotine content which affects human health (BTC, 2012; Asiyah *et al.*, 2015). Nicotine is required in the human body, however according to Mishra *et al.* (2015); excess intake of nicotine is harmful to humans as it has a rapid onset of action on peripheral and central nervous systems. Similarly, Yamamoto *et al.* (1998) and Patil *et al.* (1999) cited nicotine as having side effects on human reproductive health, as it can reduce reproductive capacity causing mutagenic consequences towards the germ cell production and maturation. Such claims and scientific evidence on the negative effects of nicotine to human being have created challenges to the honey international markets (BTC, 2012). Among the sufferers from these claims are producers who seek to provide a nutritious product to local and international markets. Despite the long-time claims regarding the association of nicotine in honey and tobacco crop, there has been no study to justify the concerns. Most of the studies dealt with honey quality in terms of hydroxymethyl furfural (HMF), water content, sugar content, pH, ash content, and honey colour (Murray *et al.*, 2011; Gidamis *et al.*, 2004; Muruke, 2014). It is against this background that the current study set out to determine nicotine amounts in honey from tobacco and non-tobacco growing areas in Kigoma Region, Tanzania. Specifically, the study determined the amount of nicotine available in honey and bee fodder plants.

METHODOLOGY

Description of the Study Area

The study was conducted in Uvinza and Kibondo Districts in Kigoma Region (**Fig. 1**). The selection of the two districts among others was based on their high production of honey (BTC, 2012; Mwakatobe *et al.*, 2016) and large coverage of tobacco farms (BTC, 2011). Beekeeping practices in the two districts differ: in Uvinza District beekeeping is done in Miombo woodlands adjacent to tobacco farms and in the forest reserves, while in Kibondo District beekeeping is carried out in Moyowosi Game Reserve, which is about 50 km away from the homesteads and agricultural activities. The majority of beekeepers engage in beekeeping activities in this reserve because of having suitable conditions for apiaries.

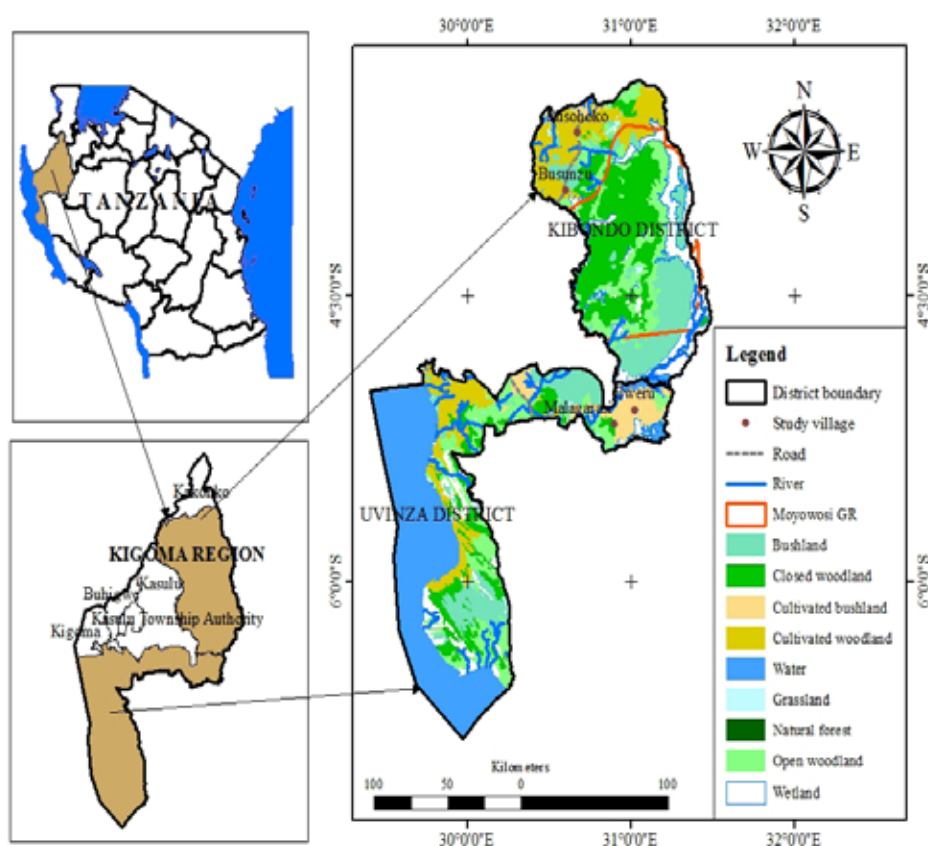


Figure 1: The map of study area

Source: NBS (2012)

Data Collection

Collection of samples for laboratory work

A total of 12 honey samples were collected from beehives and local vendors. Three beehives were sampled from tobacco growing areas (Uvinza District) and other three from non-tobacco growing areas (Kibondo District). Three samples of honey were collected from local honey vendors within the village and at the town centre (in both Uvinza and Kibondo districts) each, to make a total of six samples. These samples were collected in order to verify if there is any honey abuse along the distribution and marketing chains. Suitable bee fodder trees in the study area were identified through Focus Group Discussions (FGDs) and key informant interviews. Stakeholders involved in FGD and key informants interview were bee keepers, elders, beekeeping officers. Nine most suitable bee fodder tree species were selected where three samples from each section (bottom, middle and growing tip) from stems (of the same tree species

located at different localities). The collected samples were transported to the Department of Food Science and Technology at Sokoine University of Agriculture for laboratory analysis.

Laboratory procedures for nicotine quantification

Nicotine amount was determined following a method described by Suryan *et al.* (2012). A total of 72 triplicates of honey and leaf samples were prepared for laboratory analysis. Some 1 – 5 g of homogenized samples of honey and leaves were put into 100 ml Erlenmeyer flask then 10 ml of methanol were added to it. The mixture was stirred for 30 minutes to extract the sample. Then 25 ml of distilled water was added to dilute the sample followed by the addition of 2N NaOH. The mixture was warmed in a water bath at 80°C for 6 minutes and then left to cool. The mixture was then filtered through number 41 whatman filter paper into 50 ml volumetric flask. A 1 ml of Zinc acetate was added followed by the addition of 1 ml Potassium hexacyanoferrate (II) solution and distilled water was added to complete the volume. The mixture was centrifuged at 3600 rpm for 5 minutes. The supernatant was transferred into another clean 50ml volumetric flask and the residue was discarded. Then 5 ml of 0.01 N NaOH was added and distilled water was added to complete the volume. Absorbance was read at 602 nm using spectrophotometer, where nicotine content of the sample was recorded in µg/g units.

Data Analysis

Descriptive statistics analysis was performed for laboratory data, and the results were presented indicating the level of nicotine contents in µg/g, in honey and in the leaves. Furthermore, t-test analysis was used to compare the means of nicotine contents from the honey collected from tobacco and non-tobacco growing areas. The data which were collected through FGDs and key informant interview were analysed using content analysis. The data were categorized into meaningful units and themes and summarised into meaningful information based on the study objectives.

RESULTS AND DISCUSSION

Levels of Nicotine in Honey

The results from the laboratory analysis showed that the levels of nicotine in fresh honey samples which were collected directly from beehives in Kigoma Region were 0.46 and 0.26 µg/g for the beehives within tobacco and non-tobacco growing areas, respectively. The honey samples which were collected randomly from vendors in town had nicotine content of 0.41µg/g and the samples from village vendors had nicotine content of 2.98 µg/g (**Table 1**).

Table 1: Nicotine content in honey samples from different sources in Kigoma region

Sample name	Average sample weight (g)	Nicotine contents (µg/g)
Beehives within tobacco growing areas	5.27	0.45
Beehives within non-tobacco growing areas	5.60	0.24
Honey from vendors in Town	5.43	0.41
Honey from vendors in village	5.44	2.98

Note: µg/g is equivalent to mg/Kg

The results indicate further that all honey samples contained traces of nicotine at different amounts, which were revealed to be tolerable to human health (AEMSA, 2014). However, there was a statistical significant

difference ($p < 0.05$) between the means for the honey which was collected from beehives located within tobacco and non-tobacco growing areas (**Table 2**). The difference in means could be attributed to nectar from tobacco flowers, as some of the beehives were not harvested during June and July blossom. The flowering of tobacco plant occurs between February and March, which is the time for nectar collection for honey to be harvested during June and July blossom.

Table 2: T-test analysis for honey samples collected in beehives from tobacco and non-tobacco growing areas

Variable	Honey sample type	
	Collected within tobacco growing areas	Collected within non-tobacco growing areas
Mean ($\mu\text{g/g}$)	0.454101972	0.243522
Variance	0.009343989	0.000576
Observations	9	9
Df	8	
P-value	0.000134009	

The nicotine content of the honey from Kigoma Region was higher than that found in other regions such as Tabora and Kilimanjaro which ranged from 0.0007 to 0.0037 mg/Kg (Ilomo *et al.*, 2013). Moreover, nicotine content from the study area was higher than 0.003 to 0.005 mg/Kg which was reported by Mumbi *et al.* (2014) from Tabora, Dodoma and Kilimanjaro regions in Tanzania. This result could be attributed to factors such as the type of plants available and which bees use for forage. This study indicates that leaves from plants used by bees as fodder contained large amount of nicotine than the nicotine observed from the honey. Similar finding is reported by Mumbi *et al.* (2014) who reveal that the nicotine content in honey was also from other plant species which were found within the honey producing areas which contaminate the honey. In line to this, a study by Swaileh and Abdulkhaliq (2012) showed no statistical significant differences in nicotine concentration between honey samples from different geographic regions in Palestine. Nicotine is a natural plant product that is found in many plant species, especially tobacco. Honeybees encounter nicotine trace concentrations in the floral nectar of *Nicotiana* plant spp. Nicotine is known as a nourishing restraining owing to its bitter taste, and pollinators may encounter nicotine in both nectar and pollen. In nature, bees collect nectar from various plants, which may be mixed in the hive to reduce the concentrations of nicotine in honey.

Similarly, a study by Swaileh and Abdulkhaliq (2012), Pasquale *et al.* (2013), Muruke (2014), Ndefe *et al.* (2014), and El Sohaimy *et al.* (2015), revealed that chemical contents in honey are attributed to the type of botanical plant used. There was no established standard of the level of nicotine in honey from Tanzania Bureau of Standards. However, the American E-Liquid Manufacturing Standards Association states that the commonly allowable nicotine content in flavoured products is 36mg/ml which is equivalent to 36,000 $\mu\text{g/g}$ (AEMSA, 2014). Also data bases and safety sheets consistently state that lethal dose for adult is 30 – 60 mg (Hayes, 1982). Regarding these facts, honey samples from Kigoma Region contain small amounts of nicotine that is not lethal to human being.

Nicotine content of honey found in Kigoma was not as high as nicotine content found in other foods consumed by human on every day basis. For instance, common vegetables such as tomatoes and lettuce are known to contain large amounts of nicotine and are always consumed in large quantities (Carlos *et al.*, 2011). Fresh tomatoes are reported to contain 4.1 - 4.3 mg/Kg of nicotine (Edward *et al.*, 1999). As per

research findings, a human being requires a certain amount of nicotine through diet or supplements, to manage the smooth functioning of the body. In case there is a nicotine deficiency, the body may not be able to fulfil the basic as well as complex mitochondrial functions (Mumbi *et al.*, 2014).

Levels of Nicotine in Major Bee Fodder Plants

Most of the Miombo woodland trees have certain amounts of nicotine. **Table 3** shows some Miombo woodland tree species that were identified during FGD as most preferred by bees for honey production. Most of the tree fodder species in the study areas belong to Fabaceae family.

Table 3: Nicotine content in identified bee fodder trees in Kigoma

Local name	Botanical name	Family	Average sample weight (g)	Nicotine content (µg/g)
Mtundu	<i>Brachystegia spiciformis</i> Benth.	Fabaceae	1.092	20.966
Muba / Mlugwe	<i>Julbernardia globiflora</i> (Benth.) Troupin	Fabaceae	1.096	13.168
Myenzi	<i>Brachystegia boehmii</i> Taub.	Fabaceae	1.014	3.071
Mkambati /Mkurungu	<i>Pterocarpus chrysothrix</i> Taub.	Fabaceae	1.042	1.708
Mlembele	<i>Dalbergiella nyasae</i> Baker f.	Fabaceae	1.155	1.434
Mmbanga	<i>Pericopsis angolensis</i> (Baker) Meeuwen	Fabaceae	1.076	1.344
Mlulunguja	<i>Vernonia colata</i> (Willd.) Drake	Asteraceae	1.063	0.836
Mkoyoyo	<i>Combretum collinum</i> Fresen.	Combretaceae	1.036	0.006
Mlama	<i>Combretum molle</i> R.Br. ex G.Don	Combretaceae	1.031	0.004

Among the most important bee forage trees with the highest amount of nicotine were *Brachystegia spiciformis* Benth. (20.966 µg/g), and *Julbernardia globiflora* (Benth.) Troupin (13.168 µg/g) while *Vernonia colata* (Willd.) Drake, *Combretum collinum* Fresen and *Combretum molle* R.Br. ex G.Don had the lowest nicotine contents of 0.836, 0.006, 0.004 and µg/g, respectively. Mumbi *et al.* (2014) estimated nicotine level in tobacco (*Nicotiana tabacum* L.) leaves to range from 6 to 8 mg/Kg. These findings show that, apart from tobacco plants, there are also other plant species with nicotine that contaminate honey. However, most of the sampled tree species had low levels of nicotine concentration compared to the levels estimated in tobacco leaves. Similar findings were also reported by Singaravelan *et al.* (2006) and Adler *et al.* (2006) in Israel and USA, respectively.

CONCLUSION AND RECOMMENDATION

Honey samples which were collected from all sources contained some traces of nicotine at different amounts. However, such amounts of nicotine from all sources were tolerable to human health. The sampled bee forage tree species were also observed to have nicotine, which is also associated with nicotine in the honey. Much of the sampled honey from beehives which were within tobacco growing areas was not associated with nectars from tobacco plants as it was produced after the end of tobacco production season. However, some of the sampled honey was from beehives which were not harvested during June and July blossoms; therefore they could also be associated with the nicotine traces in the honey. Generally, the current traces of nicotine in honey could also be from other bee fodder plants apart from tobacco. It is important to establish the national standard that will indicate the allowable amounts of nicotine in honey and which are not harmful for human consumption. Further research needs to be done in other areas and vegetation types in order to address this concern country-wise.

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