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BIOCHEMICAL ENGINEERING STREAM

Characterization and Optimization of bioinsecticide extracted from Cigarette waste to control cabbages insect pests

A Final Thesis Submitted to the Graduate School of Chemical and Bio Engineering, Addis Ababa Institute of Technology, Addis Ababa University, in Partial Fulfillment of the Requirement of the Degree of Masters in Biochemical Engineering

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DECLARATION

I declare that the thesis entitled by "Characterization and Optimization of bioinsecticide extracted from Tobacco of Cigarette waste to control cabbages insect pests" is my first work and could not be submitted to any university for any level of degree or diploma program.

Approved by Examiner Board

Name	Signature	Date
Advisor		
Internal Examiner		
External Examiner		

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ABBREVIATION AND ACRONOMY

amu = atomic mass unit

ANOVA = Analysis of Variance

Bt.= Bacillus thuringiensis

CAS = Chemical Abstract Service

CB = Cigarette Butt

CCD = Central Composite Design

DDT = Dichlorodiphenyltrichloroethane

EC = Emulsified Concentrate

EPA = Environmental Protection Agency

EtOH = Ethanol

ETS = Environmental Tobacco Smoke

FAOS = Food and Agricultural Organization Statistic

FID = Flame Ionization Detector

FIFRA = Federal Insecticide, Fungicide and Rodenticide Act

GATS = Global Adult Tobacco Survey

GS-MS = Gas Chromatography – Mass Spectrometry

IPM = Integrated Pest Management

LD_{50} = Lethal Dosage of 50mg per kg

LOD = Loss on Drying

MeOH = Methanol

MoA = Ministry of Agriculture

MSD = Multiple Selection Diode

m/z = mass to charge ratio

nAChRs = nicotinic Acetylcholine Receptors

NaOH = Sodium hydroxide

NTE = National Tobacco Enterprise

P^H = Potential of Hydrogen

PIP = Plant-Incorporated-Protectants

R^2 = Regression ratio

SIM = Selective Ion Monitoring

STD = Standard Deviation

TCP = Tobacco containing portion

TIC = Total Ionic Compound

TSNAs = Tobacco specific Nitrosamines

US = United State

USA = United State of America

UV = Ultra Violet

WHO = World Health Organization

ABSTRACT

Chemical pesticides and fungicides have been in use since long period to protect the crops from insects and diseases. Plant extracts were likely the earliest agricultural bio-insecticides. Nicotine in the form of tobacco extracts was reported in 1690 as the first plant-derived insecticides. A huge mass of tobaccos is wasting in NTE during cigarette processing and within a cigarette butt disposal. Therefore, this study is aimed to characterize and optimize nicotine extract from these tobaccos wasted for insecticide application.

The samples were collected from different parts of the countries. The tobacco fillers were removed from wastes, dried and resized for better extraction efficiency and quality. The processes involve the extraction, emulsification, and testing of efficiency on cabbage aphids. The extraction process was performed by the solvent extraction method using methanol to ethanol ratio of 1:4 determined by GCMS. The independent variables analyzed for extraction parameters were temperature (30-60^{0}C), extraction time (4-6hr), and molarity of sodium hydroxide (1-3M), and their optimum conditions analyzed by design expert, Response Surface methods, Central Composite design. Quality analysis was carried out by GCMS whereas quantity was analyzed by GCMS and UV/visible Spectrophotometer.

The maximum yield and nicotine content obtained at the optimum condition of parameters were 17.749 and 3.258 % of the weight of tobacco filler. The emulsification process was carried out by mixing 10g nicotine-containing extracts (54. 4629 g) with 13.616 gram of the mixture of palm oil and surfactants prepared by 1:4 ratio of palm oil to surfactant at room temperature and atmospheric pressure. The physicochemical characteristic of the emulsified concentrated such as density, viscosity, pH, flash point, and surface tension were analyzed and their value was recorded as 1.0122 ± 0.0103 g/ml, 585.33 ± 2.52 mPas, 9.37 ± 0.03, 87.96 ^{0}C, and 34.10mN/m respectively. The best efficiency of the emulsified concentrated extract tested on the cabbage aphid was observed at the ratio of 1:100 (the emulsified concentrated to solvent).

***Key words**: Cigarette Butt, Nicotine, Emulsification, Surfactant, GC-MS, UV/Vis. RSM,*

1 INTRODUCTION

1.1 Background

Chemical pesticides and fungicides have been in use since long period to protect the crops from insects and diseases. The first strategy of pest control was conducted by the Summerian in 2500 B.C who used the sulfur compound to control insect pests and mites. Plant extracts were likely the earliest agricultural bio-insecticides. Nicotine in the form of tobacco extracts was reported in 1690 as the first plant-derived insecticides, followed by Pyrethrin from pyrethrum flowers and rotenone from derris roots in the early 1800s (Tomizawa and Casida 2005). It was isolated from the tobacco plants in 1828 by physician Wilhelm Heinrich Posset and Chemist Karl Ludwig Reimann of Germany, who considered it as an insecticide (Idrees 2016).

In 1934, DDT was discovered by Swiss chemist Paul Muler as effective insecticides, and it was used in Switzerland to control potato beetles in 1939 and commercial production began (Pesticides 2003). Biological controls for insect pests in agriculture date back as far as 1835, when Agostine Bassi demonstrated that white-muscadine fungus (Beauveria bassiana) could be used to cause an infectious disease in silkworm. Mineral oils as plant protectants were also reported in the 19th century. During the rapid institutional expansion of agricultural research during the early 20th century, an ever-growing number of studies and proposal for biopesticides were developed.

Until the early 20th Century, cultural and mechanical methods augmented by Pesticides in the Modern World – Pesticides Use and Management a diverse range of organic and inorganic substances derived from plants, animal and minerals dominated pest control. Effective and affordable synthetic pesticides gained ground by the mid-20th Century, due to the maturing chemical industry and environmental concern (Aklilu, 2017).

In 1901, bacterium Bacillus thuringiensis (Bt) was the initial microbial biopesticide isolate from a diseased silkworm by Japanese biologist Shigetane Ishiwata to kill caterpillar (Koul 2011). Over 200 biopesticide products are currently sold in the US. Approximately 45% of the total biopesticide use occurs in the USA, Canada, and Mexico whereas Asia uses around 5% of biopesticides sold (Dara, 2018)

Neem is a member of the Mahogany family Meliaceae and it contains an active ingredient known Azadirachtin, which is a proven natural anti-feedant, growth regulator, anti-ovipository and insect repellent. It is toxic to soft bodied insect larvae. Azadiracthin has proven effectiveness as a pesticide against about 200 insect species and is reported as non-toxic to humans. In 1963 Indian scientists extensively examined the chemistry of the active principles of Neem. Following the discovery of Neem kernel as a locust feeding deterrent, its chemistry has grown considerably. Several compounds have been isolated and characterized. The main feature is that most of them are chemically similar and biogenetically derivable from a Tetracyclicterpenes (Rinaldi et al. 2017).

Cedric Brines and colleagues note that for century, gardeners have used home made mixtures of tobacco juice and water as a natural insecticide to kill insect pests. Nowadays, the use of tobacco is almost all diverted to cigarette production. In Indonesia, the second biggest manufactures of tobacco, 99% of total production of tobacco are used for cigarette manufacturing (Rabinoff, 2018).

In Ethiopia, there is one huge tobacco industry known as 'The National Tobacco Enterprise' producing five types of cigarette brands at this time, namely Nyala, premium Nyala, Gissella and Delight and Elleni, which are produced from three types of commercial tobacco species. These tobaccos are farming in five farm places such as Shoa Robbit, Wolayita, Hawassa, Billate and East shoa, and cultivated twice in a year. These are: Virginia, oriental and burley. Moreover, it also uses some imported Virginia tobacco leaves. The National Tobacco Enterprise is the only industry which has mandate to organize tobacco production and processing in the country (Tassew 2007).

1.2 Problem Statement

Ethiopia, cabbage cultivations are very low compared to other fruits and cereal crops because of the insect pests affects it highly than the other crops. In addition, to control insect pests from fruit and other vegetables, farmers use chemical insecticides to control insect pests and fungicides to control diseases. They are directly applied to the them, and some of chemicals may still be present as residues on fruits and vegetables after their harvested which can cause lethal effects on the consumers. According to WHO report of 2008, 25% of the world production of chemical pesticides were applied in developing countries where 99% cause death. This problem is also significant in Ethiopia. Furthermore, chemical pesticides affect a non-target organism which are a natural insect enemy, pollinators, domestic and wild animals found there.

Therefore, production of biopesticide from these cigarette waste will decrease the problems caused by chemical pesticides on non-target organisms, economy and environment**.**

1.3 Objective of the Study

1.3.1 General Objective

The general objective of this study is Characterization and optimization of bioinsecticide extracted from Tobacco of Cigarette waste to control cabbages insect pests.

1.3.2 Specific Objectives

- To identify the ratio of methanol to the ethanol used to extracts high yields of crude oil containing nicotine
- To analysis the effect of temperature, time and concentration of NaOH on the extraction process
- To formulate emulsified concentrated insecticide and investigate its physicochemical characteristics
- To test dilution ratios of the concentrated emulsion insecticide used for spray using cabbage aphids as the experimental specimen

1.4 Significance of the Study

Still, the reusing of cigarette waste is very low compared to other wastes such as automobile batteries, water bottles, plastics shoes, bags, and jerry can in Ethiopia. So, this study initiates the use of cigarette wastes for different purposes.

The other significance is replacement of chemical insecticides. Since nicotine-based insecticide is less toxic compared to chemical insecticides, it used to replace it. Collection of cigarette butts treats the environment.

This study plays a major role for the NTE company. In NTE company, a huge number of cigarettes and tobacco blended are wasted that would not have recycled to normal cigarettes during processing. Still, they threw it to the waste storing areas. Additionally, this research may aid as a reference for other researchers.

1.5 Scope of the Study

This research was focused on characterization and optimization of bioinsecticide extracted from tobacco of cigarette wastes. The extracted insecticide was emulsified to increase penetration ability through the skin of the insects. It was extracted at a temperature of between 30 to 60^{o}C, time of extraction from 4 to 6 hours, and molarity of sodium hydroxide of 1 to 3M to find the maximum yield and quality. The result was analyzed by GCMS and UV/visible spectrophotometer. The density, viscosity, pH, surface tension, flash points, and stability of emulsified concentrated insecticide were characterized. The efficiency of the end product was tested on Cabbage aphids. Statistical significancy test was performed for methanol to ethanol ratio, parameters, stability and efficiency of end products.

2 LITERATURE REVIEW

2.1 Pesticides and its Types

Pesticides are classified according to its origin and formulation styles. Tomlin (2008) defined pesticides as natural and synthetic agents that are used in agricultural production to control pests, diseases, weeds and other plant pathogens in an effort to reduce or eliminate yield losses and enhance product quality throughout the world since middle of the last century.

According to Pimentel, Mishra and Sharma (2014) definition, the term pesticide refers to any chemical substance or mixture of substances intended for preventing, destroying, repelling or mitigating any pests. Pesticides are classified into two types based on its origin.

2.1.1 Chemical Pesticides

Chemical pesticides are pesticides formed by synthesizing inorganic and organic chemicals. It can be classified into four major groups depending on target pests. These are 1) insecticides: organochlorines, organophosphates carbamates, and insect repellents such as Diethyl toluamide, DDT and citronella. 2) Herbicides (target to weeds): paraquat, glyphosate and propanil. 3) Fungicides (target to mould and fungi) and 4) Rodenticides (used to kill mice, rat and other rodents) (Tomlin, 2008).

2.1.1.1 Environmental Effect of Chemical Pesticides

Chemical pesticides have different distribution and persistence pattern in the environment. According to Bolton (2002) stated, an agricultural chemical that is applied to a given site, representing a risk to applicators, bystander and wild animals. When it is applied to target pests, the whole site is affected including crop plants, soil, and organism potentially where as wild life affected in immediate area. Nirmal Shankar (2013) found that the chemical pesticides result in the destruction of various beneficial microbes, flora, and fauna causes serious in human. Therefore, there is a great need to develop green and cheaper alternatives for handling economically important pests. Garg (2014) estimated that only about 0.1% of the chemical pesticides reach the target organisms and the remaining bulk contaminates the surrounding environment. According to the study of Garg, pesticides affect target and non-target organisms including earthworm, predators, pollinators, human, fishes, amphibians and birds in addition to their impact on soil, water and air ecosystem.

2.1.2 Biopesticides

Bio pesticides are a pesticide almost all obtained from living things. As Bolton (2002) defined, bio pesticides are types of biochemicals extracted from certain minerals that can be used for controlling pests. According to Leahy et al (2014) definition, bio pesticides are pesticides derived from natural materials such as animals, plants and microbes.

Typically, bio pesticides have unique modes of action and considered reduced risks pesticides. Bio pesticides fall into three major classes (Leahy et al. 2014; Nirmal Shankar, 2014; Malik 1995).

i) Biochemical Pesticides:

Leahy et al. (2014) explained that biochemical are naturally occurring substance that are extract from plant to control pests by non-target mechanisms. They include insect sex pheromones which interfere with mating and various plant extract that attracts insect pests to traps. They are plant derived insecticidal compounds that control the insects either by killing or preventing them from destructive behavior by interfering with physiology of insects, affecting their nervous system, hampering normal development of insects and acting as antifeedants.

ii) Microbial Pesticides:

Although many microbes are used as bio pesticides, the most known microbe is bacteria. Nirmal Shankar (2013) reported that the most widely known microbial pesticides are varieties of the bacterium Bacillus thuringiensis, or Bt which can control certain insects in cabbage, potato and other crops. In addition to Nirmal Shanker study, Leahy et al (2014) studied the toxicity of Bt on insect pests. Bt can be applied to plant foliage or incorporated into the genetic materials of crops and as discovered it is toxic to caterpillar (larvae) of moths and butterflies.

iii) Plant Incorporated Protectants (PIP):

This is the combination of microbial and biotechnology application to control pests from plants. They are pesticidal substances that plants produce from genetic material that has been added to the plants.

2.2 Production and uses of Pesticides in Ethiopia

The startup of using pesticide in Ethiopia date back to 1960's due to the emergence of commercial farms. Pesticide use in Ethiopia is increasing (Aklilu 2017). According to MoA (2013) reports, 2973 tons of pesticides were imported in between 1996-1998, 3670 metric tons between 1999-2001, 5079 tons between 2002-2004, 8302 tons between 2005-2006, and in between 2006 -2011 a total of 27,268.73 metric tons of pesticide were imported to the country.

However, such records do not include products imported illegally. Teklu (2016) described that commercial farmers, as the main users of pesticides account for the use of about 80% of pesticide imported into the Ethiopia. The remaining 20% of the total import is used for small scale farming, household, health and industrial purposes. Of the total 4125 metric tons of active ingredients that were used in Ethiopia, the largest proportion (75%) were herbicides, followed by insecticides (15%), fungicides (9%) and the remaining was rodenticides (1%) in 2010.

The annual importation of synthetic pesticide recorded in 2014 was 4200 tons. The growth in amount of imported pesticides in use and pesticide user is requiring a better choice of subsidized and appropriate pesticide with good pesticide management system. In Ethiopia, still only one chemical pesticide is fabricated in Adami Tulu. From a website of www.cic.gov.et, Adami Tulu pesticide processing factory has the capacity to formulate 1500 tons of dust and 1500000 liters of liquid formulation in each year.

2.3. Consumption of Cigarettes

Global consumption of cigarette has increased rapidly since 1900s. In 1920, 300 billion cigarettes were consumed, and in 2009, cigarette consumptions had reached 5.88 trillion units. The western Pacific region consumes about half of the world's cigarettes, while about 11percent is consumed in the Americas. Tobacco and tobacco products are produced, traded and consumed legally as all other products, and their production and trade are subject to the same rules and regulations as all other products. Thus, although many countries take active measures to reduce smoking and other tobacco use as a policy for reducing tobacco-related social costs, economies of other countries have to depend heavily on tobacco growing and tobacco-related manufacturing for employment and income.

Table 2.1 The smoking prevalence rate[1] of the world in 2015

Continent	**Male smoking rate, %**	**Female smoking rate, %**
Africa	18	2
America	21	12
Eastern Mediterranean	34	2
Europe	38	21
Southeast Asia	32	2
Western Pacific	46	3

(Source: Tobacco Atlas, 2016)

Table 2.2 Total global cigarette consumption from 1880-2017 in the world

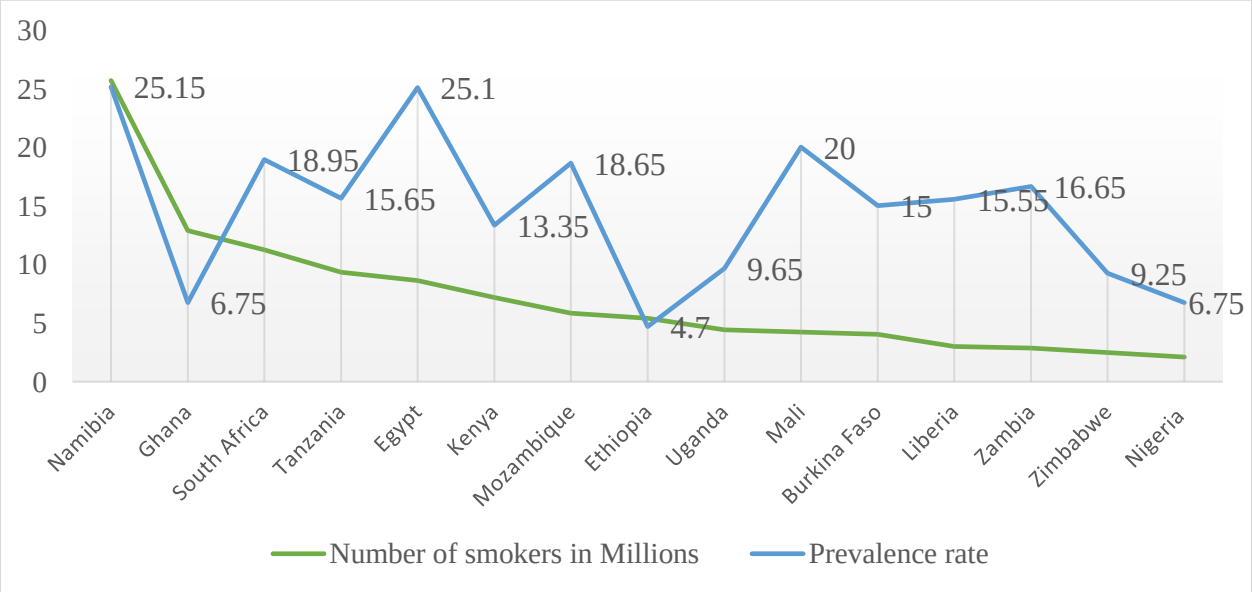
Year	Billions of Cigarette consumed	Year	Billions of cigarettes consumed
1880	10	1970	3262
1890	20	1980	4453
1900	50	1990	5328
1910	100	2000	5711
1920	300	2009	5884
1930	600	2014	5800
1940	1000	2016	5505
1950	1686	2017	5416
1960	2150		

(Source: World Statista, 2020)

[1] Smoking prevalence rate is the proportion of the smokers in the population for a specified period of time.

According to a Lancet Survey (2019), the prevalence rate of cigarette smoking in Africa is low compared to America and eastern Mediterranean. Currently, smoking rates have generally declined in developed world, but continue to rise in some developing nations. In sub-Saharan Africa, cigarette consumption increased by 52% between 1980 and 2016, means that, in 1980, 164 billion cigarettes consumed while in 2016, 250 billion cigarettes were consumed (Wind, 2018).

Lesotho had the highest prevalence of tobacco users in Africa as of 2019. That year, 26.7 percent of Lesotho's population consumed tobacco. Morocco followed close with 23.4, then South Africa with a prevalence of 20.3 percent. West African countries like Nigeria and Ghana had the lowest prevalence rankings in Africa that year. Although the prevalence rate of smoking is decline, the actual number of smokers increasing because of population growth. Figure 2.1 shows the number of cigarette smoker for some African countries.



(Source: Prevalence of Tobacco smoking, 2020)

Figure 2.1 The number of cigarette smokers in African countries in 2019

Low and middle-income countries like Ethiopia represents over 80% of cigarette smokers (Jan, 2020). Ethiopia has an increasing number of population and prevalence rate of smoking cigarette. It was ranked at 21th and 130th from Africa and world respectively in 2019 by rate of smoking. From year to year, the numbers of smokers are increases. According to GATS (2016), 1.9 million adults smoked manufactured cigarettes in 2016/17.

In 2017/18, the number of cigarette smokers older than 15 years old are increased to 2.42 million, and in 2018/19, they became 6.3 million. Due to the country's increasing rate of smoking and demand, National Tobacco Enterprise (NTE), expanded its market from 4 billion cigarettes to 6 billion annually across each brand. Some of cigarettes were also imported from china, Lithuania, Kenya and Germany. As the demand of cigarette was increase, a huge number of cigarettes enters the country from Djibouti and Somalia into the southeast illegally (Contraband) (Global Data, 2019).

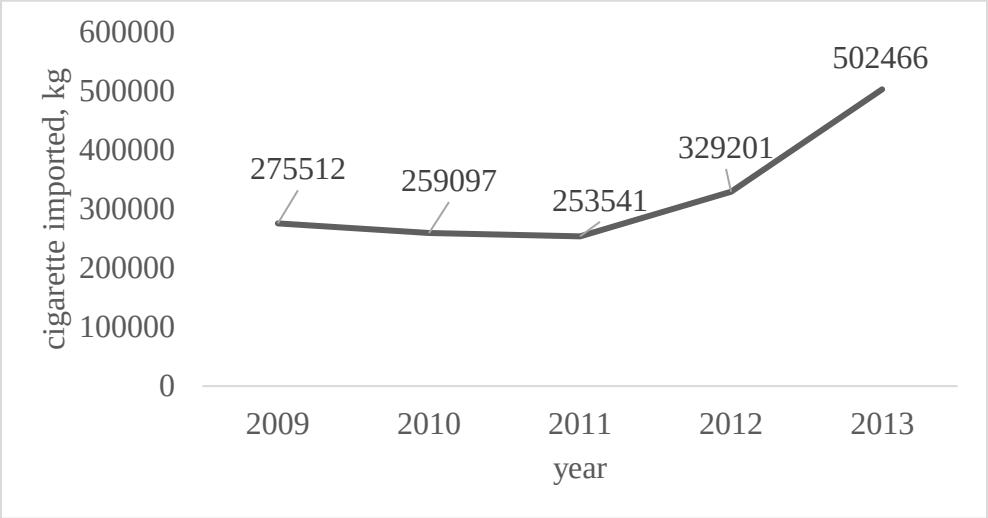


Figure 2.2 Amount of cigarette imported in Ethiopia from 2009 - 2013

2.4 Cigarette Waste

There are two ways of cigarette wastes such as cigarette wasted at factory level and cigarette butts. A cigarette butt is a waste produced when a cigarette is smoked and discarded to any part of the environment by smokers. Cigarette butts and other tobacco product waste items are the most ubiquitous form of litter worldwide. In 2012, the global tobacco industry produced an estimated 2262 million kilograms of manufacturing waste. In addition, total nicotine waste produced in the manufacture of reduced nicotine cigarettes was estimated at 300 million kilograms (Oguh et al. 2019).

China produced and consumed 400 to 500 million metric tons of tobacco yearly, and more than 200 million tons of tobacco waste pieces of stuff are produced per annum in tobacco farming and cigarette manufacturing industries. From these wastes, more than 20% (> 40 million tons) of the tobacco leaves are discarded during cigarette processing (Novotny and Zhao 1999).

2.4.1. Weight of Cigarette Butts

The butt is typically about 30% of the cigarettes' original length. It consists of a tissue tube which holds a filter and some remains of tobacco mixed with ash. No one knows the exact number of cigarette butts which are littered annually (Rabinoff, 2007). According to tobacco Atlas (2018), the weight of cigarette waste was estimated as; from 1pack, 3.4 grams; 15 billion (approximated daily sold), 2551000 kilograms; and 5.6 trillion (approximated yearly consumed) 950000000 kilograms were obtained. As 4.5 trillion cigarette butts are discarded, 765.4 million kilograms of filter paper could generate. This quantity does not include the weight of the tobacco still attached to the filter, or the packaging, matches, disposable lighters, and other collateral waste that is generated by smoking.

2.4.2 Cigarette wastes in Ethiopia

A huge number of cigarettes are produced, consumed and wasted in NTE. Data regarding trends in cigarette use are limited in Ethiopia since it is confidential. A few studies regarding the prevalence, distribution and social determinants of cigarette smoking and the level of nicotine present in Ethiopian cigarette were conducted. Dereje et al. 2014, Eticha and Kidane 2014, Reda et al. 2012, Rudatsikira et al. 2007, Schoenmaker 2005 were conducted a research on prevalence, distribution and social determinants of smoking cigarette while Geto et al 2012 and Kasa et al 2013 conducted on nicotine level of Ethiopian cigarettes.

Even if there no numerical quantitative data of cigarette butt weights as a national level, there is a number of smoked cigarettes per year. According to data 2012/13 estimates, over six billion stick of cigarettes were smoked. According to Global Data (2019) reports, 6.2 billion stick cigarette in 2011, 7.5 billion cigarette in 2016 and 7.4 billion stick cigarettes in 2017 were smoked. Since all people discard the butts to the environment, it is easy to calculate the weight of the cigarette from data of Census of United States in 2005. According to this data, in united states 360 billion cigarettes smoked in 2007 and weighted a total of 135,000,000 pounds (61.29 million Kg) of discarded butts. Hence, in Ethiopia in 2017, around 1259850 kg of cigarette butts were thrown on the environment (i,e weight of $CB_{2017} = \frac{7.4}{360} * 61.29$ million = 1259850 kg of cigarette paper only.

The weight of cigarette butts in each year is varies based on the size of the butts, number of smokers and the average amount of cigarette smoked in a day by individual person. According to GATS (2016), an individual person averagely smoked 10.3 manufactured cigarette daily.

2.4.3 Components of Cigarette wastes

Tobacco

Cigarette butt contains tobacco fillers, additive chemicals, papers and inks. Shevchenko (2012) stated that there are many known species of tobacco, one of which is Nicotiana tabacum. It is a tobacco containing a powerful alkaloid drug called nicotine. Wulan (2014) reported that tobacco is the major raw material for cigarette manufacturing.

Non-Tobacco Components of a Cigarette wastes

According to Dustin, Shahana, and Steven (2016) reports, cigarette butts have the following components in addition to tobacco.

i) Cigarette Paper: it is made of pure cellulose pulp from textile fibers such as flax or hemp or from wood. It may be porous to varying degrees (i.e. capable of letting through a stream of air that regulates how the tobacco burns.

ii) Filter: cigarettes are made of tow cellulose acetate. They are designed to trap nicotine and tar to varying degree.

iii) Side seam adhesive: is used in small amounts to secure the cigarette paper around the tobacco rod.

iv) Monogram ink: a small brand identifying mark (monogram) is often printed either on the cigarette paper towards the filter end of the cigarette or on tipping paper. The monogram is printed on the paper using a minute amount of ink which contain e.g black, blue, red and/or yellow pigment

v) Filter adhesives:

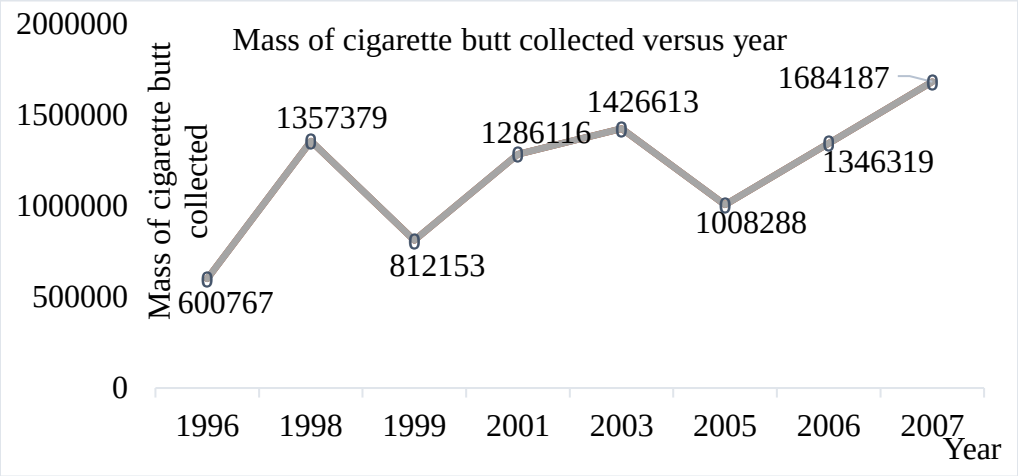
2.4.4 Environmental Effects of Cigarette Butts

Cigarette butts are the most common form of urban litter in the world. In Australia, 7 billion cigarette butts are discarded to the ground, of which 10% ends up in water bodies (Novotny et al. 2009). In the world, this figure rises to more than 5.6 trillion. Cigarettes and their wastes deposited as discarded filters with remnant tobacco contain many chemicals that may be harmful to the environment. These chemicals are sourced from agricultural treatment of tobacco plants, uptake from contaminated soils, addictive instilled in the manufacturing process, the attached cellulose acetate filter and combustion products generated in the course of smoking cigarette. This comprises the largest percentage of waste approximately 38% of the total waste products (Novotny and Zhao 1999). Novotny et al. (2011) reported that cigarette butts are commonly discarded onto beaches, sidewalks, streets, parks and many other public places where children, domestic animals and wildlife may be exposed to risk of ingestion. Lozano-rivas et al. (2015) estimated an amount of cigarette butts tossed on the street and sidewalk in Bogota. He concluded that an estimate of 94.9 million cigarette butts with a weight of 16 tons, are annually dropped on streets and sidewalks of the nightlife areas of Bogota. Figure 2.3 shows the cigarette butts discarded to the waterways

Source: (Cigarette Butt Litter, Beachapedia.html, 2019)

Figure 2.3 Cigarette butts disposed on waterways

About 680000 tons of cellulose acetate was used in the production of filtered cigarettes. With 5.6 trillion filtered cigarettes consumed worldwide in 2007, and nine trillion expected by 2025, the global environmental burden of cigarette filters is also significant. It is estimated that 1.69 billion pounds (845000 tons) of butts wind up as litter worldwide per year (Novotny and Slaughter 2015). This weight of cigarette butts is not include the weight of tobacco attached to the butts, and fill the volume of 2.8 million metric cube (Census of US, 2005).



Source: (Novotny and Slaughter 2015)

Figure 2.4 Cigarettes and Cigarette Filters Collected (tons) in the United States in the International Coastal Cleanup, 1996-2007.

Novotny (2011) studied that smoked cigarette filters can cause harm in the marine environment in several ways. They present a vector for the transport and introduction of toxicants, including heavy metals, nicotine and known carcinogens to aquatic habitats. According to Slaugher and collagues (2011) report, the chemical leached from smoked cigarette butt with remnant tobacco was the most toxic to marine water compared to smoked cigarette with no remnant tobacco and unsmoked cigarette filter with no tobacco. Shevchenko (2012) explained that Smoked cigarettes butts contain numerous chemicals, such as ammonia, formaldehyde, butane, acrylonitrile, toluene, benzene, alkaloid nicotine. Small animals are often the receiving end of leached chemicals and toxins. They may be especially harmed by nicotine, ethyl- phenol, and other organic compounds in cigarettes. Ethyl-phenol, which is commonly used to flavor tobacco, can accumulate to such high levels in small animals that they exceed the concentration in the surrounding environment.

2.5 Products of cigarette wastes

Cigarette waste could be reused/ recycled to produce several products. In China, plastic pyrolysis plants, TerraCycle company, recycles millions of cigarettes wastes into industrial plastic products such as shipping pallets, railway sleepers, frisbees, bricks, and ashtrays. Since they launching in the early 2000s, now they have more than 7000 cigarette recycling bins in nine countries around the world including the United States, the United Kingdom, and Australia, and people can also send their old cigarette butts in for free to have them recycle (Fiona, 2016).

In India, the study conducted by Vahidhabanu et al. (2014) suggested that crude extracts from littered cigarette butts is used as a corrosion inhibitor for J55 oil well tubular steel. According to Rahman, Mohajerani, and Giustozzi (2020) studied, cigarette butts is used for fiber modifier in bitumen as asphalt concrete. Aeslina Abdul Kadir and Abbas Mohajerani, had worked research on the Possible Utilization of Cigarette Butts in Light Weight Fired Clay. Assres and Abate (2019) recycled cigarette butts to home decor and shoulder pads for making of suits and jackets in Ethiopia.

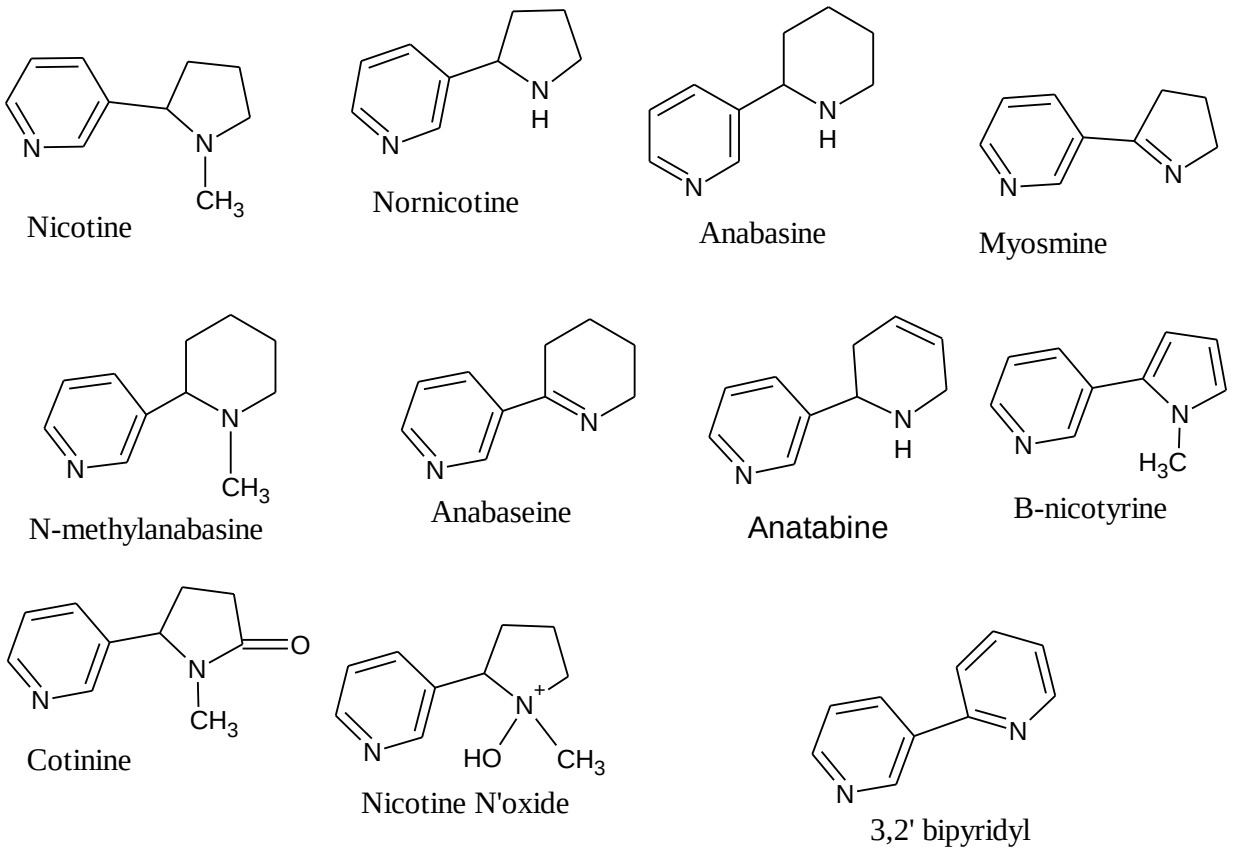
Any cigarette wastes contain unburned tobacco and papers. Discarded tobacco leaves with cigarette butts and at industry are not used for other purposes and pollute the environment despite containing an abundance of potentially useful compounds such as protein, polysaccharides, and aromatic compounds (Zhang 2015). Therefore, it is important to study and utilize the tobacco leaves discarded for reprocessing. Approximately 0.6 % to 5.0 % of the dry weight of tobacco is nicotine, which has been used as a plant pesticide (Novotny and Zhao 1999). Cigarette butts are one of the most common garbage worldwide, as an estimated 4.5 trillion cigarette butts are deposited somewhere into the environment every year. Barnes and Francisco (2011) studied that typically discarded cigarette butts consist of unsmoked remnant tobacco (including partially smoked tobacco on the end), the filter and a paper wrap. The tobacco parts of these cigarette wastes could not be reused to produce insecticide. So, this study try to extract an insecticide chemicals called nicotine, characterize and optimize from these cigarette wastes.

2.6 Nicotine

Nicotine is named by JeanNicot, French ambassador in Portugal, who sent tobacco and seeds from Brazil to Paris in 1960 and promoted their medicinal use. It is the most abundant insecticidal compounds in a tobacco leaf. As Asthana et al. (2004) definition, nicotine (pyridine, 3-(1-methyl-2-pyrrolidinyl), (S) is one of the highly toxic tobacco alkaloids present in tobacco leaves and cigarette smoke. Its CAS registry number is 54115. Nicotine appears to be a promising tracer for environmental tobacco smoke (ETS) because of its specificity for tobacco. It is also a systemic and contact insecticide and is also used as a drug. Yildiz (2004) defined nicotine as a naturally occurring alkaloids found primarily in the Solanaceous plant family such as potato, tomato, green pepper in addition to tobacco.

According to US EPA report of 2008 explanation, nicotine is acutely toxic by all routes of exposure (oral, dermal, and inhalation). The LD_{50} of nicotine is 50 mg/kg for rats and 3 mg/kg for mice. A dose of 40–60 mg can be a lethal dosage for adult human beings and doses as low as 1-4 mg can be associated with toxic effects in some individuals. Nicotine is an agonist at nicotinic receptors in the peripheral and central nervous system. Xing, Li, and Ping (2009) recorded that nicotine accounts approximately 95% of total alkaloids in tobacco.

Olsauskaite (2015) reported that tobacco contains more than 20 different alkaloids that are biologically active substances and structurally related to nicotine. The minor tobacco alkaloids include nor-nicotine, anabasine, anatabine, N-methylanabasine, anabaseine, cotinine, and some others which are also pharmacologically active but less potent than nicotine. The concentration of anatabine, anabasine and nornicotine equals approximately 5 percent of nicotine concentration (Dwoskin, 2016).



Source: sketched by ACD/chemsketch freeware version 12

Figure 2.5 Chemical structure of Nicotine and related compounds

2.6.1 Level of Nicotine in Tobacco and Cigarettes

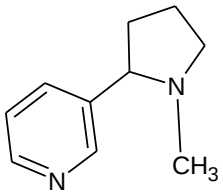
The nicotine content of tobacco leaves generally ranges from 0.3 to 5% and sometimes 7 % have been recorded in some heavy bodied tobaccos (Zhang, 2015). Tassew (2015) reported that in China, the nicotine level of tobacco ranging from 0.78 to 3.26 % depending upon different leaf position and different treatment. A normal cigarette contains 10-30 milligram of nicotine while a cigarette butt contains 5-7mg (Takematsu, 2020).

Geto et al. (2012) reported that the nicotine level of Premium Nyala and Gissila of Ethiopian cigarette are 3.84 and 4.26 %, while Kassa et al. (2013) reported the nicotine levels in Nyala and Delight as 2.04 and 2.54 % respectively (Kassa and Admassie 2013).

2.6.2 Toxicity of Nicotine to Insect Pests

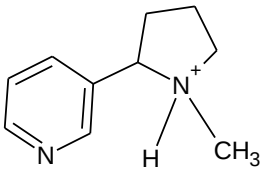
Nicotine is occurred in three forms. Free nicotine, monoprotonated and diprotonated forms as shown in Figure 2.6. Free nicotine is the most toxic to insect pests. It causes ascending motor paralysis of the nerve cord. Injected nicotine caused almost immediate reaction in the fly phormia. The abdomen, legs and wings quired violently. Silkworm larvae showed violent convulsions followed by paralysis when injected by nicotine. In the case of phormia, the nicotine was the more effective as the point of injection approached the central ganglion. The roach heart was much more responsive to nicotine showing a marked stimulation at concentrations as low as 0.0005% without subsequent depression. At higher concentrations, this stimulation was followed by partial depression or complete depression and paralysis, and the heart stopping in stole.

A maximum toxicity is apparently associated with the relative points of attachment of the two nitrogen containing rings linkage in the 3,2 or beta, alpha position as in the naturally occurring alkaloids. Quarantine (2018) studied that a concentration of 0.01% nicotine resulted in no loss in contractility, a concentration of 0.1% paralyzed the entire body with the exception of the heart and reduced the alary muscle responses where as 1% fresh nicotine stopped the heart and paralyzed the alary muscle. Kimura-kuroda et al., (2014) developed a theory that nicotine acted by interfering with the acetylcholine receptors in the synapse.



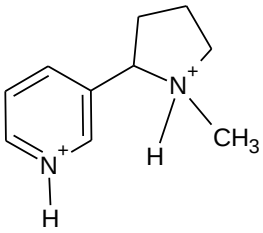
Free base Nicotine

3-(1-methylpyrrolidin-2-yl) pyridine



Monoprotonated Nicotine

1-methyl-2-(pyridin-3-yl) pyrrolidinium



Diprotonated Nicotine

3-(1-methylpyrrolidinium-2-yl) pyridinium

Figure 2.6 Forms of Nicotine structure

According to Zhang et al., (2000) reported, Nicotine was used for centuries in pest control, and synthetic neonicotinoids have in the past 10 years come to play a major role in integrated pest management, particularly for sucking insects such as aphids, leafhoppers, and whiteflies.

2.6.3 Efficiency of Free Nicotine compared to its Salts and other Alkaloids

Addition of salts may increase the effectiveness of insecticidal properties. Feinstein, (n.d.) tested, salts of nicotine with acids and metallic compound as stomach poison and contact insecticide. He found that water soluble salts such as laurate, oleate, stearate and naphthenate are not effective by stomach poison but highly effective by contact insecticides. Since a very large proportion of nicotine utilized for insecticidal purpose is in the form of nicotine sulfate, studies of the relative toxicity of nicotine as the free alkaloid and the nicotine sulfate are interest. Feinstein found that the efficiency of nicotine and nicotine sulfate to aphids as both as spraying and as fumigant, increased with the pH of the solutions.

Zammit et al., (2014) compared the toxicity of anabasine, nornicotine and nicotine to several species of mites and insects. Anabasine was the most toxic to the cabbage aphid, pea aphids, nasturtium aphid and citrus red mite whereas nornicotine and nicotine being equal, and nicotine was the most toxic to the large silkweed bug. Larvae et al., (2014) found that nicotine was much more effective than anabasine and nornicotine when tested against the codling moth. All three compounds gave approximately equal results against the celery leaf tier and red spider. A basic structure of the two six membered nitrogen containing rings as in anabasine seems to be more active than the combination of a six membered with five membered rings as in nicotine. The presence of the methyl group on the pyrrolidine nitrogen does not seem important as toxicity as nornicotine and anabasine, which are slightly more effective than nicotine and hence, anabasine and nornicotine have been recorded as being more effective insecticides than nicotine. Joseph and his collageous (1935) have shown free nicotine to be more toxic than ionized nicotine in the solution. They investigated that at pH = 5, the free nicotine base was 5 to7 times as toxic as nicotine sulfate. The toxicity of free base nicotine is increased with increasing pH and reaching the maximum at the highest pH. Also, they found that anabasine sulfate is five times as toxic as nicotine sulfate to aphis while anabasine nearly ten times as effective as nicotine to the same insect. According to Soloway (1976) studies, the effects of nicotine on aphis rumicis is based on optical isomerism. Yokotani (2014) decided that natural l-isomers are appreciably10 times more toxic than the d-isomers. Of equal or greater significance is the effect of having a hydrogen in place of methyl joined to nitrogen of the saturated heterocycle; the N-H compounds are more active.

This difference is particularly striking when the saturated heterocycle is the six-membered piperidine; Anabasine is ten times as active as nicotine. Conversely, when the aromatic pyrrole ring is joined to pyridine, as in nicotyrine, the activity is one-tenth that of nicotine (refer figure 2.5 for all compounds).

2.6.4 Properties of Nicotine

The chemical properties of pure nicotine are on UV light or various oxidizing agents, nicotine is converted to nicotinic acid, nicotine oxide and methylamine. As the active stimulant in tobacco products, it is a highly addictive drug when absorbed and exhibits neurotoxic properties in the body, and as a nitrogenous base, nicotine forms salts with acids that are usually solid and water soluble. The physical properties are summarized in Table 2.3.

Table 2.3 Physical Properties of Nicotine

Molecular formula	$C_{10}H_{14}N_2$
Molecular mass	162.23g/mole
Odor	Colorless to pale yellow liquid
Appearance	Weak amine-like, stronger at high temperature
Density	$1.01 g/cm^3$
Melting point	-79°C
Boiling point	247°C
Solubility in water	Miscible
Solubilities	Very soluble in alcohol, chloroform, kerosine, diethyl ether, oil and petroleum ether
Vapor pressure	0.038mmHg@ 25°C
Flash point	95°C
Optical Density	-168.5@25°C
Refractive index	1.53

2.7 Extraction Technology

Extraction is the first step to separate the desired natural products from the raw materials. Many researcher extract nicotine from tobacco by solvent extraction to investigate the nicotine contents of cigarettes. Bernardo-Gil (2017) extracts nicotine from tobacco leaves by steam distillation, ultrasonic, Soxhlet and microwave extraction method to compare their efficiency, advantage and disadvantages on extraction.

Rabinoff (2018) studied the extraction of nicotine from tobacco leaves of Gold Live Classic Brand cigarettes by liquid-liquid extraction method. As a general, the extraction processes of nicotine from its feedstock needs selected extraction method, suitable solvents and appropriate characterization instruments.

2.7.1 Selection of Extraction methods

As discussed above, several extraction methods were used to extract nicotine from tobacco. However, one has an advantage over the other based on several factors such as availability, easy to operate, smaller consumptions of energy, solvent and times, yielding higher quality and quantity of products and etc. According to Mishra, Sharma, and Sharma (2014) study on the comparison of ultrasonic bath, Soxhlet and microwave method of pesticide extraction from botanical parts, the extraction technique with the best efficiencies was the ultrasonic extraction. The maceration, solvent extraction method, have been suggested by Novotny, (2013) as more applicable, convenient and less costly methods for small and medium enterprises compare to other modern extraction methods. According to Myroxylon Balsamum (n.d), hot maceration process is mainly used to decrease the duration of extraction of active compounds. In this process, the long effleurage time is reduced by the immersion of samples in heated material at 45 - 60^{o}C for 3 to 5hr, depending upon the plant species.

2.7.2 Selection of Solvents

The solvent is one of the determining factors in the extraction process. The selection of the solvent is crucial for solvent extraction. Selectivity, solubility, cost and safety should be considered in selection of solvents. Based on the law of similarity and inter-miscibility (like dissolves like), solvents with a polarity value near to the polarity of the solute are likely to perform better and vice versa. Solubility of the solvent so as to produce the extract as much as possible. The effectiveness of organic solvents can be achieved by mixing different polarity.

Zhang (2018) stated that based on the principles of inter-miscibility, nicotine is soluble to some types of solvents such as alcohol, chloroform, ether, petroleum ether, kerosene and water for the basis of nicotine extraction from free tobacco using solvent extraction methods. Alcohols (EtOH and MeOH) are universal solvents in solvent extraction for phytochemical investigation.

2.7.3 Selection of Analytical Analyzer

There are a variety of methods/techniques used for the determination of nicotine and related compounds in biological samples. Dustin, Shahana and Steven (2016) stated that nicotine is more volatile than most TSNAs, making a wider range of analytical methods available. The most common analytical methods used to determine the nicotine from the tobacco extracts are thin layer chromatography, gas chromatography coupled with mass spectrometry (GC-MS), and high-performance liquid chromatography. Compared to other currently utilized methods for the detection of nicotine in tobacco extracts, GC-MS provided advantages of high sensitivity, nicotine specific detection and lower instrumentation cost.

2.8 Emulsification of Insecticides

A pesticide formulation is a combination of active and inert ingredients that forms an end use pesticide product. An emulsion occurs when one liquid is dispersed (as droplets) in another liquid. Each liquid retains its original identity. Emulsifiable concentrates are liquid formulations where the active ingredient is dissolved in oil and an emulsifier is added so that the formulation may be mixed with water for spraying. ECs typically contain 0.24 to 0.72 gram/ml (2 to 6 pound/galloon) of active ingredient. They are adaptable to many types of application equipment, from small, portable sprayers to hydraulic sprayers, low-volume ground sprayers, mist blowers, and low-volume aircraft sprayers. Its advantages are; relatively easy to handle, transport, and store, little agitation required

2.9 Mode of action of Nicotine Insecticides

Nicotine exerted a more pronounced blocking effects on the nerve impulse transmission than did eserine. It acts primarily on the ganglia of the insects' central nervous system, possibly at the synapse, causing excitation at low concentration and depression or paralysis at high concentrations. Nicotine is highly lipophilic and can pass through dermal tissues as well as the blood brain barrier (Oguh et al. 2019).

In both insects and mammals, nicotine is an extremely fast-acting nerve toxin. It competes with acetylcholine, the major neurotransmitter, by bonding to acetylcholine receptors at nerve synapses and causing uncontrolled nerve firing. This disruption of normal nerve impulse activity results in rapid failure of those body systems that depend on nervous input for proper functioning.

The receptors in mammals are located in the central and peripheral nervous systems, while in insects they are placed in the central nervous system only. All animals with nervous system have nAChRs. Nicotine is agonists of the α4β2 (alpha-4 beta-2 nicotinic receptor) that make up acetylcholine receptors. The difference between insects and vertebrates is that all nAChRs of insects contain these subunits, whereas in vertebrates only about 8–10% of nAChRs have them. The susceptibility of vertebrates to nicotine, therefore, is much less than that of insects.

2.10 Nicotine Pesticide Reregistration

As described above, nicotine that derived from the tobacco plant has been used as a pesticide since at least the 17th century. Its use in the United States began expanding in the 1950s and 1960s. After World War II, over 2,500 tons of nicotine insecticides were used worldwide, but by the 1980s the use of nicotine insecticide had declined below 200 tons due to the availability of other insecticides that are cheaper and less harmful to mammals. Formerly, nicotine is prohibited as a pesticide for organic farming in the United States. However, it is used as minor insecticides in other countries such as China, Canada, European union, Hindi, Latin America, Australia, New Zealand, Philippe and other (Tomizawa and Casida 2005). In US, there was a complain on the cancellation of registration of nicotine-pesticide, and the reregistration was started for special crops. The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) was amended to accelerate the reregistration of products with active ingredients registered prior to November 1, 1984. Nowadays, there are many commercial products of tobacco extract which used advanced technology to develop formulation of the extracts as concentrated emulsion which shows good physicochemical characteristic and good effects in order to scale up for commercial purpose (Puripattanavong et al. 2013).

3 MATERIAL AND METHOD

3.1 Materials and Chemicals

The experiment was conducted at Addis Ababa Institute of Technology, School of Chemical and Bio Engineering, Biochemical Engineering Laboratory. Physicochemical characterizations such as viscosity, density, pH, stability study and UV/vis. analysis were done in AAiT laboratory while GC-MS analysis, bomb calorimeter and du Nouy ring were performed in Addis Ababa Science and Technology University.

Chemicals: The chemicals used for this study were standard nicotine 98% pure, ethanol (96% pure), methanol (99.5 % pure), sodium hydroxide Chrystal, distilled water, Palm oil, sodium lauryl sulfate, ethoxylated sorbitan ester and cigarette and cigarette butt.

Materials: Oven, Digital balance, cylinder, flask (6), water bath, magnetic stirrer, filter paper, aluminum foil, vacuum evaporator, vacuum filter, GC-MS 6800, UV visible Spectrophotometry 3200, ruler, 3.15mm mesh sieve, scissor, pH meter, viscometer, Bomb calorimeter, du Nouy ring, Incubator, Autoclave, microscope, sprayer, petri dish, syringe, infested cabbage

3.2 Methods

The experimental processes of this research involve four basic steps. These were raw material acquisition and preparation (characterization), extractions, emulsification and efficiency testing. The detailed description was discussed below. The following figure shows the entire steps of overall processes.

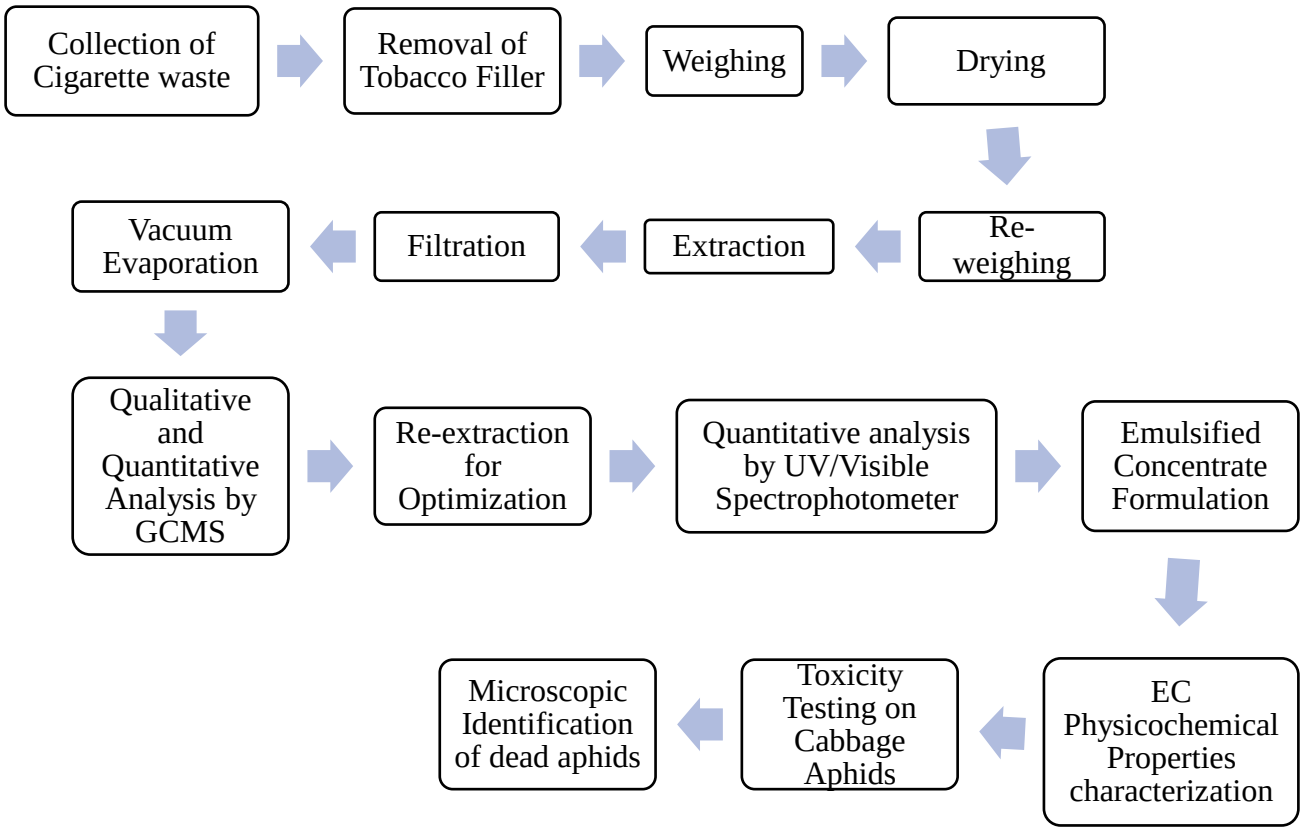


Figure 3.1 Block flow diagram of experimental processes

3.2.1 Raw Material Acquisition and Preparation

3.2.1.1 Sample and Reagent Collections

Pure standard nicotine was borrowed from NTE company, Pure 96% ethanol and NaOH were purchased from MTET laboratory chemical trading PLC, 99.5% pure methanol was purchased from Heparin laboratory chemical trading PLC, emulsifier (sodium lauryl sulfate and ethoxylated sorbitan ester were taken from ABIM Soap and Detergent Factory, waste cigarettes were collected from residence home and around Addis Ababa Universities (Sidist Kilo, Amist Kilo and Arat Kilo), and normal cigarettes of seven brands (Nyala, Premium Nyala, Delight, Winston, Marlboro, Oris, and BR) were purchased from shops.

3.2.1.2 Determination of weight of Tobacco of Cigarette wastes

The tobacco remain in the cigarette butt is varies based on the length of cigarette butts remain after smoking. In order to approximate the remnant tobacco and weight of cigarette butts discarded on the environments, the sizes of the cigarettes were taken at different sizes as shown in the figure 3.3. The sizes of full cigarette, filter paper, full tobacco-containing part, ¼, 1/3, ½, 2/3, and 3/4 of tobacco-containing parts were measured, and their masses were weighed for each length with and without cigarette papers.

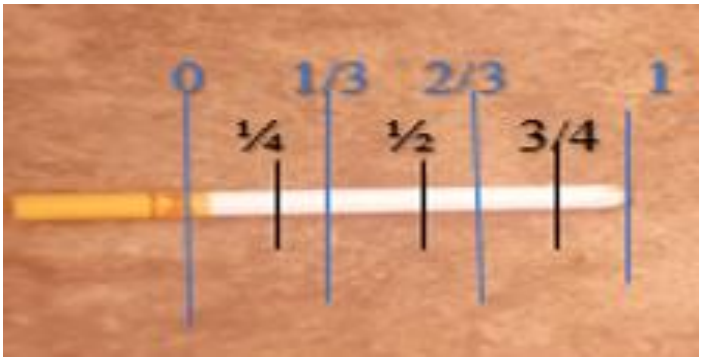


Figure 3.2 Possible length of cigarette butts contain remnant tobacco

3.2.1.3 Determination of Moisture Content

The method of determining the moisture content was based on loss on drying (LOD) moisture meter method (Bogart 2013). According to this method, the weight of tobacco filler removed from butts and raw cigarettes was measured before and after drying at 65^{o}C for three and a half an hour.

Figure 3.3 Moisture content determination a) cigarette butt; b) tobacco filler; c) Drying of tobacco filler under oven

The percent of moisture removed from tobacco filler was calculated by equation 3.2;

$$Moisture\ removed\ (\%) = \frac{W,initial - W,dried}{W,initial} * 100 \quad \ldots\ldots\ldots\ldots\ldots \quad 3.1$$

Where W,initial = weight of sample before drying; W, dried = weight of sample after drying.

The percent of total solid was simply calculated as;

Total solid (%) = 100 - % moisture removed ……..……3.2

3.2.2 Extraction process

The extraction process had two stages. The first stage was extraction done for analysis of qualitative and quantitative values of nicotine extracted at different ratio of methanol to ethanol (v/v) using GCMS whereas the second stage was extraction performed by using the selected ratio to investigate the effects of variables (temperature, time and molarity of NaOH) on extracts' yield and nicotine contents.

3.2.2.1 Extraction for Determination of Methanol to Ethanol ratio by GC-MS

The sample was ground to pass through 3.15mm mesh, and 6 g of the sample was put into a series of 250 mL round bottle flasks. Then different proportion of methanol to ethanol ratios as shown in Table 3.2 was added to separate flask, and 80 ml of 5M NaOH was equally added to each flask. The extraction was carried out in a water bath (Grant, precision, and immersion thermostat bath and circulation TX 150 and TXF 200) at a temperature of 65^{o}C and a speed of 5 for 3:30 hours.

Table 3.1: Ratio of methanol to ethanol (v/v) used during extraction.

Sample code	Methanol:ethanol ml:ml	Percent used, (MeOH; EtOH), %	Distilled water	p^H
A	60:0	100; 0	0	12.31
B	45:15	75; 25	0	12.18
C	30:30	50; 50	0	12.01
D	15:45	25;75	0	11.84
E	0:60	0;100	0	11.53
F	0:0	0;0	60ml (100%)	12

After the extraction was over, the nicotine-containing solution was filtered by filter papers with the help of vacuum filtration. Then the solvent was recovered by rotary evaporator at the temperature of 100^0C and speed of 65 RPM. Finally, the extract was analyzed by GC-MS 6800.

(a) (b) (c) (d)

Figure 3.4 Series of extraction process a) water bath extraction; b) filtration using vacuum filter; c) solvent recovers by vacuum evaporator and d) extracted samples

Qualitative Analysis

Qualitative analysis is used to identify the target molecules from the samples. The samples injected into the analyzer. Then an unknown spectrum is compared to reference spectra registered in spectral libraries and the similarity is estimated for each reference. A library search routinely informs the matched compounds with a greater similarity.

The user identifies the unknown spectrum by taking the pre-specified conditions such as compound name, molecular weight, chemical formula, and CAS registry number. The qualification, quantification, and confirmation ion of nicotine were 84, 133, and 162. The peak at m/z = 84 indicates the qualification of nicotine, the peak at m/z = 133 represents the quantification of nicotine and the peak at m/z =162 indicates the identification/confirmation of nicotine (Social, Insurance, and Unfallversicherung, 2013).

Quantitative Analysis

To quantify the nicotine in the sample, standard nicotine was first run (brief description is given in chapter 4, 4.3.2). The peak area versus concentration of nicotine was plotted to get slope. Using this slope, the concentration of nicotine in each sample was determined as equation 3.4.

$$Concentration\ of\ Nicotine(i)\left(\frac{mg}{ml}\right) = \frac{Peak\ area\ of\ standard\ Nicotine \pm Intercept}{Slope\ of\ standard\ calibration\ curve} \quad \ldots\ldots\ldots\ldots 3.3$$

Where i – stand for sample (A, B,…F)

Limit of detection (LOD) and Limit of quantification (LOQ) was calculated as 3.3 and 10 times standard deviation of the intercept divided by slope of the standard curve of the nicotine respectively according to Hossain and Salehuddin (2013) method.

Working condition for GC-MS 6800

GC-MS 6800 (rectangular with a length of 105 cm, width 60 cm, height 50 cm) analyses were performed with a flame ionization detector (FID). Helium 99.999% was used as carrier gas (mobile phase) with a 2 ml/min flow rate. The stationary phase was prepared by mixing methanol and ionized water with the ratio of methanol to distilled water (80:20 v/v). One microliter sample injection volume by split less mode was programmed from 70 to 200 ^{0}C (1 min hold) and raised to 300^{o}C at a rate of 10°C /min. Oven temperature is programmed 200-300^{o}C @ 10^{o}C/min. The selection ion monitoring (SIM) mode was used in the analysis. The operation of the MSD was in the electron impact (EI) mode at 70 eV, 230°C. The major ions peaks using the scan mode (10-200 a.m.u). The total retention was 25 minutes.

3.2.2.2 Extraction for Investigating the effects of factors on Responses

Using the ratio of methanol to ethanol at which maximum nicotine content was obtained, further extraction was taken to determine the influence of factors on responses. The procedure was similar to the previous one except for the variation of factors. Tobacco fillers removed from the collected cigarette butts were dried under an oven to remove moisture content at 65^{o}C until the mass of the dried tobacco became constants.

Two (2) grams of dried tobacco were measured and introduced to flasks. The extraction was performed at different variables such as temperature (30 – 60^{o}C), time of extraction (4 - 6 hr), and Molarity of sodium hydroxide (1 – 3 M). This molarity was prepared by dissolving 5 - 15 grams of solid sodium hydroxide in 125 ml of distilled water and stir using a magnetic stirrer to make homogeneity. Then 20ml (Djaafar, 2017) of each molarity was added for each sample. The 75% of ethanol and 25% methanol, which is determined by GC/MS Analysis, were used as an extraction solvent with 1:10 solid to the solvent proportion (Greene et al. 2013) under a water bath (Grant, precision, and immersion thermostat bath and circulation TX 150 and TXF 200). After extraction over, the extract and the filter cake were separated by filter paper with the help of vacuum filtration. The filtration was done twice by rinsing the cake with 10 ml ethanol to remove the nicotine remain in the filter cake. The solvent was removed by vacuum evaporator at a **t**emperature 100^{0}C and speed of 45 revolutions per minute for 2 hours from the filtrate to get viscous crude extracts.

The extracts contain several compounds that were present in tobacco leaves in nature and added to tobacco during cigarette processing. Generally, more than 4000 compounds were extracted with nicotine as GC/MS results indicate during the qualitative and quantitative determination of nicotine in this study. All compounds are useful since some of them are increased the efficiency of the nicotine (i,e anabasine, nornicotine, menthol, heavy metals, DDT, and other minor alkaloids) whereas the remaining compounds would use as inert ingredients. The crude extract was considered as yields. The technique to determine the nicotine contents in the extracts was performed by using UV/Visible 3200 spectrophotometer.

The standard curve used for calibration was graphed by plotting the absorbance versus concentration of standard nicotine at a wavelength of 602nm according to the method of Aldarmoon et al., (2015). The nicotine standard stock solutions were prepared by appropriate dilution of standard stock solution with methanol and ionized water.

The dilution was prepared by diluting the concentrated nicotine solution using the range between 0.213 to 1.624 g/L concentrated nicotine in order to get a linear curve for the calibration of the sample. The ratio of methanol to distilled water used was 1:1. The UV gives the absorbance of the sample.

Using this absorbance, the concentration of nicotine in the extracts was calculated by substituting the absorbance value into the standard curves.

$$X\ (concentration\ of\ Nicotine\ (\frac{g}{l}) = \frac{Y(Absorbance) \pm Intercept}{Slope} \quad \text{.....3.4}$$

The percent of nicotine content was determined by;

$$Nicotine\ content, \% = \frac{Concentration\ of\ nicotine\ \left(\frac{g}{l}\right) \times Volume\ of\ the\ extracts(l)}{Mass\ of\ the\ tobacco\ leaves\ (g)} \times 100 \text{.....3.5}$$

3.2. 2. 3 ANOVA Analysis

The design experiment was done by design expert version 12 using surface response method with central composite design (CCD). The ANOVA analysis was used to estimate the fit model and optimum conditions for independent variables.

Table 3.2 Factors and its levels

Factors	Level	
	Low	High
Temperature, ^{o}C	30	60
Time of Extraction, Hr	4	6
Molarity of NaOH, M	1	3

3.2.3 Emulsified Concentrate Formulation Processes

The concentrated emulsion formulations were prepared by emulsification methods at room temperature. The palm oil and emulsifiers (sodium lauryl sulfate (anionic surfactant) and ethoxylated sorbitan ester (nonionic surfactant)) were mixed first by the ratio of 1:4 (wt/wt). Then 13.6157gram of the mixture was added to 54.463 grams of crude extracts (10-gram nicotine) according to (Uri and Katepalli 2014). Finally, the mixture of all concentrated emulsion was homogeneously mixed by a homogenizer.

3.2.3.1 Physicochemical Analysis of Emulsified Concentrate (EC)

Specific gravity, viscosity, surface tension, flash point, pH, and emulsion stability are the most important physicochemical characteristics of the successful EC that have to be analyzed before spraying.

A) Specific gravity and Density

They were calculated by pycnometer balance methods using water as a reference. This was conducted at room temperature and pressure. Specific gravity is the measurement of the relative density of the sample with respect to water. This indicates that, the physical property of the sample immersion or flotation when it gains an opportunity to contact with water.

$$SG(sample) = \frac{mass\ of\ sample}{mass\ of\ reference\ (distilled\ water)}, for\ equal\ volume\ of\ sample\ and\ water. \quad 3.6$$

$$Density\ of\ sample = specific\ gravity\ of\ sample \times density\ of\ water\ at\ STP \quad \ldots\ldots\ldots\ldots 3.7$$

B) Flash point

Flashpoint is the lowest temperature at which a liquid can give off vapor to form an ignitable mixture in air near the surface of the liquid. The lower the flashpoint, the easier it is to ignite the material. Flashpoints are determined experimentally by heating the liquid in a container and then introducing a small flame just above the liquid surface. The temperature at which there is a flash/ignition is recorded as the flashpoint by a bomb calorimeter in the presence of oxygen. Generally, there are three main categories of product flammability. These are; extremely flammable (Flashpoint below 0^oC), highly flammable (Flashpoint below 21^oC), and flammable (Flashpoint below 55^oC).

C) Water solubility

Solubility is expressed in terms of the maximum volume or mass of the solute that dissolved in a given volume or mass of a solvent. This property is very crucial for this test. Since the final extract is going to be used by dissolving in water and spray on the field, it should not have left a residue over the crop. The more soluble mater in the solvent means the more dispersing material on the environment. The solution was left for an hour at standard temperature and pressure to get the maximum settled (undissolved) particles.

$$solubility, \% = \frac{Wi - Wj}{wk} * 100 \quad \text{.....3.10}$$

Where w_i- of sample before dissolution (g)

w_j- mass of settled sample (g)

w_k- mass of solvent (water, g)

D) pH value

pH is a numeric scale used in specifying the acidity or basicity of an aqueous solution. The acidity or basicity value of a sample would increase and decrease when dissolved and approached to the neutral scale. Due to this reason, the pH value of the extract was measured by dissolving 5ml of extract in 10ml of deionized water then directly immersing the rod to the solution and read the quantity directly at room temperature and 1atm. The pH of the formulations was evaluated using a pH meter at room temperature (25°C). The device was calibrated at pH 7.0 with distilled water.

E) Viscosity

Like specific gravity, viscosity can be used for predicting the type of creaming and the degree of spontaneous emulsification of emulsified concentrate. The viscosity of formulation was determined by using a viscometer for temperature of (18 - 25°C) and shear rate.

F) Surface tension

It was found necessary to measure surface tension at the field dilution to predict the field wettability. The leaves could be easily wetted by spray liquid through the reduction of surface tension for increasing the spreading properties of droplets.

Surface tension was determined by the interaction of the platinum ring with the surface of the formulations utilized according to the du Nouy ring method, using a Kruss K6 tension meter to determine the surface tension.

Deionized water was used for calibration, with a surface tension of 72–73 mNm^{-1}. The leaves could be easily wetted by spray liquid through the reduction of surface tension for increasing the spreading properties of droplets.

G) Stability of Emulsified Concentrate (EC)

Stability studies were conducted under cold, room, and hot temperature under incubator for a time of between 1 and 18 hours. In each cycle, each sample was kept at 4°C for 18 h and at 45°C for 18h. The sample was then evaluated for its stability after physical properties such as changes of physical appearance, pH, and density are re-evaluated according to (Puripattanavong et al. 2013).

3.2.4 Efficiency Testing for EC

The concentrated emulsion was diluted to ratio of 0.5:100, 1:100, 1.5:100, 2:100, 3:100 and 4:100 (w/w). the prepared dilution was sprayed to the aphids selected for experiments. The aphids died at a specified dilution ratio and time was identified naked eye and checked by microscope for confirmation. The efficiency was calculated in terms of counted and percent of died as expressed by equation 3.10 for dilution ratio, i.

$$Efficiency(i), \% = \frac{Number\ of\ died\ aphids}{Total\ aphids\ present} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.9$$

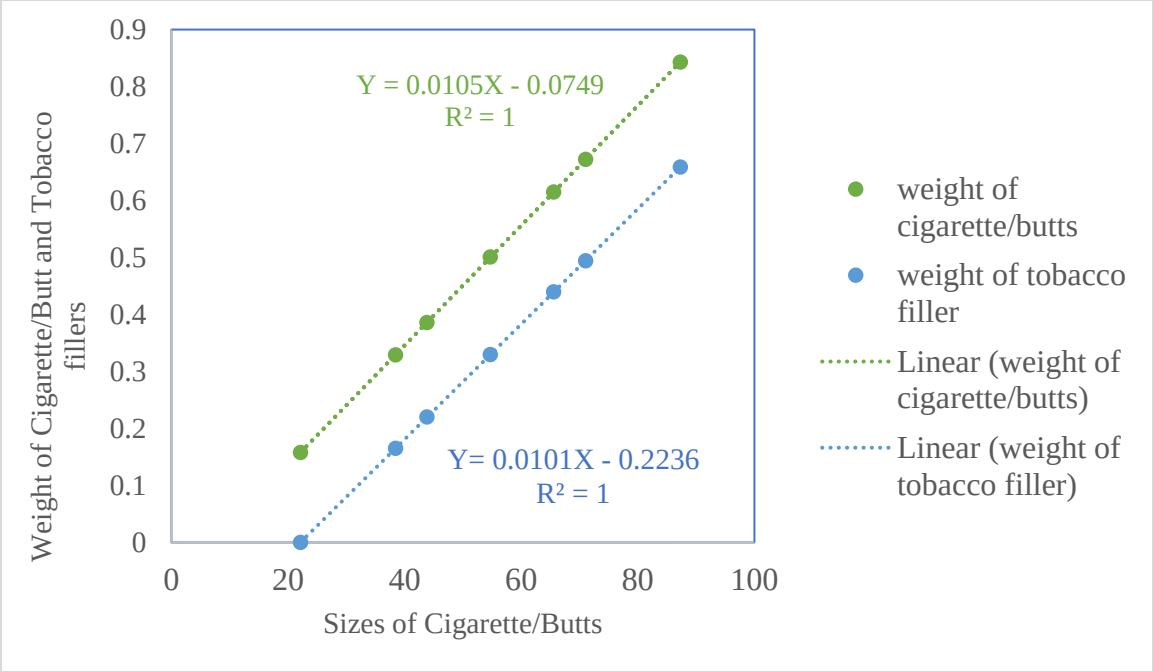
4 RESULT AND DISCUSSION

4.1 Estimated weight of Tobacco of Cigarette wastes

Different brands of cigarettes have different sizes, internal diameter, and length of filter paper. The sizes of the cigarettes are different as the companies differ. The purpose of knowing the size of a cigarette is to estimate the amount of tobacco in the cigarette and cigarette butt. The average length of all cigarette brands was estimated at 87.286 ± 6.396 mm and 0.843 ± 0.159 gram. The approximate range of sizes of cigarette butts mostly discarded to the environment was between 28.473 and 61.413mm. At this range, 0.225 to 0.571 gram of cigarette butts, and 0.0166 to 0.3864 gram of remnant tobacco was obtained from a single cigarette butt. The weight of a single cigarette butt and remnant tobacco in a smoked cigarette could be obtained from the size of cigarette butts by regression models. Both weight of cigarette butts and remnant tobacco fit the maximum regression ratio (R^2) for linear model. Equations on graph show the models used to calculate the average weight of cigarette butts and remnant tobacco of cigarette butts. The size below second row is the size of cigarette butts whereas the remain indicates the size of full cigarette.

Table 4.1 Weight of remnant tobacco remained in different possible sizes of cigarette/ butts

Average length of cigarette/ butts of 7 brands, mm	Weight of paper + tobacco, gram	Weight of tobacco, gram
87.286	0.843	0.659
71.043	0.672	0.494
65.586	0.615	0.437
54.714	0.501	0.329
43.843	0.386	0.221
38.429	0.329	0.165
22.143	0.158	0



The graph was plotted using the length of cigarette butts as x-axis and weight of cigarette butts and its respective remnant tobacco as the y-axis.

Figure 4.1 Relation of weight of cigarette waste, remnant tobacco and sizes of cigarette waste

4.2 Determination of Moisture content

Moisture content determination of tobacco filler is one of the most important in improving both the quality and quantity of yields. It is true that the moisture content of fresh cigarettes of all brands is not the same. The average moisture content was given as

$$Moisture\ content, \% = \frac{4.941 - 4.498}{4.941} \times 100 = \frac{0.443}{4.941} \times 100 = \ 8.966\%$$

The content of moisture in the cigarette filler is low compared to green leaf threshing tobacco because it dried under the drying chamber during cigarette processing. The moisture content of green leaf threshing of tobacco is from 17 to 22%.

The total solid becomes 100-8.966 = 91.034%

4.3 GC-MS Analysis

GCMS used to determine both qualitative and quantitative values of nicotine in the samples extracted by different proportion of ethanol and methanol.

4.3.1 Qualitative Determination of Nicotine Extracts

Taking the structure of the nicotine molecule, the cleavage of some fragments at one or more bonds gave different mass to charge ratio. The molecular ion of m/z 162 indicates the identity of nicotine, and the intensity that occurred at m/z of 133 shows the abundance of nicotine molecules in the sample. When the bond between the two-nitrogen heterocycle was broken, two fragments were formed and their peaks displayed on the graph. The base peak (BP) appears at m/z = 84 (99.99%) and the peak at m/z = 78 (2.99). The intensity at m/z = 84 would be a qualitative determinant of nicotine.

4.3.2 Quantitative Determination of Nicotine

The quantitative value of nicotine was analyzed using standard nicotine. The concentration of standard nicotine was prepared by dissolving nicotine in methanol (HPLC grade, 99.5%) as shown in Table 4.2, and the peak area was taken for each concentration at specified retention time. Then the graph was plotted as peak area versus concentration of standard nicotine for calibration. Based on this calibration, the concentration of nicotine in a sample would be calculated.

Table 4.2 Concentration of standard nicotine for GCMS calibration

Concentration of standard nicotine µg/ml	Observed Peak area
100	3841739
200	8189565
300	12537391
400	16885217
500	21233043
600	25580870
700	29928696
800	33276568

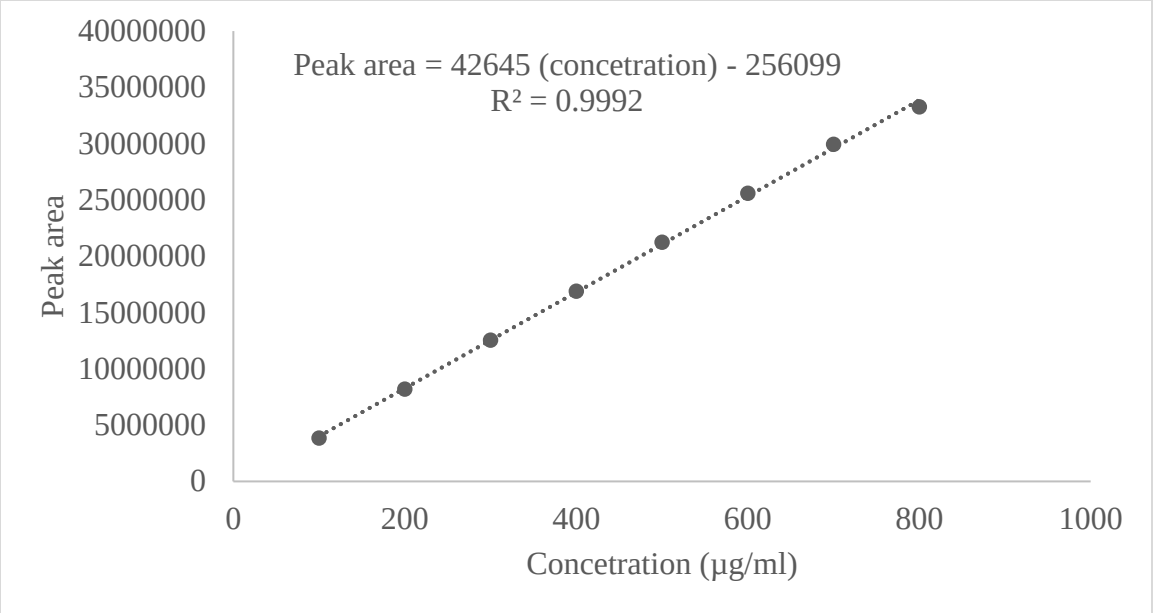


Fig 4.2 Calibration curve of standard nicotine for GCMS analysis

4.3.3 Determination of Methanol to Ethanol ratio

To determine the critical ratio of methanol to ethanol, the concentration of nicotine extracted by the mixture of them was calculated using the calibration curve given above. As shown in Table 4.3, the maximum concentration was obtained for 75% ethanol and 25% methanol extraction and selected for further processes.

Table 4.3 Concentration of Nicotine in a Sample extracted by ethanol to methanol ratio

Sample code	Ethanol: Methanol (ml/ml)	Peak area	Concentration of Nicotine in a sample (µg/ml)
A	0:60	13332876	318.65
B	15:45	16022570	381.73
C	30:30	15407084	367.29
D	45:15	16646084	396.35
E	60:0	13456900	321.56
F	Distilled water	12962560	309.97

4.4 ANOVA Analysis of Factors on Responses

The influences of temperature, time, and concentration of sodium hydroxide on the extraction of crude extracts and the contents of nicotine in the extracted yield were analyzed by design expert version 12 with central composite design. Actually, the yield and content of nicotine have a direct proportion unless there was an effect of parameters on one of the responses over the other during the process.

The yield was calculated by; $yield,\ \% = \frac{m1-m2}{m1} \times 100$.....4.1

Where m_1 – mass of dried tobacco filler before extraction

m_2 – mass of filter cake (dried and measured until the mass became constants)

The concentration of nicotine in the extract for each factor was determined by Ultra Violet Visible (UV/Vis 3200) Spectrophotometer 3200 after the standard curve was plotted for calibration as shown in Figure 4.3. The yields and nicotine content results were given in Appendix B, Table B1.

Table 4.4 Concentration of standard nicotine for UV/Vis

Run	Concentration of diluted standard Nicotine (g/L)	Absorbance
1	0.213	0.102
2	0.401	0.391
3	0.624	0.603
4	0.802	0.782
5	1.032	1.003
6	1.262	1.262
7	1.401	1.398
8	1.624	1.604

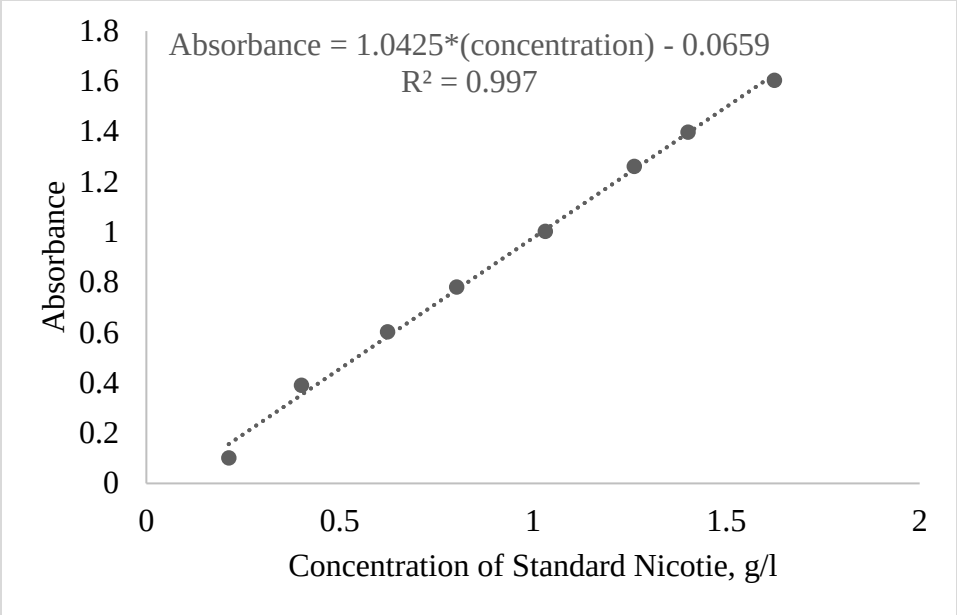


Figure 4.3 Absorbance versus concentration of standard Nicotine

4.4.1 Experimental Design Analysis Results

Response Surface Method with Central composite design at alpha (α = 2) design expert 12 software was used to determine the effects of different parameter and their optimum condition for extraction of active compounds from tobacco fillers of cigarette butts. Different criteria should be fulfilled to check the adequacy of the model such as p-value, lack of fit F-value, Regression ratio, and others after the software did them. Statistical significance of the model and the factors would be determined at a probability (p) value of less than 5% at (α = 5%). Lack of fit F-value is a measure of all the factors' effects on the response. The higher value of lack of fit is good and implies the highest fitting possibility of the model. All analyzed data were shown in Table 4.5.

Table 4.5 Experimental Design of parameters
A: Design summary

Study type	Response Surface	Run	20
Initial design	Central composite (α =2)	Blocks	No
Design model	Quadratic		

B: Factors

Name	Units	Type	Min.	Max.	Coded Low	Coded High	Mean	Std. Dev.
Temperature	Deg. C	Numeric	15.00	75.00	-1 ↔ 30.00	+1 ↔ 60.00	45.00	13.76
Time of Extraction	Hour	Numeric	3.00	7.00	-1 ↔ 4.00	+1 ↔ 6.00	5.00	0.9177
Molarity of NaOH	M	Numeric	0.0000	4.00	-1 ↔ 1.00	+1 ↔ 3.00	2.00	0.9177

C: Responses

No.	Name	Units	Obs.	Analysis	Min.	Max.	Mean	SD	Ratio	Trans.	Model
R1	Crude Extracts' yield	%	20	Polynomial	14.204	17.958	16.28	0.9720	1.26	None	Quadratic
R2	Nicotine Content	mg/ml	20	Polynomial	2.6508	3.4786	3.08	0.1866	1.31	None	Quadratic

D: Fit summary for both responses

Response 1						**Response 2**					
Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	**Remark**	Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	**Remark**
Linear	< 0.0001	< 0.0001	0.7371	0.6095		Linear	0.0008	< 0.0001	0.5704	0.3149	
2FI	0.0024	< 0.0001	0.8895	0.8550		2FI	0.0564	< 0.0001	0.6982	0.5785	
Quadratic	**< 0.0001**	**0.9864**	**0.9987**	**0.9986**	**Suggested**	**Quadratic**	**< 0.0001**	**0.5832**	**0.9979**	**0.9953**	**Suggested**
Cubic	0.9740	0.7200	0.9980	0.9954	**Aliased**	Cubic	0.4691	0.5411	0.9980	0.9887	Aliased

E: ANOVA fit for Yield of Crude Extracts

Source	Sum of Squares	df	Mean Square	F-value	p-value	Remark
Model	17.94	9	1.99	1614.91	< 0.0001	Significant
A-Temperature	13.17	1	13.17	10669.01	< 0.0001	
B-Time of Extraction	0.6712	1	0.6712	543.80	< 0.0001	
C-Molarity of NaOH	0.1378	1	0.1378	111.67	< 0.0001	
AB	0.7110	1	0.7110	576.10	< 0.0001	
AC	0.0092	1	0.0092	7.44	0.0213	
BC	1.90	1	1.90	1536.51	< 0.0001	
A^2	0.1780	1	0.1780	144.21	< 0.0001	
B^2	0.0257	1	0.0257	20.85	0.0010	
C^2	1.11	1	1.11	901.70	< 0.0001	
Residual	0.0123	10	0.0012			
Lack of Fit	0.0012	5	0.0002	0.1051	0.9864	Not significant
Pure Error	0.0112	5	0.0022			
Cor Total	17.95	19				

F: ANOVA fit for Nicotine Content

Source	Sum of Squares	Df	Mean Square	F-value	p-value	Remark
Model	0.6608	9	0.0734	1025.19	< 0.0001	Significant
A-Temperature	0.2177	1	0.2177	3040.19	< 0.0001	
B-Time of Extraction	0.1760	1	0.1760	2457.43	< 0.0001	
C-Molarity of NaOH	0.0285	1	0.0285	397.72	< 0.0001	
AB	0.0895	1	0.0895	1250.04	< 0.0001	
AC	0.0034	1	0.0034	47.69	< 0.0001	
BC	0.0098	1	0.0098	136.15	< 0.0001	
A^2	0.0343	1	0.0343	478.68	< 0.0001	
B^2	0.0004	1	0.0004	6.04	0.0338	
C^2	0.0738	1	0.0738	1030.29	< 0.0001	
Residual	0.0007	10	0.0001			
Lack of Fit	0.0003	5	0.0001	0.8206	0.5832	Not significant
Pure Error	0.0004	5	0.0001			
Cor Total	0.6615	19				

G: Fit summary

Response	Std. dev.	Mean	C.V, %	R2	Adjusted R^2	Predicted R^2	Adeq. Precision
1	0.0351	16.26	0.2158	0.9993	0.9987	0.9986	151.5219
2	0.0085	3.08	0.2747	0.9989	0.9979	0.9953	138.2832

The Model F-value of 1614.91 for response 1 and 1025.19 for response 2 (Table 4.5 (E) and (F)) implied the model is significant for both. P-values less than 0.0500 indicate model terms are significant. In these case, A, B, C, AB, AC, BC, A^2, B^2, C^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. The Lack of Fit F-value of 0.11 and 0.82 for response 1 and 2 respectively implied that Lack of Fit was not significant relative to the pure error. There are 98.64% and 58.32% chances that a Lack of Fit F-value these large could occur due to noise for responses 1 and 2 respectively. A non-significant lack of fit is good; the model was best to select. The sum square for each response is type III- partial.

4.4.2 Development of Model Equation

A model equation is a representative equation that mathematically relates the responses with factors. A model of this study would be quadratic for both responses that were suggested by design expert software. Final Equation:

Yield of Crude Extracts $= +16.49 +0.9072*A +0.2048*B +0.0928*C -0.2981*A*B -0.0339*A*C - 0.4869*B*C -0.0841*A^2 +0.0320B^2 -0.2104C^2$ ……4.2

Nicotine Content $= +3.10 +0.1167*A +0.1049*B +0.0422*C -0.1058*A*B -0.0207*A*C - 0.0349*B*C +0.0369*A^2 -0.0041*B^2 -0.0542*C^2$……4.3

Where A – coded for temperature (^oC); B – extraction time (hr) and C – molarity of NaOH (M)

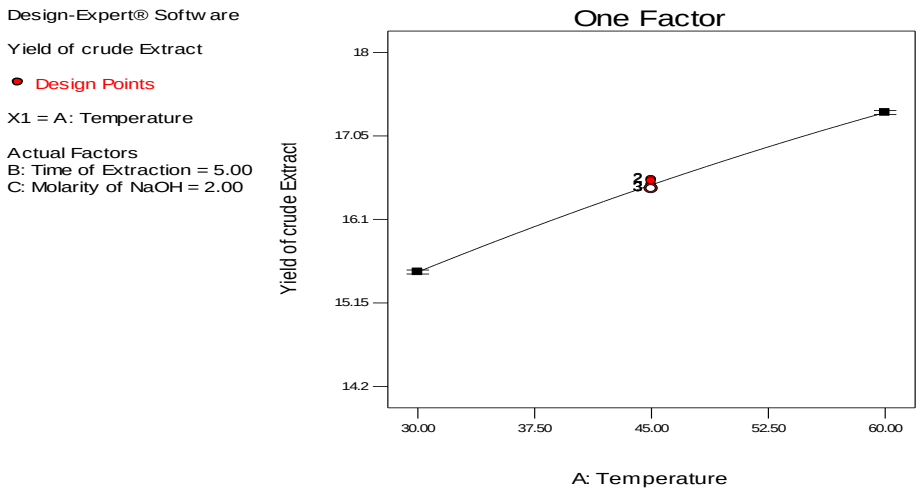
4.4.3 Model Adequacy Checking

From ANOVA results for responses that confirm the adequacy of the quadratic model was the probability p-value of less than 5%. The adequate precision measures the signal to noise ratio. The ratio greater than four (4) shows good desirability. The difference between Adjusted R^2 and predicted R^2 should be less than 0.2 for reasonable agreement for the fitness of statistics. Therefore, for both responses, the model could be navigating the design space as the difference of adjusted R^2 and predicted R^2 are 0.0001 and 0.0026, and adequate precessions are 151.5219 and 138.2832 for response 1 and 2 respectively as shown in Table 4.5 (G).

4.5 Effects of Individual Parameters on Yields and Nicotine contents

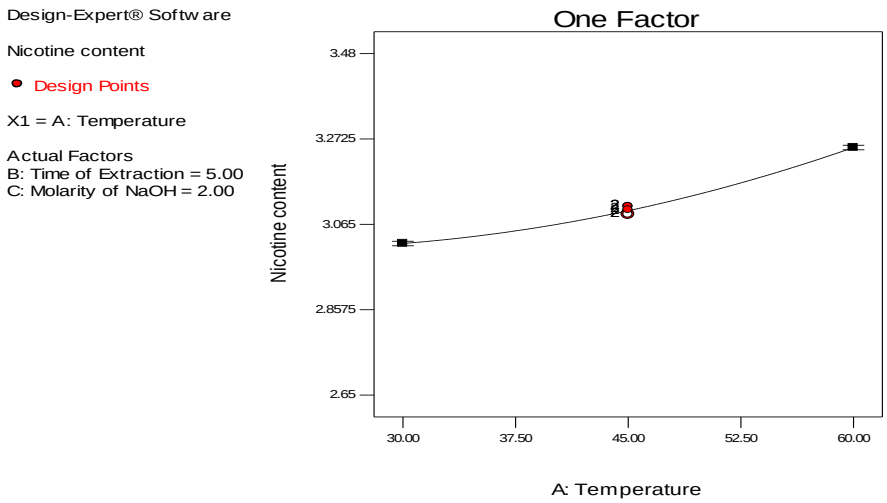
4.5.1 Effects of Temperature

The temperature has positive effects on both yields and nicotine contents as shown in Figure 4.4. As the temperature increased, the yield as well as the concentration of nicotine were increased. At low temperature (30^0C), 15.5014 and 3.01835 while at high temperature (60^0C) 17.3158 and 3.25166% yields and nicotine contents respectively were resulted. Since the boiling point of methanol and ethanol was 65 and 78^0C respectively, the temperature below their boiling point is selected.



m

(a)

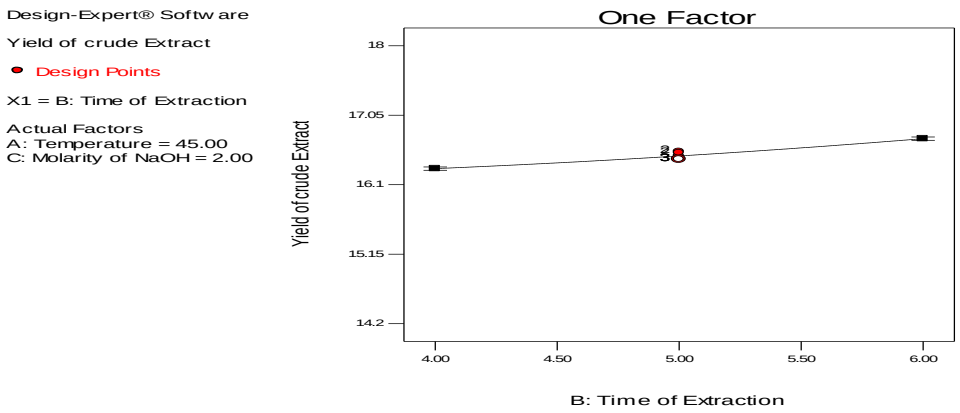


(b)

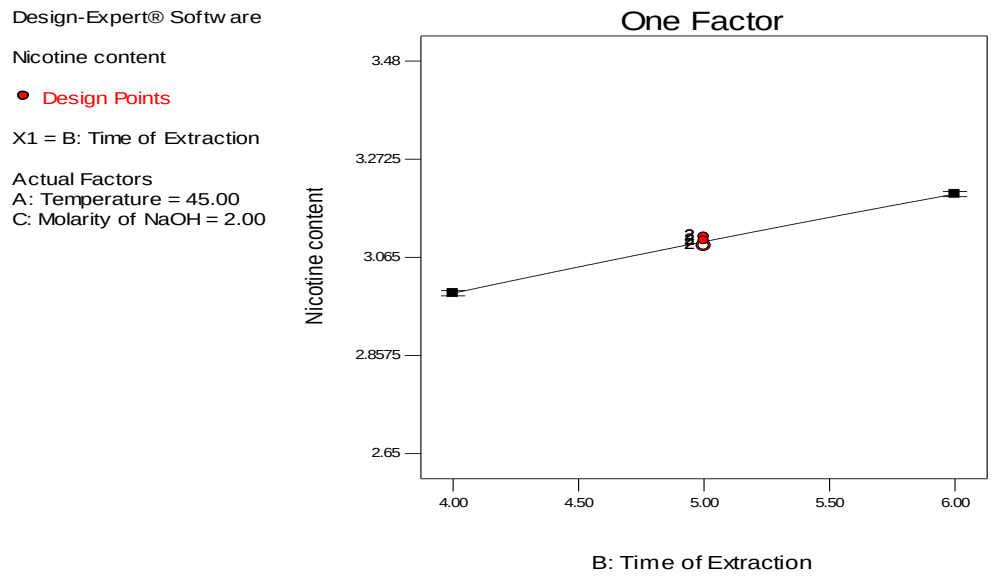
Figure 4.4 Effects of Temperature on responses a) Yields of extracts, b) Nicotine contents

4.5.2 Effect of Extraction Time

The time of extraction has a linear relationship with both yields of extracts and its Nicotine contents. As time increased, the nicotine and other compound were removed from the tobacco filler via solvents. After 5hr, the yield slightly increased while the nicotine content was linearly increased. The removal of solvents from the extracts increased the concentration of the nicotine. Figure 4.5 shows the influence of time on yield and its nicotine content.



(1)

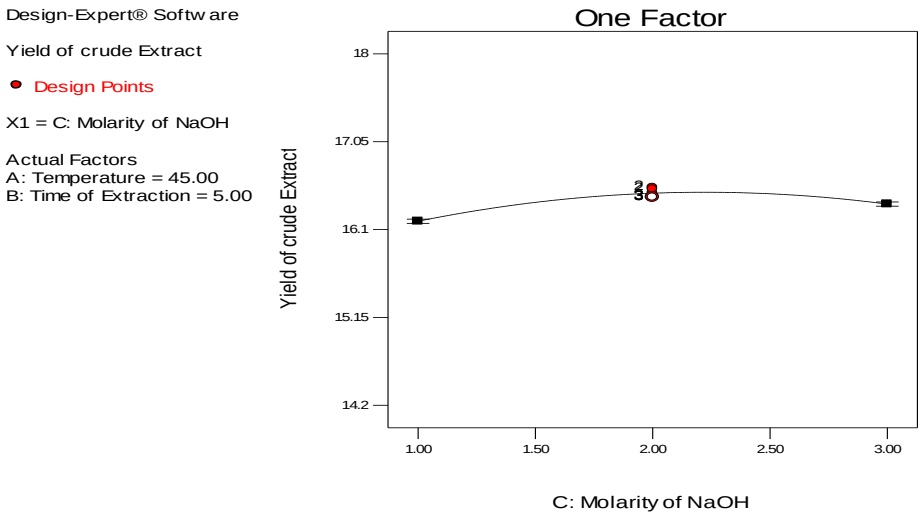


(2)

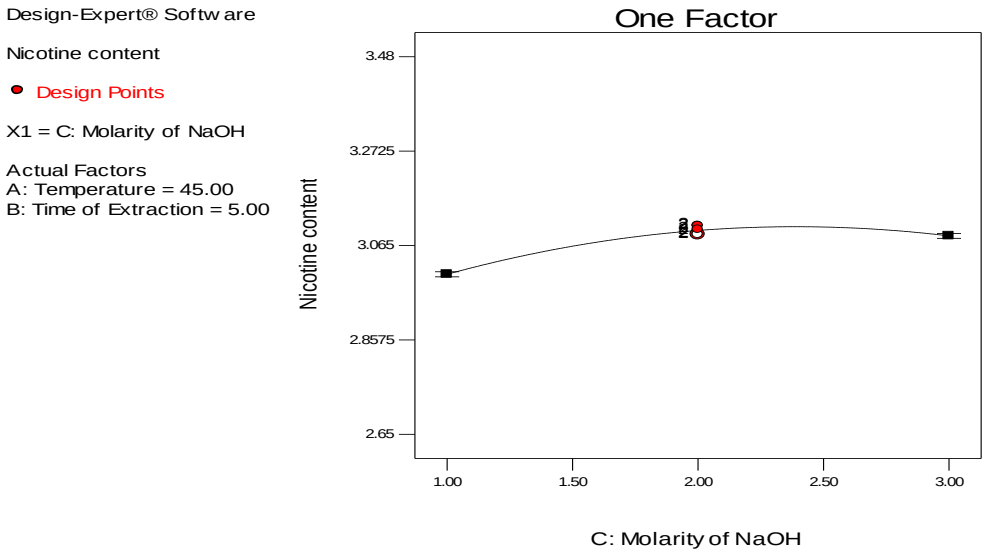
Figure 4.5 Effects of Time on Responses 1) yields of extracts 2) nicotine contents

4.5.3 Effects of Molarity of NaOH

The aim of adding sodium hydroxide was to increase the pH of the solution. As the pH increased, the removal of free nicotine from its salts also increased. At the pH above 8, the nature of nicotine present in the extract would be freebase nicotine. At various molarities, the yield and nicotine content in the extracts was plotted as shown in graph 4.6. Both yields and nicotine contents increased with increasing molarity of sodium hydroxide up to 2.5M. Beyond this molarities, the yield and nicotine content slowly decreases. This shows that both yields and nicotine contents reach the maximum and then decreased due to the formation of a complex mixture.



(i)



(ii)

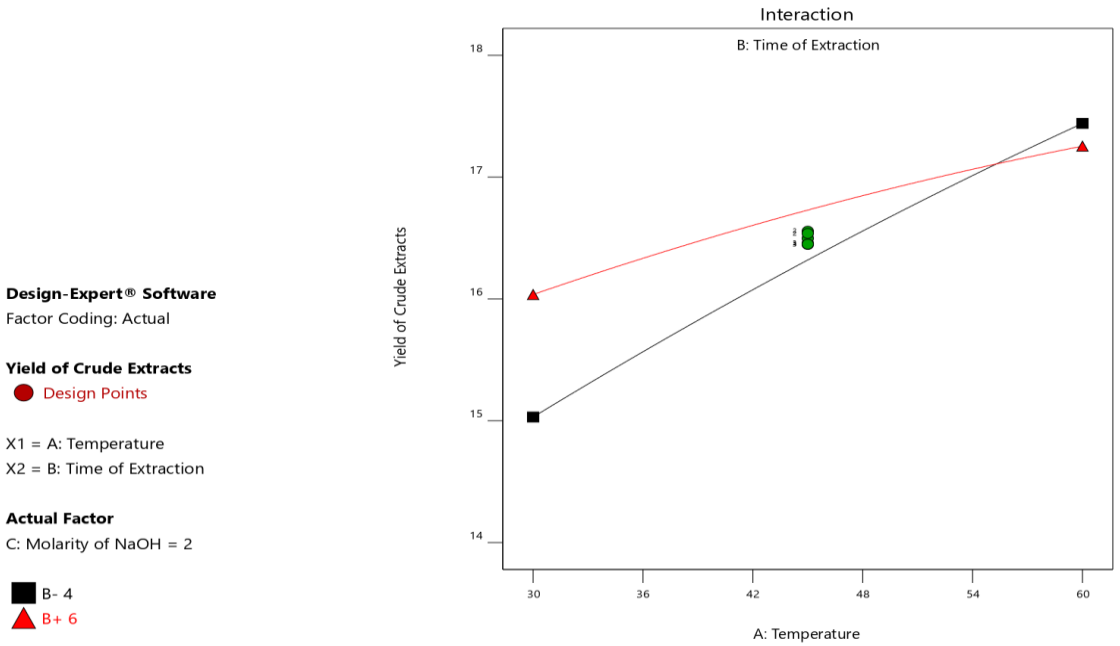
Figure 4.6 Effects of concentration of NaOH on Responses i) yields of extracts and ii) nicotine content

4.6 Interaction Effects of Parameters on Yields and Nicotine contents

Two or more parameters could influence the yields and nicotine contents simultaneously**.** The effects might be more or less than the effects of individual factors, and became significant when the probability level (p-value) should be less than 0.05.

4.6.1 Interaction Effects of Temperature and Extraction Time

Both temperature and extraction time had the significant effects on both responses. At low level of both factors, yields as well as nicotine contents were low. But as temperature and time increased, both responses increased as shown on Figure 4.7. However, at high temperature and low extraction time, the yield was becoming the maximum. The maximum nicotine content was observed when the temperature was high and time was either at maximum level or minimum level as shown figure 4.7(b). Therefore, to bring the maximum of both responses, the time has no effects when the temperature is increased.



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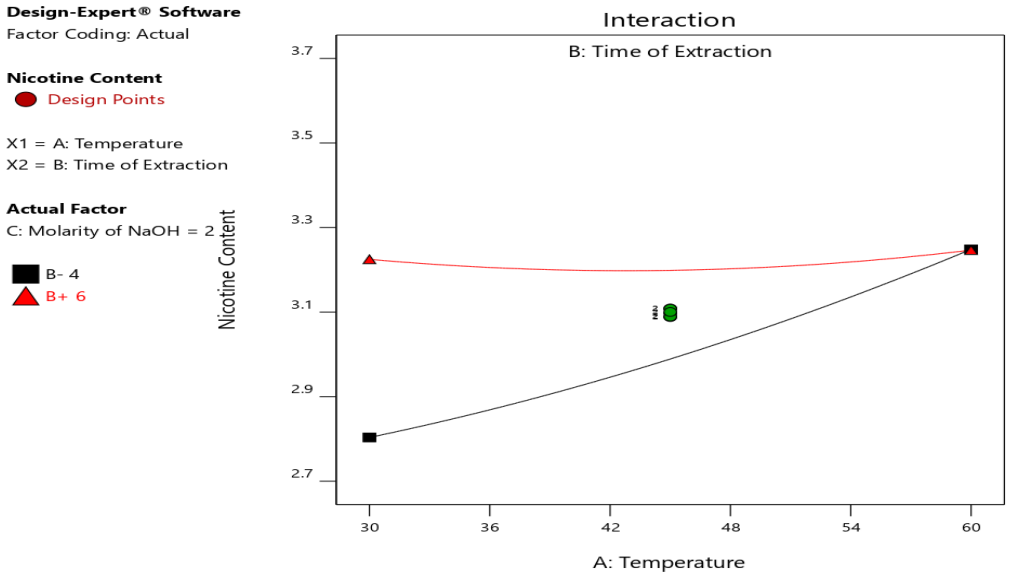
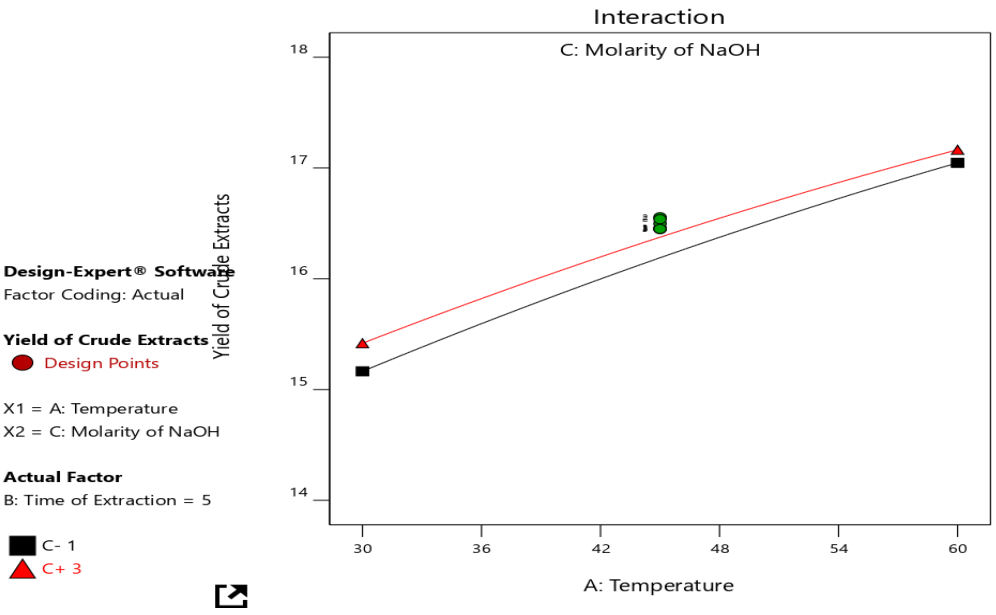


(b)

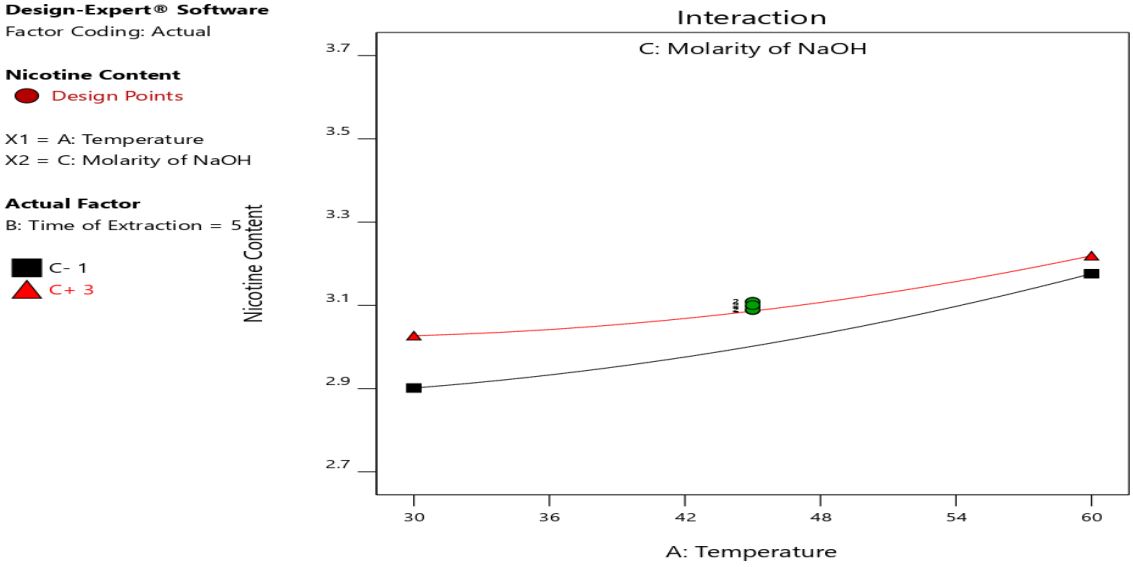
Figure 4.7 Interactive effect of Temperature and Time on Responses a) Yields b) Nicotine contents

4.6.2 Effects of Temperature and Molarity of Sodium Hydroxide

Both yield and nicotine content were increased as temperature and molarity increased. Both factors had significant effects as the p-value is less than 0.05. When both factors were at low level, both responses were low. As temperature increased to maximum level at constant molarity of NaOH, the yield and nicotine content became increase linearly. When the temperature was at low level and molarity increased, again they are increased but the increment was less compared to increasing temperature at constant molarity, and when both factors are at high level, the values of responses were highest. Therefore, increasing both temperature and molarity would bring the maximum products as in Figure 4.8.



(1)

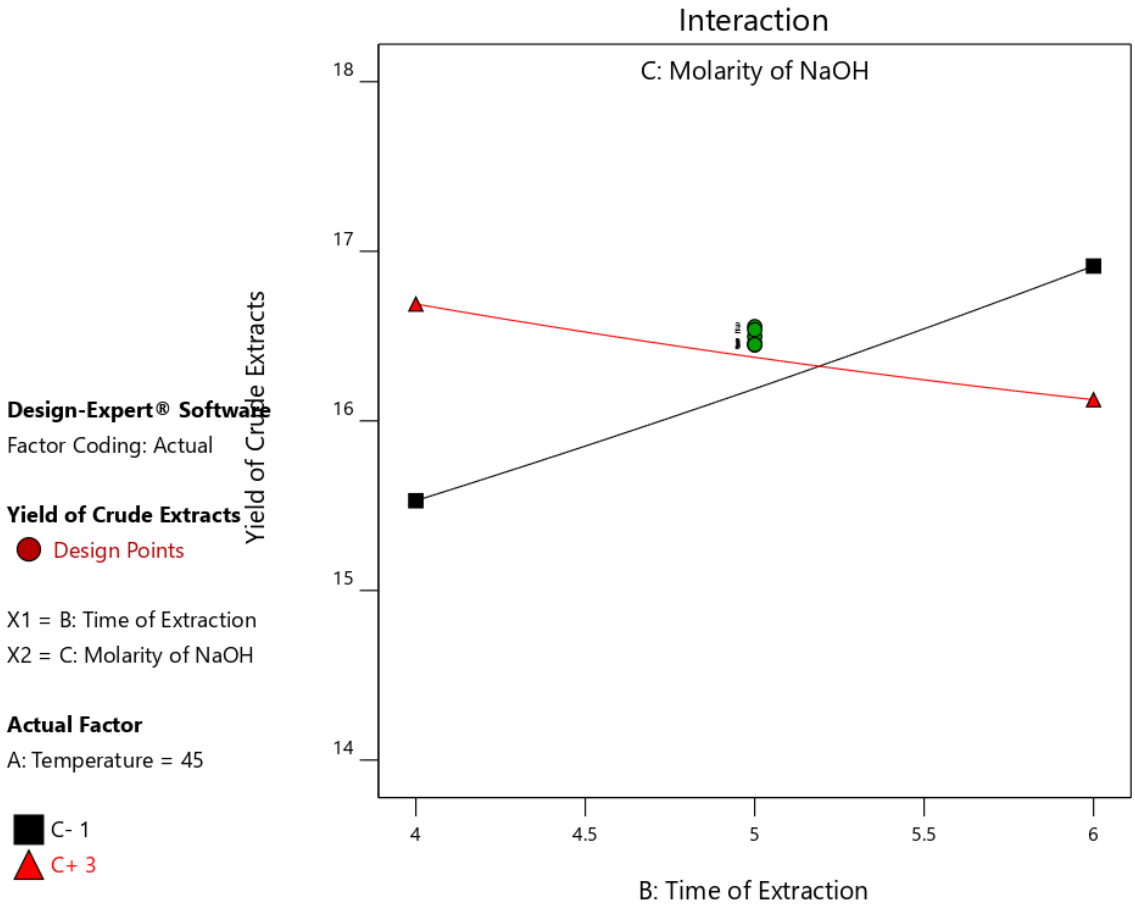


(2)

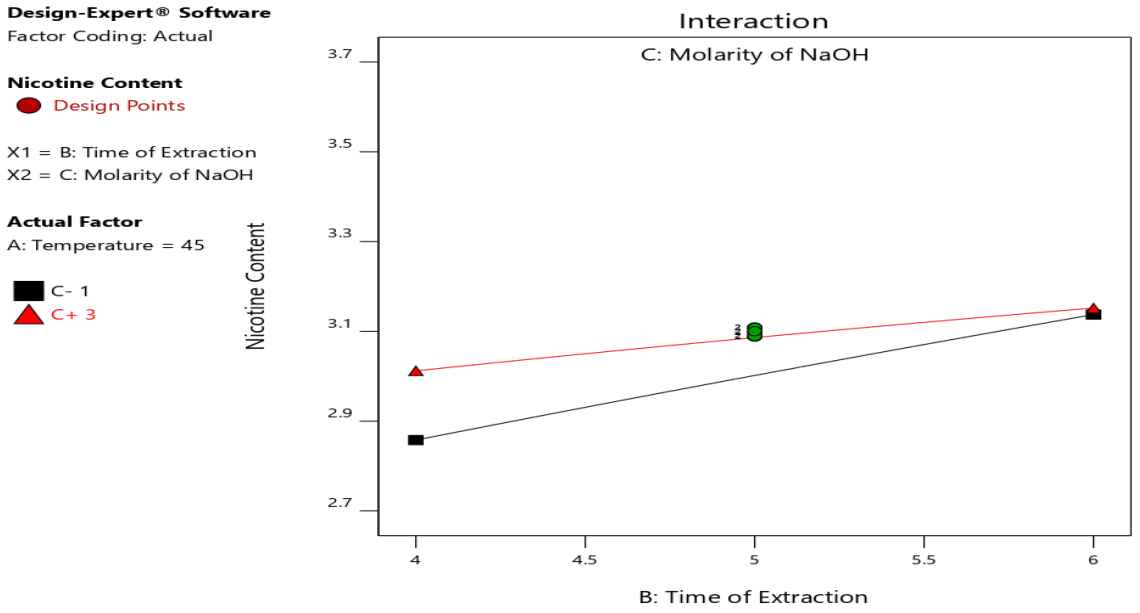
Figure 4.8 Interactive effects of Temperature and Molarity of NaOH on responses 1) Yields 2) Nicotine contents

4.6.3 Interaction Effects of Extraction Time and Concentration of NaOH

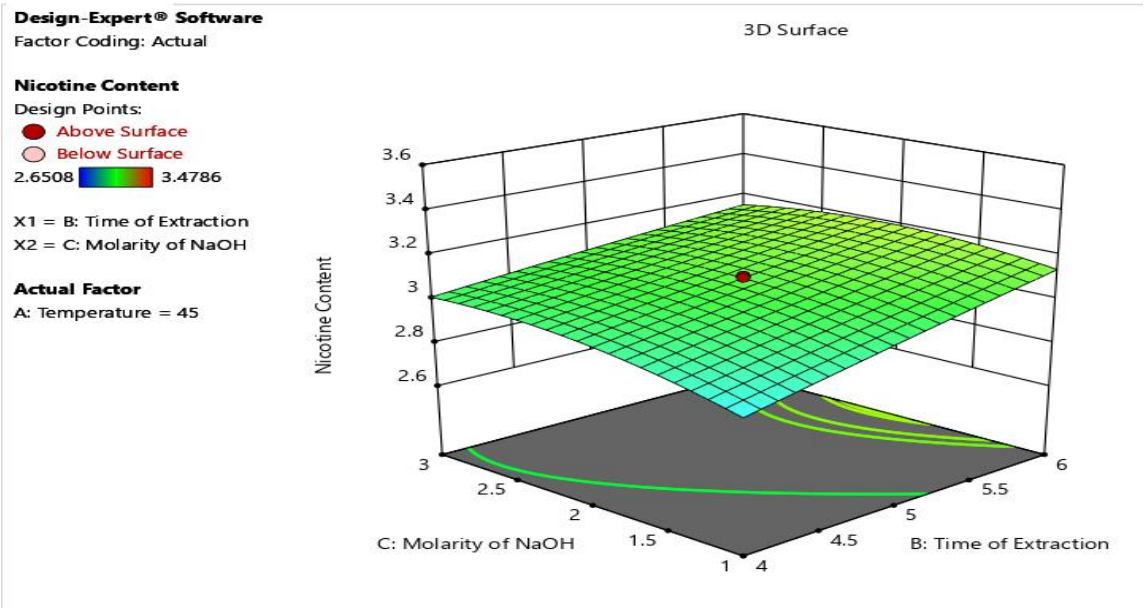
Figure 4.9 (i) show the interaction effects of time and molarity of sodium hydroxide on the crude extract yields. Both factors had a significant effects on it as the p-value was less than 5% (i,e < 0.0001). As both factors increased to reach the maximum, the yield was decreased. Generally, at high level of extraction time and low level of NaOH molarity, highest yield was obtained. Like yield of extracts, nicotine content was affected by the combination effects of time and molarity of sodium hydroxide significantly. However, the influence level of time was greater than that of molarity of NaOH. The high nicotine was observed at high level of time and high level of molarity of sodium hydroxide as depicted on graph 4.9 (ii).



(i)



(ii)



(iii)

Figure 4.9 Interaction effects of time and concentration of NaOH on responses i) yield ii) nicotine contents and iii) their 3D representation

4.7 Optimizations of Parameters

The aim of optimization is to minimize or maximize responses and factors according to requirements. In this case, the goal of optimization was maximizing the responses in the range of factors. For this purpose, the software selects the factors required to achieve the goals with the highest desirability. Around 78 possible solutions were obtained by RSM-CCD of Design Expert 12 for both responses. The top 30 were tabulated under Appendix B, Table B2. The maximum results of crude extracts, yield, and its nicotine contents were 17.749 and 3.258% respectively at optimum conditions of temperature 60^{o}C, time 4 hours and molarity of sodium hydroxide 2.830 M with a maximum desirability of 0.832 or 83.2% shown in Figures 4.10.

Table: 4.6 Constraints of optimization

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A: Temperature	is in range	30	60	1	1	3
B: Time of Extraction	is in range	4	6	1	1	3
C: Molarity of NaOH	is in range	1	3	1	1	3
Yield of Crude Extracts	Maximize	14.204	17.958	1	1	3
Nicotine Content	Maximize	2.6508	3.4786	1	1	3

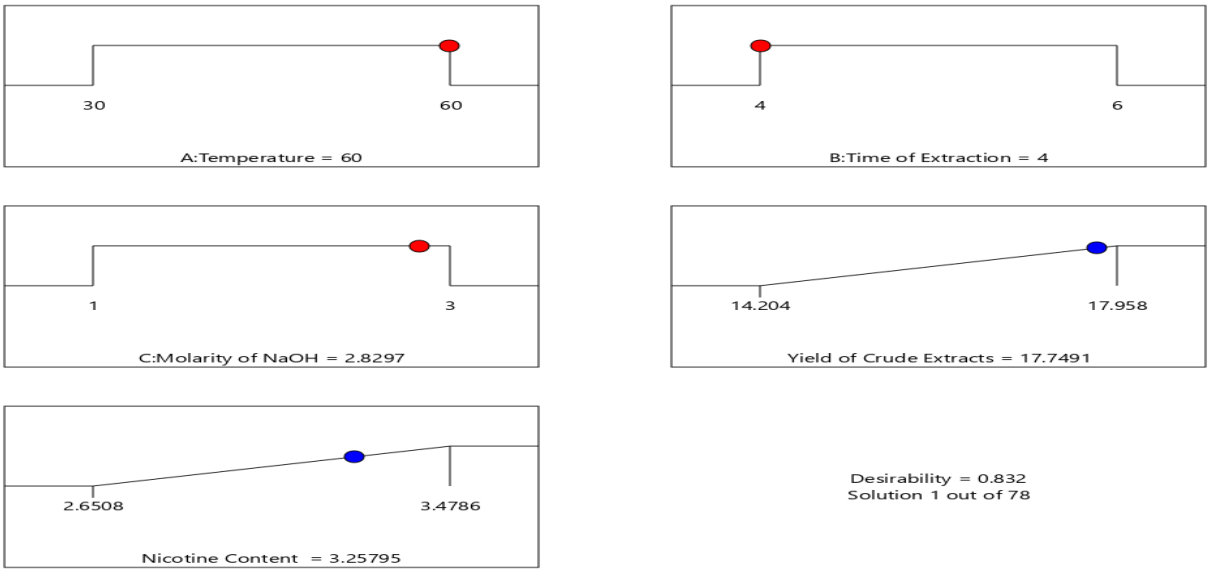


Figure 4.10 Ramp plot of optimization condition

4.8 Physicochemical Characterization of Emulsified Concentrates

Many physicochemical properties were crucial to estimate the efficiency of concentrated emulsified nicotine insecticide. Some of the basic important were descried below.

Specific gravity and density

Specific gravity can be used for predicting the type of creaming and the degree of spontaneous emulsification of EC. It was calculated with respect to distilled water. Measuring was taken place thrice by varying the volume. Although the density of palm oil is less than density of pure nicotine ($1.01 g/cm^3$), the density of concentrated emulsified nicotine was greater than density of pure nicotine due to density of emulsifier.

Table 4.7 Specific gravity and Density of EC

Replication/volume	Mass of sample	Mass of distilled water	Specific gravity	Density(g/ml)
1/10ml	12.8091	12.4978	1.0249	1.0218
2/15ml	19.0573	18.7472	1.0165	1.0135
3/20ml	25.1064	24.9987	1.0043	1.0013
Mean ± STD			1.0152 ± 0.0104	1.0122 ± 0.0103

Viscosity

Viscosity can be used for predicting the type of creaming and the degree of spontaneous emulsification of emulsified concentrate. The viscosity was measured thrice by varying the temperature between 18 to 25^0C. The calculated mean value was 585.33 ± 2.52 mPas.

pH

The pH is a very important parameter because it can reflect a chemical change in the components present in the formulation. Also, the change in pH over long storage periods can indicate degradation of the active components or instability or incompatibility of certain compounds. The average pH of the concentrated emulsified nicotine measured became 9.37 ± 0.03.

Solubility

The solubility was determined after the mass of the solute dissolved in volumes of water and left for an hour. At the last, the mass settled at the bottom of the flask was reweighted.

Table 4.8 Solubility of EC in tape water

	Initial mass	Undissolved mass	Mass difference	Solubility, %
1	5.3500	0.0010	5.3482	99.966
2	7.5403	0.0026	7.5377	99.965
3	10.0046	0.0041	10.0005	99.959
Mean ±STD				**99.963 ±0.004**

Surface Tension

The surface tension is another important parameter to be considered for the formulation of emulsified concentrated products. The formulated emulsified concentrated nicotine had a surface tension of 34.10mN/m. The lower surface tension is a desirable property for most agricultural sprays because it facilitates the spreading of droplets upon impact on crops or target surface-active area and improves penetration as well as uptake of the products into the plants.

Flash Point

The flashpoint is a measure of the tendency of a formulated to form flammable mixtures with air in controlled laboratory conditions is a parameter for storage and handling when considering as flammable materials. The prepared formulation in all the storage conditions showed high flash point values. It was measured once, and the value was obtained 87.96^0C. This value indicates that the emulsified concentrated nicotine was inflammable within the category of flammable scales.

Stability Study of Emulsified concentrated Nicotine

The most important factor in emulsion preparation is based on the selection of appropriate surfactants that will emulsify the selected ingredients satisfactorily and preserve their stability. A mixture of surfactants plays a major role in surface chemical applications and often exhibit interfacial properties more pronounced than those of the individual surface-active components of the mixtures. Stability studies were conducted by varying the temperature and time. For these factors, the pH, density, color change and homogeneity were re-evaluated as shown in the Table 4.9. The temperature change was taken place under Incubator.

Table 4.9 Stability study of EC

Temperature	After 18 hours of initial testing			
	Color change	Homogeneity	pH	Density
At 4^{o}C	No color change	Same	9.34 ± 0.01	1.0131 ± 0.0105
At room temperature				
At 45^{o}C				

From this, the product was stable if it stored any place for many months. According to the study of Jindaporn (2013), the formulated emulsified concentrated nicotine was stable for six months without any physicochemical property changes.

4.9 Efficiency Testing of Emulsified Concentrate

Different masses of the active ingredients of EC were dissolved in 100 ml of tap water to evaluate the efficiency of the dilution ratios used for field application. The experimental species (cabbage aphids) were collected from residence home and transported to the laboratory place with the help of plastic bags. The selection of dilution ratio was depending on the time interval at which the insecticides kill all insect pests, the season of application, place (area) of application, types of crops, and nature of insect pests. If the amount of active ingredient is high, the time taken to kill all pests will low. But it affects the crops and other useful vertebrate species and pollinators found around there.

During the summer, the rain can wash the insecticide from crops before it fully contacted with insects. So, the active ingredient present in the solvent should be higher than that of spring, winter, or autumn season. According to Jindaporn (2013) reported, the concentration of nicotine used as an insecticide was 0.5-1% w/w for vegetables. As the experience obtained from farmers, the effective insecticides have to control or kill the pests for two to three days. An insecticide that kills all pests in a day is very toxic and may cause a problem in a food chain. According to Richardson (2015) reported, a concentration of nicotine used for controlling or killing aphids more than 50 % and 95% was 0.003 and 0.007 gram per 100 ml of water respectively. But in this study, 1g of EC to 100 g of water dilution ratio was became an efficient.

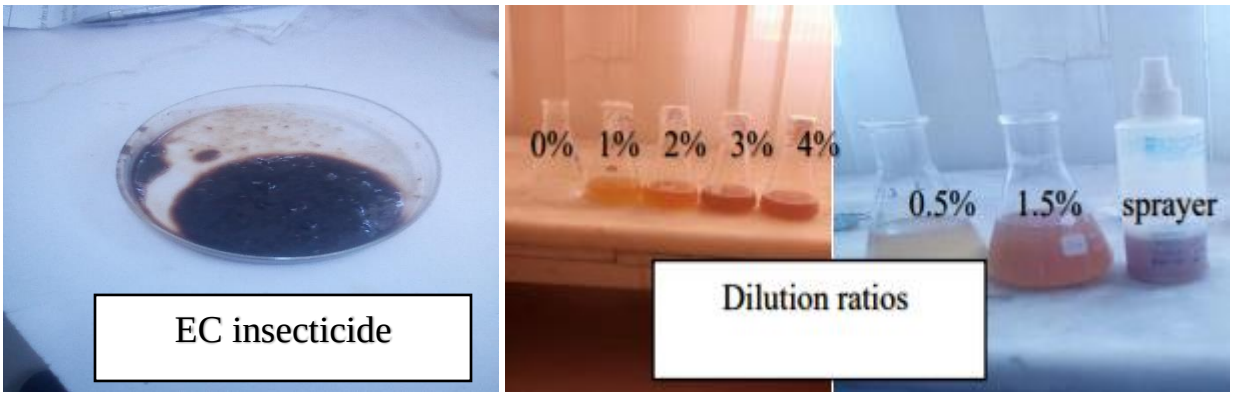


Figure 4.11 Efficiency testing of Emulsified concentrate

Table 4.10 Dilution ratio of EC to solvents

Dilution ratio (EC: Water)	Total aphids selected	Total numbers of aphids died		Percent of aphid died	
		After 8hr	After 24hr	After 8hr	After 24hr
0.5:100	34	16	31	47.058	91.177
1:100	36	23	34	63.889	94.444
1.5:100	27	21	26	77.778	96.296
2:100	29	24	28	82.759	96.552
3:100	32	29	32	90.625	100
4:100	27	25	27	92.593	100
0:100 (pure water)	22	1	3	4.546	13.636

5. CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Bioinsecticide has several advantages over conventional pesticides as it is specific to the target insects and less harmful to non-target species. As the cigarette wastes contain remnant tobacco which has an insecticidal chemical such as nicotine, other minor alkaloids and toxic additive chemicals, the produced insecticide was effective to control cabbage insect pests.

According to the experiment, from a single cigarette butt at average sizes of seven brands of cigarettes that were smoked mostly in Ethiopia, 0.2304 ± 0.166 gram of tobacco filler and 7.506 ± 5.409 mg of nicotine were calculated.

To extracts, the insecticidal chemicals from tobacco fillers of cigarette butts at maximum yields, temperature, time, and concentration of sodium hydroxide have great effects. The temperature had the most impact while the molarity of NaOH had the least impact on yield and nicotine contents. The maximum yields of the crude extracts and nicotine content 17.74 9 % and 3.258 % respectively were observed at a high level of temperature (60^0C), low level of time (4hr), and about high level of molarity of sodium hydroxide (2.83 M). An emulsified concentrate formulation of insecticide is more efficient than other forms of formulation because of high penetration capacity through derma. The emulsified concentrated nicotine insecticide has a physicochemical property of an inflammable, viscous, basic, stable, wettable and highly solubility in water.

Generally, recycling of cigarette butt to bioinsecticide product has multifunction since the bioinsecticide is used for agricultural application and the waste collection treats the environment from wastes.

5.2 Recommendation

The cigarette wasted at cigarette factories should be supplied for insecticide production since it contains a huge amount of tobacco that cannot recycle for fresh cigarette production. For quantitative analysis of nicotine by GCMS, in addition to nicotine standards, other internal standards should be applied to get a better calibration curve. During the testing of insecticide on insects directly, the number and classes of insects selected for study should be many.

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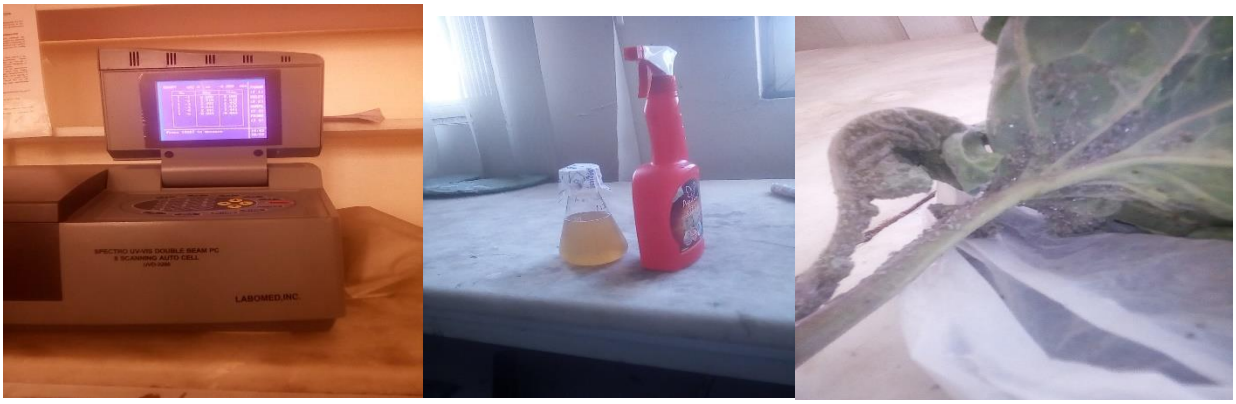
Appendix A: Experimental Activities, Measured and Calculated Data



(a) (b) (c)

(d) (e) (f)

Figure 1 Photo taken during experimental activities a) UV/Visible spectrophotometer analysis of concentration of nicotine b) 1% diluted insecticide selected for application c) cabbage aphids specimens d) identifying dead and live aphids by naked eye e) microscopic checking for confirmation f) counted dead aphids

Figure A1 Experimental Activities

Table A1 Quantitative Analyzed Samples for Factors at all Levels

STD. Run	Mass of Filter Cake, (g) *	Yield of extracts, (%) **	Absorbance *	Concentration of Nicotine, (g/L) **	Volume of extracts, (ml)*	Nicotine content (%) **
1	1.716	14.204	2.51	2.451	21.63	2.6508
2	1.666	16.689	2.492	2.434	25.73	3.1318
3	1.676	16.194	2.612	2.548	24.65	3.1408
4	1.650	17.489	2.441	2.386	26.94	3.2131
5	1.691	15.426	2.508	2.449	23.16	2.8368
6	1.644	17.778	2.499	2.441	26.63	3.2496
7	1.691	15.471	2.648	2.583	24.79	3.2016
8	1.667	16.628	2.46	2.404	26.43	3.1768
9	1.713	14.345	2.512	2.453	24.59	3.016
10	1.641	17.958	2.654	2.588	26.88	3.4786
11	1.676	16.218	2.548	2.487	23.15	2.8793
12	1.660	17.014	2.627	2.563	25.65	3.2867
13	1.691	15.457	2.479	2.422	23.09	2.7962
14	1.683	15.836	2.496	2.438	24.36	2.9696
15	1.671	16.457	2.502	2.444	25.35	3.0968
16	1.669	16.556	2.526	2.467	25.21	3.1087
17	1.670	16.498	2.524	2.465	25.22	3.1081
18	1.671	16.448	2.528	2.468	25.02	3.0882
19	1.671	16.45	2.529	2.469	25.02	3.0893
20	1.669	16.538	2.532	2.472	25.08	3.1004

***-** measured data; **-** calculated data

Appendix B: Experimental Design Data

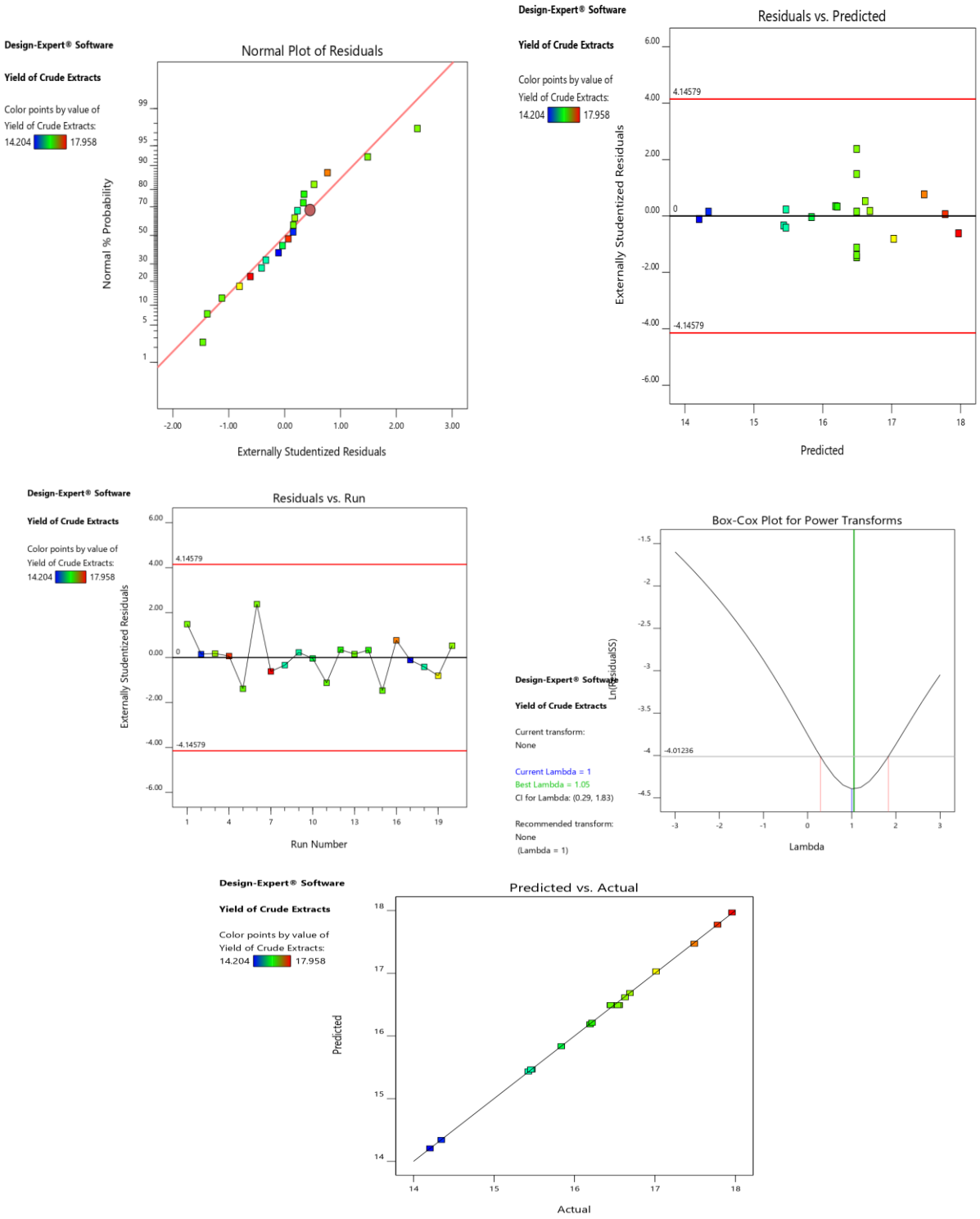


Figure B1 Diagnostic graph of yields

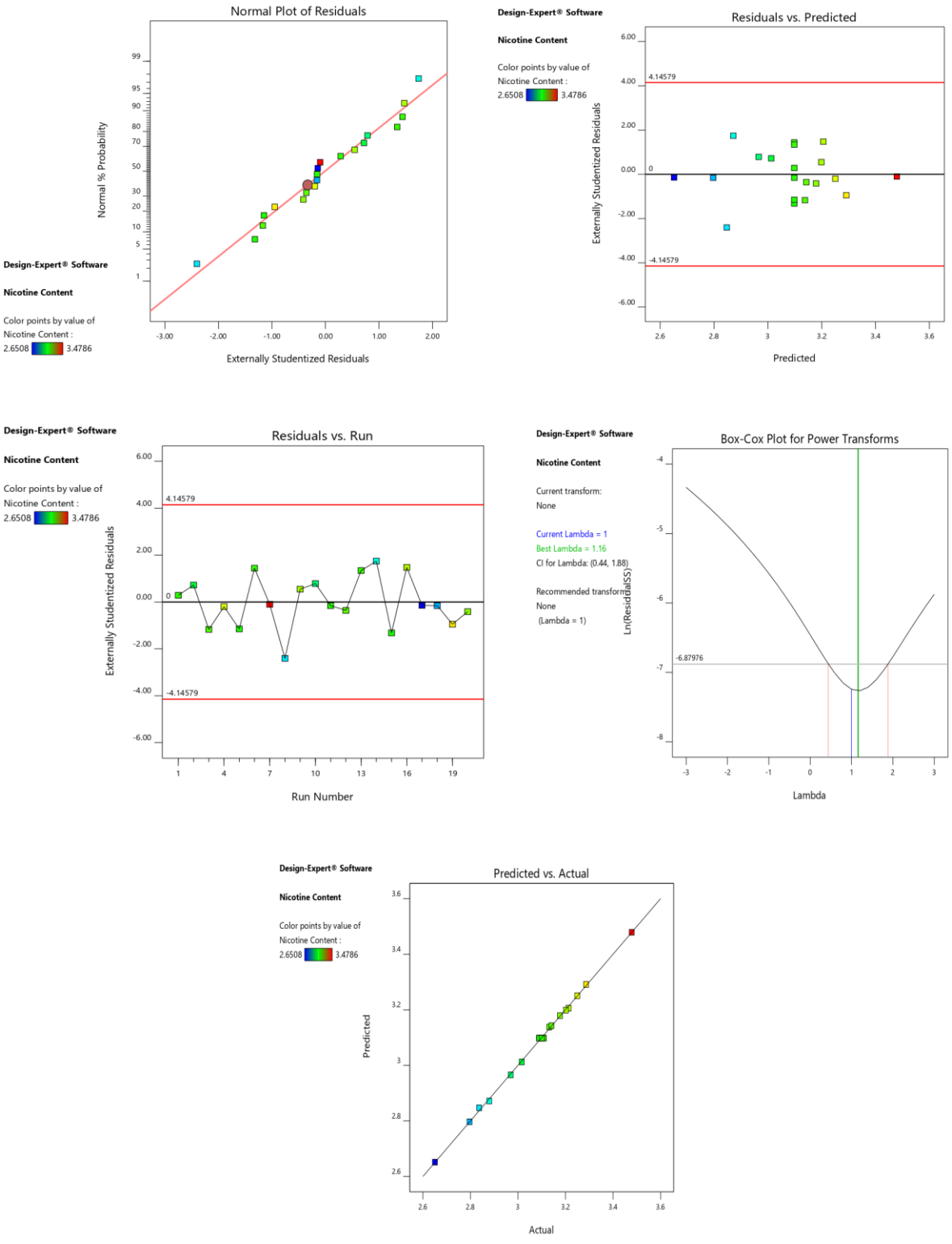


Figure B2 Diagnostic graph of Nicotine Contents

Table B1 Designed Variables analyzed by Central Composite Design

Std	Run	Factor 1 A: Temperature Deg. C	Factor 2 B: Time of Extraction Hour	Factor 3 C: Molarity of NaOH M	Response 1 Yield of Crude Extracts %	Response 2 Nicotine Content %
1	17	30	4	1	14.204	2.6508
2	3	60	4	1	16.689	3.1318
3	12	30	6	1	16.194	3.1408
4	16	60	6	1	17.489	3.2131
5	8	30	4	3	15.426	2.8368
6	4	60	4	3	17.778	3.2496
7	9	30	6	3	15.471	3.2016
8	20	60	6	3	16.628	3.1768
9	2	15	5	2	14.345	3.016
10	7	75	5	2	17.958	3.4786
11	14	45	3	2	16.218	2.8793
12	19	45	7	2	17.014	3.2867
13	18	45	5	0	15.457	2.7962
14	10	45	5	4	15.836	2.9696
15	11	45	5	2	16.457	3.0968
16	6	45	5	2	16.556	3.1087
17	13	45	5	2	16.498	3.1081
18	15	45	5	2	16.448	3.0882
19	5	45	5	2	16.45	3.0893
20	1	45	5	2	16.538	3.1004

Table B2 Optimized solution of top 30 of 78

Number	Temp.^{o}C	Time, hr	M. of NaOH	Yield of Crude Extracts, %	Nicotine Content, %	Desirability	Remark
1	**60.000**	**4.000**	**2.830**	**17.749**	**3.258**	**0.832**	**Selected**
2	60.000	4.000	2.827	17.749	3.258	0.832	
3	60.000	4.000	2.802	17.743	3.259	0.832	
4	60.000	4.000	2.867	17.756	3.257	0.832	
5	60.000	4.000	2.886	17.759	3.256	0.832	
6	60.000	4.000	2.754	17.733	3.260	0.832	
7	60.000	4.006	2.816	17.743	3.258	0.832	
8	60.000	4.005	2.784	17.737	3.259	0.832	
9	60.000	4.000	2.737	17.729	3.261	0.832	
10	60.000	4.000	2.958	17.771	3.253	0.831	
11	60.000	4.000	2.678	17.714	3.262	0.831	
12	60.000	4.000	2.643	17.705	3.262	0.830	
13	59.837	4.000	2.839	17.740	3.255	0.829	
14	60.000	4.053	2.762	17.707	3.259	0.828	
15	60.000	4.000	2.542	17.675	3.263	0.827	
16	59.755	4.000	2.740	17.713	3.256	0.827	
17	59.761	4.000	2.970	17.757	3.248	0.826	
18	59.688	4.000	2.832	17.729	3.252	0.826	
19	60.000	4.000	2.503	17.662	3.263	0.825	
20	59.950	4.055	2.569	17.657	3.261	0.824	
21	59.568	4.000	2.823	17.719	3.250	0.823	
22	60.000	4.066	2.394	17.600	3.262	0.817	
23	60.000	4.000	2.299	17.585	3.260	0.814	
24	59.135	4.000	2.816	17.688	3.242	0.814	
25	60.000	4.000	2.259	17.568	3.259	0.812	
26	60.000	4.000	2.212	17.547	3.258	0.808	
27	60.000	4.000	2.164	17.525	3.256	0.804	
28	60.000	4.000	2.087	17.487	3.253	0.798	
29	58.867	4.000	2.389	17.544	3.241	0.796	
30	60.000	4.379	2.184	17.445	3.257	0.795	

Appendix C: GCMS Results

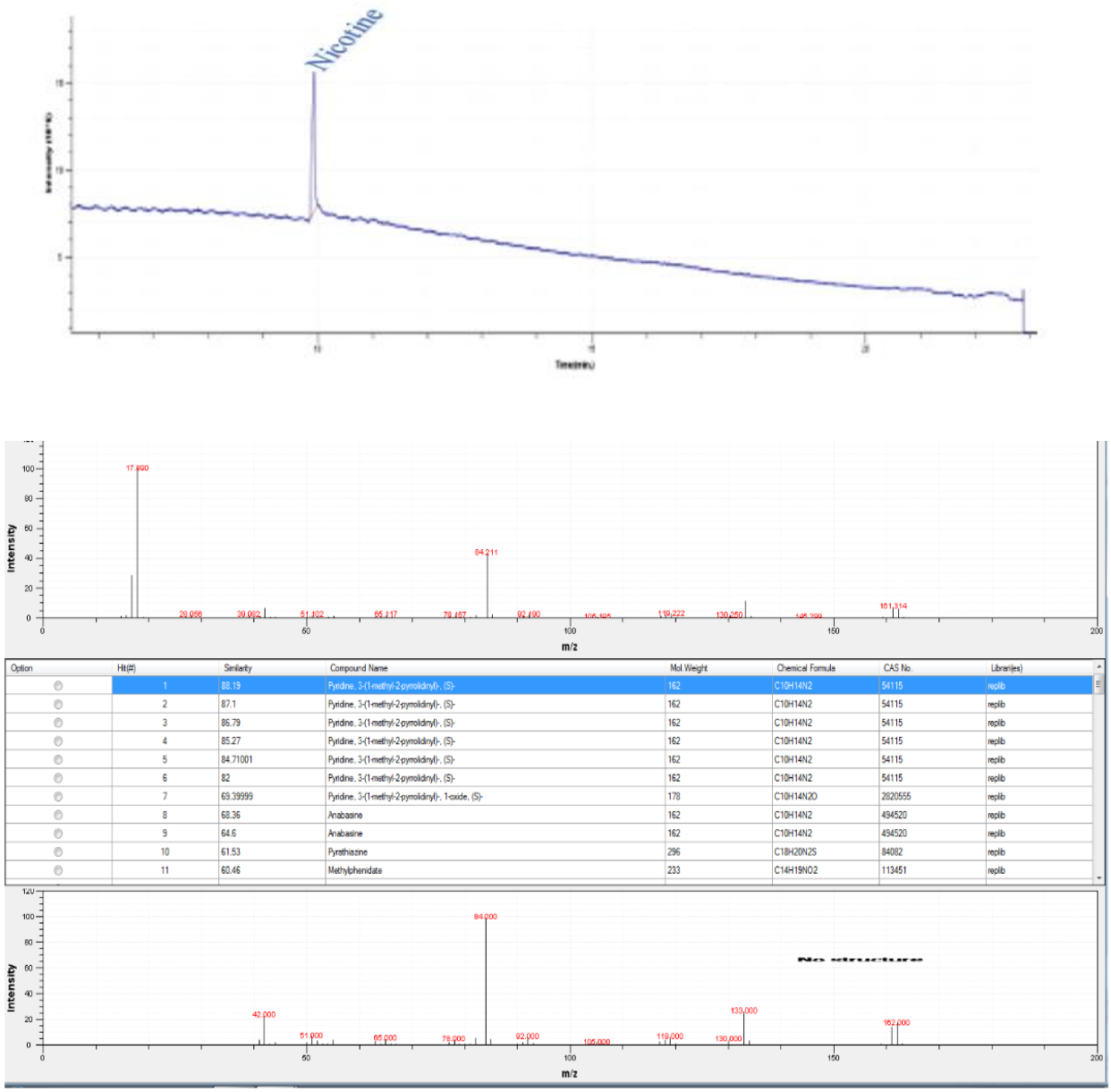


Figure C1 GC-MS scanned sample for qualitative analysis

Table C1 Model performance validation of GCMS and UV/Visible Spectrophotometer

Criteria	**GCMS**		**UV/Visible Spectrophotometer**	
Graph plot	Peak vs Concentration, µg/ml		Absorbance vs Concentration, g/L	
Slope	42645		1.0425	
Correlation factor	0.9995		0.9985	
Intercept	- 256099		- 0.0659	
Intercept SE	242945.089580844		0.033810926	
Intercept SD	687152.47954		0.09563	
LOD (µg/ml)	18.79983		0.303	
LOQ (µg/ml)	56.96919		0.9173	
Regression Statistics	Multiple R	0.999618396	Multiple R	0.998495
	R Square	0.999236937	R Square	0.996992
	Adjusted R Square	0.99910976	Adjusted R Square	0.99649
	Standard Error	311790.3579	Standard Error	0.030726
	Observations	8	Observations	8

ANOVA[2]

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	7.63809E+14	7.64E+14	7857.049	1.38885E-10
Residual	6	5.83279E+11	9.72E+10		
Total	7	7.64392E+14			

Standard Error	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
242945.090	-1.05414	0.0332406	-850563.7406	338366.7	-850564	338366.7
481.1029671	88.64	1.39E-10	41467.74954	43822.18	41467.75	43822.18

ANOVA [3]

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1.877248	1.877248	1988.445	8.51781E-09
Residual	6	0.005664	0.000944		
Total	7	1.882913			

Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
0.024093829	-2.73368	0.03402	-0.124820276	-0.00691	-0.12482028	-0.00690933
0.02337913	44.59199	8.52E-09	0.985315195	1.099729	0.9853152	1.099728537

[2] ANOVA for Model Validation of GCMS
[3] ANOVA for Model Validation of UV/Visual Spectrophotometer

Table 2 Data of fresh cigarette and cigarette butts

Brand Name	**Weight of cigarette at specified parts**						
	Full	Filter	1/4TCP	1/3TCP	1/2TCP	2/3TCP	3/4TCP
Nyala	0.95	0.155	0.2375	0.317	0.475	0.633	0.751
Delight	0.95	0.155	0.2375	0.317	0.475	0.633	0.751
Prem N.	0.95	0.155	0.2375	0.317	0.475	0.633	0.751
Winston	0.95	0.155	0.2375	0.317	0.475	0.633	0.751
Marlboro	0.85	0.235	0.2125	0.283	0.425	0.567	0.696
BR	0.55	0.135	0.1375	0.183	0.275	0.367	0.446
Oris	0.7	0.115	0.175	0.233	0.35	0.467	0.554
Mean ± SD	**0.843± 0.159**	**0.158 ± 0.037**	**0.211± 0.040**	**0.281± 0.053**	**0.421± 0.080**	**0.562± 0.106**	**0.672± 0.123**

Brand Name	**Weight of tobacco at specified size**					
	Full	1/4TCP	1/3TCP	1/2TCP	2/3TCP	3/4TCP
Nyala	0.85	0.2125	0.28333	0.425	0.354	0.638
Delight	0.75	0.1875	0.25	0.25	0.288	0.563
Prem N.	0.8	0.2	0.267	0.267	0.307	0.6
Winston	0.75	0.188	0.25	0.25	0.288	0.563
Marlboro	0.55	0.138	0.183	0.183	0.211	0.413
BR	0.365	0.091	0.122	0.122	0.140	0.274
Oris	0.55	0.138	0.183	0.183	0.211	0.413
Mean ± SD	**0.659 ± 0.175**	**0.165 ± 0.044**	**0.220 ± 0.058**	**0.240 ± 0.096**	**0.257± 0.073**	**0.495 ± 0.131**

Size	**Size of Cigarettes**							
	Nyala	Delight	Prem. N	Marlboro	Winston	Oris	BR	**Mean ± SD**
Full	84	84	84	82	84	95	98	**87.286 ± 6.396**
Filter	20	20	20	25	20	20	30	**22.143 ± 3.934**
TCP	64	64	64	57	64	75	68	**65.143 ± 5.429**
1/2(TCP)	32	32	32	28.5	32	38	34	**32.571 ± 2.715**
1/3(TCP)	21.3	21.3	21.3	19	21.3	25	22.7	**21.7 ±1.818**
1/4(TCP)	16	16	16	14.25	16	19	17	**16.286 ±1.357**
2/3(TCP)	42.7	42.7	42.7	38	42.7	50	45.3	**43.443 ± 3.612**
3/4(TCP)	48.0	48.0	48.0	42.8	48.0	56.3	51.0	**48.9 ± 4.1**

Sizes (≤ 2/3 TCP+ filter paper)	Average length of cigarette butts of 7 brands, mm	Average weight of cigarette butts, gram	Average weight of tobacco filler, gram
2/3TCP + filter paper	65.586	0.615	0.437
1/2TCP + filter paper	54.714	0.501	0.329
1/3TCP + filter paper	43.843	0.386	0.221
¼ TCP + filter paper	38.429	0.329	0.165
Filter paper	22.143	0.158	0
Mean ± SD	**44.943 ± 16.470**	**0.3978 ± 0.173**	**0.2304 ± 0.166**