Effect of Natural Based Bio-inhibitors and N (n-butyl) Thiophosphoric Triamide (NBPT) for Slow Release Fertilizer (SRF) Application

by

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14996

Dissertation is submitted in partial fulfilment of
the requirements for the
Bachelor of Engineering (Hons)
(Chemical)

January 2015

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CERTIFICATION OF APPROVAL

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A project dissertation submitted to the
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In partial fulfilment of the requirement for the
BACHELOR OF ENGINEERING (Hons)
(CHEMICAL)

Approved by:

_____
(Dr. Nurlidia Binti Mansor)

UNIVERSITI TEKNOLOGI PETRONAS
TRONOH PERAK
January 2015

CERTIFICATION OF ORIGINALITY

This is to certify that I am responsible for the work submitted in this project, that the original work is my own except as specified in the references and acknowledgements, and that the original work contained herein have not been undertaken or done by unspecified sources or persons.

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NG SUI NEE

ABSTRACT

The application of N (n-butyl) Thiophosphoric Triamide (NBPT) as urease inhibitor with urea fertiliser have shown presence of chlorosis and necrosis on plant leaves that decreases the amount of chlorophyll pigments essential for photosynthesis reaction. Three types of vegetables, Spinach (*Spinacia oleracea*), mustard green (*Brassica juncea)* and water spinach (*Ipomoea aquatica*) are cultivated in soil lasting 4 weeks and irrigated with deionized water daily and fertilized with urea, NBPT and Thiosulfinates (TS) at different concentrations (0%, 0.012%, 0.062%, and 0.125%). The physical changes and the chlorophyll concentration were analysed using Trichromatic method. Besides that, inhibition studies to show the potential of TS in garlic extract as a bio based inhibitor is conducted to compare the inhibition performance with chemical based inhibitor, NBPT. The plants treated with 0.125% of NBPT had the least chlorophyll concentration compared to control plants treated with only urea. This might be due to ammonium toxicity experienced by the plants which then led to the decrease in chlorophyll pigments. In addition, both NBPT and TS exhibit inhibition abilities but showed different trends. Inhibition by TS began earlier but lasted only for 20 minutes while NBPT showed a much longer period but began after 60 minutes of application. As a conclusion, the chlorophyll results showed that NBPT did effect the plant growth which is proportional to the concentration of NBPT applied. Hence, bio based urease inhibitor like TS should be considered as one of the alternative to replace chemical based inhibitors for a more sustainable future in the agriculture sector.

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ABBREVIATIONS AND NOMENCLATURES

AHA	Acetohydroxamic acid
EDTA	Ethylenediaminetetraacetic acid
EIS	Electrospray ionization
FYP	Final Year Project
HPLC	High Performance Liquid Chromatography
MSc	Master of Science
NBPT	N (n-butyl) Thiophosphoric Triamide
PhD	Doctor of Philosophy
PPE	Personal Protective Equipment
UNESCO	United Nations Educational, Scientific and Cultural Organization
SRF	Slow Release Fertilizer
TS	Thiosulfinates
TWA	Time - Weighted Average
UTP	Universiti Teknologi PETRONAS
UV-VIS	Ultraviolet – Visible light

CHAPTER 1: INTRODUCTION

The background and principle of fertilization with the application of existing chemical based urease inhibitor known as N (n-butyl) thiophosphoric triamide (NBPT) are explained in Chapter 1. In addition, the potential of thiosulfinates (TS) in garlic extract as potential urease inhibitor is also being discussed with clearly stated problem statements, objectives and scopes of study for this research.

1.1 Background of Study

Over the years, agricultural fertilisation activities have improved significantly due to the development of science and technology. Basically, urea is the most common fertiliser applied in agriculture with reduced cost and promising productivity. However, it can contribute to environmental pollution due to ammonia emission into the atmosphere [1]. Thus, this has encouraged various research studies to carry out in order to have more in depth understanding about processes associated with fertilisation to mitigate such problems and provide sustainable solutions in agriculture field.

Generally, N – (n-butyl) thiophosphoric triamide (NBPT) is known as the most effective urease inhibitor in fertilisation process for delaying the hydrolysis of ammonia [2]. It is usually applied with urea fertilisers on crops and absorption will gradually take place from plant roots to shoots [3]. However, in previous studies the application of chemical based urease inhibitor like NBPT has showed some adverse effects on the plants growth such as leaf – tip scorch and chlorosis. This may due to the excessive accumulation of nitrogen in plant cells which can result in ammonium toxicity [4].

Thus in this project, chlorophyll is used as one of the most reliable and important indicator for quantifying the damages on leaves as it is related to the photosynthesis activity within plant growth [5]. With that, Trichromatic Method is used in UV-VIS spectrophotometer to determine the chlorophyll results presence in day 3, 5 and 7. The results indicated that plants applied with different concentrations of NBPT have lower chlorophyll concentration than control plants applied with urea only.

Unlike NBPT, potential bio-inhibitor like garlic, *Allium Sativum L* can be used as a sustainable solution in fertilisation process. For thousands of years, garlic is utilised in food and medication purposes due to the presence of S-alk-(en)yl-L–cysteine–sulphoxides compounds with allicin most abundant. This compound is responsible for its bioactivities such as antioxidant, antibacterial, anti-carcinogenicity and etc [6]. The experiment in this project is carried out by using a standard garlic extract that has thiosulphinates (TS) from allicin compound as the urease inhibitor to modify the urease enzyme's activation site [7].

With that, the inhibition studies of NPBT and TS are carried out to compare the effectiveness of inhibiting urease. The study results indicated that both exhibited inhibition properties at different time with similar concentration using UV-VIS spectrophotometer. TS demonstrated its inhibition properties in a short time before losing its inhibition ability while NBPT took a longer time for full inhibition to occur but maintained its inhibition properties longer than TS. Overall, the application of urease inhibitors are mainly depending on the ability of inhibition over time.

1.2 Problem Statements

The application of NBPT, a chemical based urease inhibitor in conjunction with urea had caused visible changes to plant growth such as transitory yellowing of leaf tips which will eventually lead to necrosis. Thus, it can cause the decrease of chlorophyll in plant shoots that is essential for plants to carry out photosynthesis for further growth. Besides that, NBPT is non-biodegradable and is a chemical component that is not environmental friendly. Therefore, this research is attempting to study the potential of TS in garlic as an alternative inhibitor in comparison with the existing chemical based inhibitor NBPT.

1.3 Objectives of Study

The main focus of this research is to study the visible changes on the plant growth fertilized with NBPT with urea solution and TS with urea solution. This research also aim to carry out a comparison study on the inhibition performance between TS in standard garlic extract as bio-based inhibitor and NBPT as chemical based urease inhibitor to prevent ammonia volatilisation. Overall the objectives of this research are:

- To study the effects of NBPT and TS on plant growth through the presence of chlorophyll.
- To investigate the inhibition studies of urease using Thiosulfinates and NBPT.

1.4 Scopes of Study

- To conduct relevant experiments using NBPT, TS and urea solutions on *Ipomoea Aquatic*, *Brassica Juncea* and *Spinacia Oleracea* plants.
- To analyse the effects on plant after applying NBPT as chemical based urease inhibitor and TS as bio-based urease inhibitor with urea solution.
- To provide an overview and analysis on the comparison between bio and chemical based urease inhibitors through inhibition studies.

1.5 Relevancy of Research

This project is highly feasible to carry out due to the availability of resources such as garlic and vegetable seeds which can be easily obtained from current market at reasonable cost. Furthermore, the NBPT is readily available in the laboratory while the preparation of TS from garlic extract can be done easily in a short period of time. The laboratory is also well-equipped with the equipment needed and procedure to operate for this experiment.

CHAPTER 2: LITERATURE REVIEW

Throughout this chapter, latest comprehensive literature review in accordance with the problem statement and objectives are carried out to have in depth understanding about the research. The description of ammonia volatilisation, types of urease inhibitors and chlorophyll are discussed in each subtopics of Chapter 2.

2.1 Ammonia Volatilisation

The process involving the exchange of ammonia gas, NH_3 from the surface of the soil to the atmosphere is known as ammonia volatilisation. In the United States and Europe, this process had contributed to countless environmental pollutions such as destruction of crops and contamination of drinking water. This is because during fertilisation, urease will act as a natural catalyst to promote the hydrolysis of urea fertilizer in soil into unstable carbamic acid followed by immediate reaction without the presence of urease into carbon dioxide and ammonia gas as shown in equation (1). From the equation, the ammonia gas will either escape into the atmosphere or react with water to form ammonium ions in equation (2) which will result in high pH of soil[2, 8].

$$(NH_2)_2CO_{(s)} + H_2O_{(l)} \xrightarrow{urease} NH_{3(g)} + H_2NCOOH_{(l)} \longrightarrow 2NH_{3\,(g)} + CO_{2(g)} \quad (1)$$

$$NH_{3(g)} + H_2O_{(l)} \longrightarrow NH_4^{+} + OH^{-} \quad (2)$$

On the other hand, there are also several important factors need to be considered that can affect ammonia volatilisation. These factors included surrounding temperature, soil pH and moisture, wind velocity and so on are summarized in Table 1 [9].

Table 1: Factors Affecting Ammonia Volatilisation [9]

No.	Factors	Descriptions
1)	Temperature	The ammonia gas released into the atmosphere is directly proportional to the increase of surrounding temperature.
2)	Soil pH	The greater the amount of urea fertilisers dissolved in soil, the higher the soil pH.
3)	Soil moisture	Higher moisture in soil dissolved the urea fertiliser forming more ammonium ions.
4)	Soil bioactivities	The greater population of urease presence in soil, the higher ammonia volatilisation rate.
5)	Soil content	Clay in soils adsorbs ammonium ions reducing loss into atmosphere.
6)	Soil buffer capacity	The amount of clays in soil can act as a medium to alter the soil pH.
7)	Wind velocity	Higher wind velocity can promote ammonia volatilisation.
8)	Rainfall	Urea fertilisers dissolved readily into soil when contacted with rain.
9)	Residues	Residues act as a filter to strand fertilisers from soil to lower the exposure of urease enzyme.
10)	Calcium carbonate	Lime in the soil will react with ammonium ions.

2.2 Urease Inhibitors

Generally, the application of urease inhibitors can be found in medical and agricultural fields. For agriculture usage, urease activities can be inhibited by lowering the amount of ammonia gas released into the atmosphere. This mechanism followed the enzyme catalysed reaction to modify the active site of urease which also known as metalloenzyme. There are four types of chemical structures that this enzyme can be classified into as shown in Table 2 [10, 11].

Table 2: Types of Urease Inhibitors based on Chemical Structures [10]

Group	Description
First	Thiolic compounds which contain anions react with the active site of urease.
Second	The derivatives and hydroxamic acid itself that will bind to the enzyme.
Third	Phosphorodiamidates substituition on active site.
Fourth	Average inhibition that consists of nickel, chelators and lugands from fluoride ion and certain peptides.

2.2.1 Chemical Based Urease Inhibitor (NBPT)

The most effective chemical based urease inhibitor is known as N (n-butyl) Thiophosphoric Triamide (NBPT), in short NBPT. Normally, it has a trade name called AGROTAIN® with formulation of 25% NBPT, 60% to 65% of unspecified nontoxic substances and 15% of N-methyl pyrrolidone. In previous toxicological studies, NBPT is listed as hazardous chemical with the ability to cause eye irritation and respiratory problems which has been proven in one of the incident report that the workers do suffered such illness after exposed to AGROTAIN® [12]. The general physical properties of NBPT are listed down in Table 3 [13, 14].

Table 3: Physical Properties of NBPT [13]

No.	Physical Properties	Description
1)	**Molecular Formula**	$C_4H_{14}N_3PS$
2)	**Molecular weight**	167.2 g/mole
3)	**Appearance**	White crystalline solid
4)	**Boiling point**	264.0°C
5)	**Melting point**	59.1°C
6)	**Density**	1223.2 kg/m^3

Many researchers had performed studies on the application of NBPT and other chemical based urease inhibitors with different types of plants. The results obtained were depending on various factors such as methodology, concentration of urease inhibitors applied with urea fertiliser, plant's species and etc. Most of the plants are vegetables that human consume daily. In the following Table 4 showed the summary of previous literature reviews.

Table 4: Summary of Literature Reviews on Different Types of Plants with NBPT

Plant Name	Methodology	Results	References
Hordeum vulgare L.	1. Hordeum vulgare L. is experimented with NBPT under Mediterranean conditions to evaluate the effectiveness of N losses after applying urea. 2. The N concentrations in soil, dissolved organic carbon (DOC), denitrification potential, NO_x fluxes were and crop yield are determined.	Ammonia gas emission is reduced significantly after NBPT applications. Crop yield increased by 5% followed by N uptake up to 6%. In the experimental conditions, the results showed the potential of NBPT in abating NO_x emissions from soils with urea fertilizers.	[1]
Pisum sativum and Spinacea Oleracea	1. Cultivation is done in hydroponic culture with urea. 2. Application of NBPT is done after 2 to 3 weeks. 3. At days 0, 1, 2, 4, 7 and 9 the NBPT content in these tissues were determined. 4. Urea, urease, amino acid and ammonium contents were determined in shoots and roots.	Pisum sativum is more affected by the NBPT absorbed by inhibiting the urease activities in leaves and roots. The leaves was observed to have necrotic leaf margins. Reduction of ammonium and amino acid content which caused by changes in N assimilation are determined. Spinacea Oleracea is 35% less affected compare to	[2]

		Pisum sativum and no obvious inhibition of urease activities.	
Lolium perenne	1. Four application with 40 kg Nha^{-1} urea fertilisers • 'Green Urea 14'containing 45.8 % N as urea • 'Agrotain_' consists of NBPT with 5 L of urea • 'Nhance' a fine particle spray consists of 46 % N as urea • 'Agrotain' with 1 L of urea and gibberellic acid. 2. In autumn and spring the ammonia loss was determined.	In Autumn, Green Urea and Nhance reduced NH_3 emmisions to 9 and 23 %. During spring the ammonia loss only 2% due to 4 mm of rain fell within 1 day after application onto wet soil. Overall, 72.8 % of the applied N is recovered in the plants and soil.	[15]
Triticum aestivum L.	1. For 4 weeks the plants were grown in a greenhouse with urea fertilisers and NBPT at concentrations of (0, 0.012, 0.062 and 0.125% w/w). 2. Each NBPT concentrations were replicated 6 times. 3. A control plant with no treatment was also cultivated. 4. At the end of growth period, the N metabolism were determined.	Physical effects like transitory yellowing of the leaf tips were observed. A greater amount of urea in plant tissues were detected with decrease of amino acid glutamine synthetase and urease activities. At the end of study period the physical and metabolism effects had gradually recovered.	[16]

2.2.2 Bio Based Urease Inhibitors (Allium Sativum L.)

Sustainable development in agricultural field has favoured the used of organic substances instead of chemicals in fertilisation process. With this, many studies are carried out to determine the potential of inhibition abilities from different types of plant extracts. So far, previous research determined that garlic (Allium Sativum L.) which had its own unique odour and flavour from other vegetables appeared to be one of the most effective bio-based urease inhibitor. Below table showed the comparison on the inhibition study results obtained from garlic, onion, leek, cabbage and Brussel sprouts extracts in terms of thiosulfinates concentrations [7].

Table 5: Comparisons between Allium and Brassica on Inhibitory Properties [7]

Types of Extracts	Methodology	Results
Allium (garlic, onion, leek)	1. Firstly, the thiosulfinates concentrations are identified using spetrophotometric method. 2. The inhibitions mechanisms are analysed using phenol-hypochlorite method and graphs are plotted accordingly.	All plant extracts showed inhibition abilities and the TS concentration in the extracts determined the inhibition strength. In this case, garlic juice is the most efficient compare with others.
Brassica (cabbage, Brussels sprouts)		Brussels sprouts extract appeared to be the second most efficient whereas the least efficient is cabbage extract.

2.3 Chlorophyll as Plant Growth Indicator

The leaves of a plant contained essential photosynthetic pigments like chlorophylls, carotenoids and etc. that are responsible for the normal growth of plants. They are very useful for photosynthesis reaction to supply glucose for the plants as main source of food while regulating the oxygen in atmosphere. In general, chlorophylls are green in colour due low green light absorption in the spectrum whereas absorption of blue-violet light are by carotene and lutein Previously, the correlation between the leaf conditions in terms of chlorophylls absorption and nitrogen content has been used as analysis study [17]. In addition, literature review on leaves assessment of winter wheat using chlorophyll fluorescence parameters in China has shown promising results as plants indicator on nitrogen contents [18].

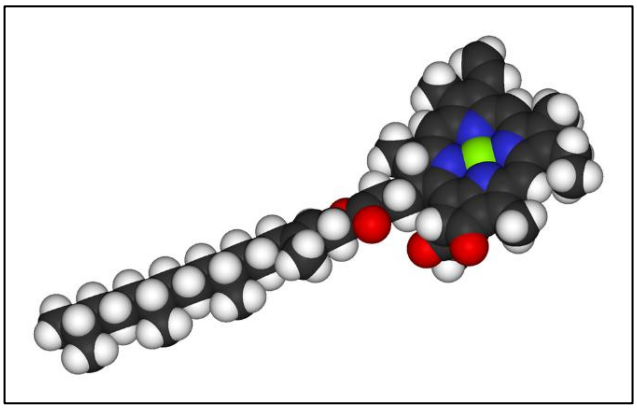


Figure 1: Chlorophyll Molecule in 3D [19]

Besides that, a research was done at Christmas Hills in Tasmania on a half-year old *E. globulus* and one and a half – year-old *E. nitens* trees that experiencing leaf necrosis due to foliar pathogen using SPAD as chlorophyll indicator to keep track of the growth resulted in approximately 80% of E. globulus leaves were affected with an average affected area per leaf of 15% whereas E. nitens were less affected as only 30% of leaves affected with an average affected area per leaf of 5% [20].

CHAPTER 3: RESEARCH METHODOLOGY

In chapter 3, sequences on the methodology are planned to execute the project with the aim to achieve the objectives as defined earlier. For this project, the experiments will be conducted with two separate methodologies. The first part is to determine the effects of plant growth applied with urea solution, NBPT and TS by measuring the concentration of chlorophyll, follow by, second part to compare the performance of TS in garlic extract and NBPT as urease inhibitors.

3.1 Set-up of Experimental Plants

The seeds of spinach (*Spinacia oleracea*), mustard greens (*Brassica juncea)* and water spinach (*Ipomoea aquatic*) in Figure 2 were obtained from the current market and ensured to be in good condition before being sown in soil: perlite (1:1 v/v) separately and irrigated with deionized water as shown in Figure 3.

Figure 2: Seeds of (from left) Spinacia Oleracea, Brassica Juncea and Ipomoea Aquatic

Figure 3: Experimental Plants Set-up

The growth rate of the plants varied according to its species as germination period of Ipomoea Aquatic seed is the shortest compare with Spinacia Oleracea and Brassica Juncea seeds which need a longer time. After 2 months, the plants are fully grown with leaves (Figure 4). Then, urea (180 kg N ha^{-1}) solution with different concentrations of 0N (n-butyl thiophosphoric triamide) (NBPT) and Thiosulfinates (TS) are applied to each plants separately with three replicates of concentrations 0.012%, 0.062% and 0.125% w/w. Control plants are also prepared with only urea solution. After that, physical observations on the leaves are carried out throughout 7 days after application and any visible changes such as leaf-tip scorch or necrosis are recorded [16].

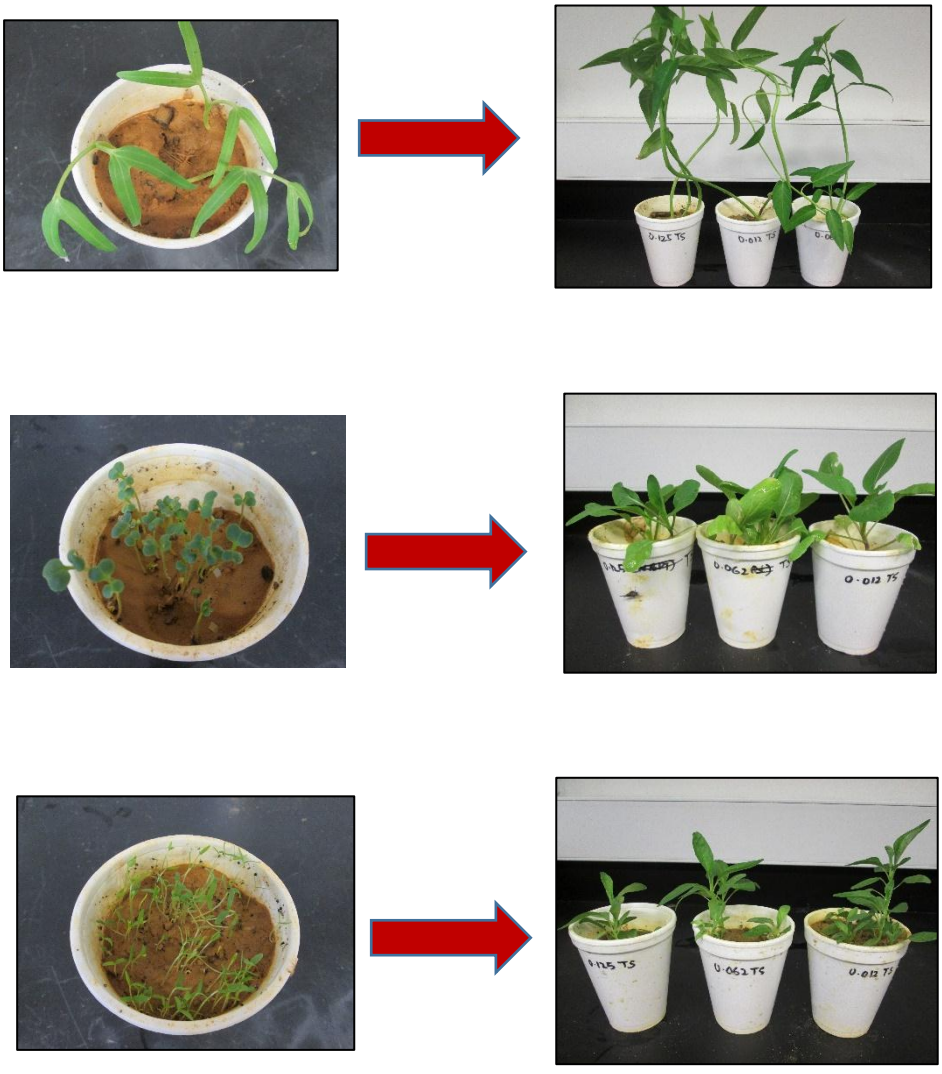


Figure 4: Images of Ipomoea Aquatic (top), Brassica Juncea and Spinacia Oleracea Plants after two weeks (left) and after 2 months (right)

3.2 UV-VIS Spectrophotometer Device Start-Up

The concentrations of chlorophyll for the experimental plants are determined using Trichromatic method in the UV-VIS spectrophotometer device. The range of wavelength is from 200 to 700 nm in detecting the absorbance of various samples. Before conducting the analysis, internal calibration need to be performed by placing a blank sample in the UV-VIS spectrophotometer device for 15 minutes to set the baseline. Once the calibration is completed, sample extract solution in cuvette will be inserted into the sampling slot to measure the absorbance at different wavelengths. With that, by using the UV Winlab Software a graph of absorbance against wavelength can be plotted as shown in Figure 4 [21].

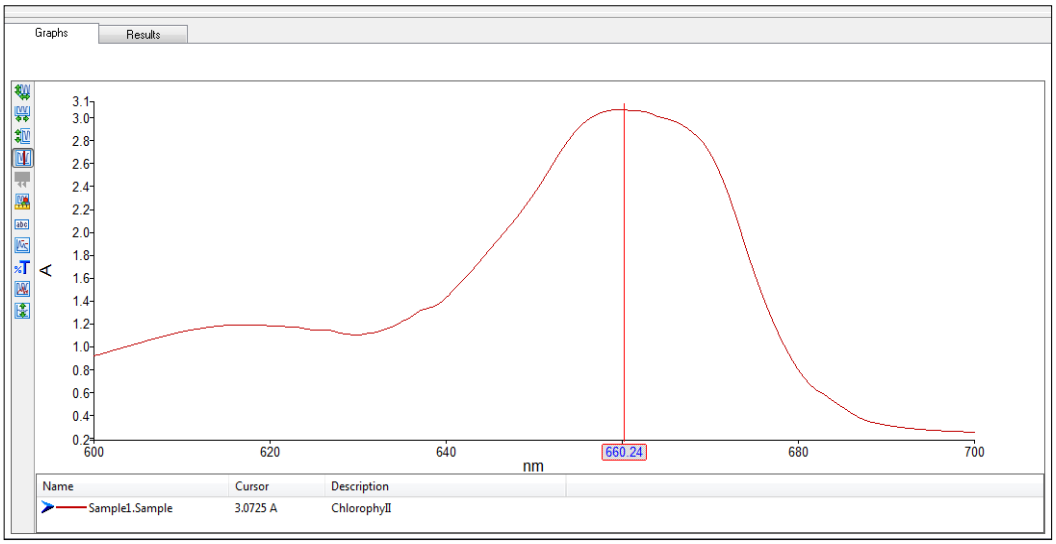
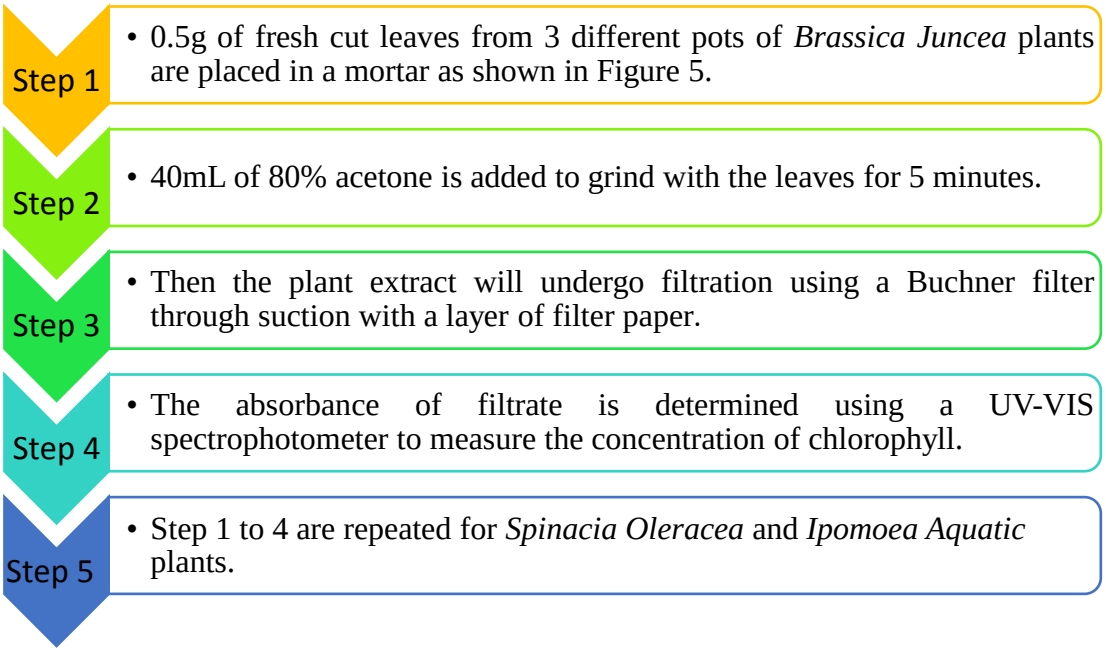


Figure 5: Graph of Absorbance vs. Wavelength in UV Winlab Software

3.3 Chlorophyll Analysis

The chlorophyll of experimental plants are determined at day 3, 5 and 7 after the application of urea solution with different concentrations of urease inhibitors which are NBPT and TS. This is to ensure sufficient time for fertilization to take place before analyzing the concentration of chlorophyll in plants' leaves. Therefore, the methodology to prepare the plant extract solutions for chlorophyll analysis are described as below:



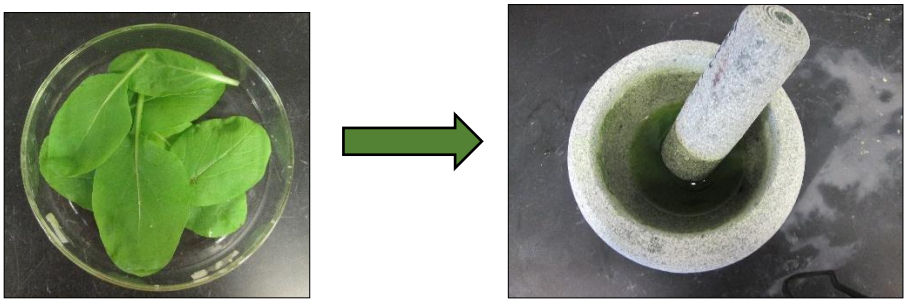


Figure 6: Preparation of Plant Extract

The total chlorophyll a, b and c of mustard greens (*Brassica juncea),* spinach (*Spinacia oleracea*) and water spinach (*Ipomoea aquatic*) plants are determined from the leaves extract using Trichromatic method (SCOR-UNESCO) in the UV Winlab Software with the equations below [21]:

$$C_a[\text{mg m}^{-3}] = (\ 11.85\ D_{663-665} - 1.54\ D_{647} - 0.08\ D_{630}\ )\ v\ l^{-1}\ V^{-1} \quad (3)$$

$$C_b[\text{mg m}^{-3}] = (\ -5.43\ D_{663-665} + \ 21.03\ D_{647} - 2.66\ D_{630}\ )v\ l^{-1}\ V^{-1} \quad (4)$$

$$C_c[\text{mg m}^{-3}] = (\ -1.67\ D_{663-665} - 7.6\ D_{647} + 24.52\ D_{630}\ )\ v\ l^{-1}\ V^{-1} \quad (5)$$

Table 6: Description of Symbols in Trichromatic method equations

Symbol	Description
C_a	Chlorophyll a
C_b	Chlorophyll b
C_c	Chlorophyll c
$D_{663-665}$	Data of absorbance 663 – 665nm
D_{647}	Data of absorbance at 647 nm
D_{630}	Data of absorbance at 630 nm

3.4 Preparation of Standard Garlic Extract

The standard garlic extract used in this experiment is 1g/30ml by diluting 1g of garlic powder in 30mL of deionized water. The overall preparation of garlic extract is summarized as below:

Fresh garlic cloves are sliced into approximately 3mm thick and dried at 50℃ for 24 hours.

After that, pulverized into powder form and the standard solution is prepared at 1g/30ml (Figure 6).

The stadard solution then undergo filtration and centrifugation for approximately 4mins to remove impurities.

Then the garlic extract is stored at 5°C.

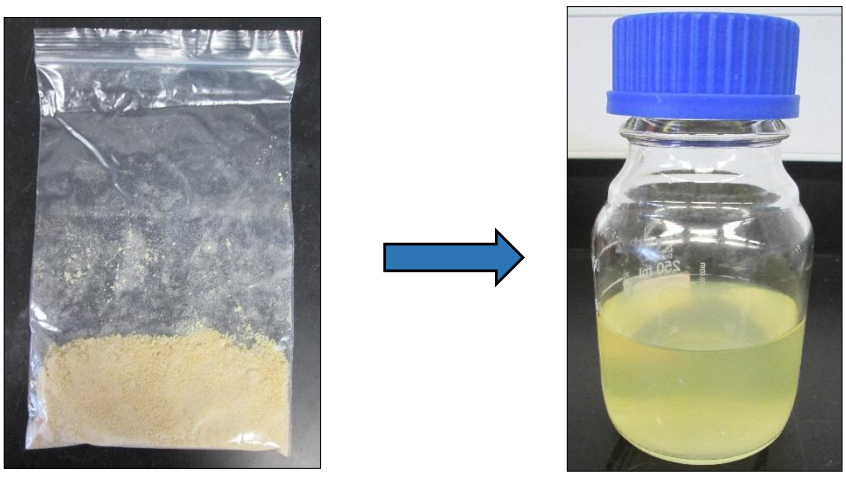


Figure 7: Preparation of Standard Garlic Extract

3.5 NBPT Solution Analysis

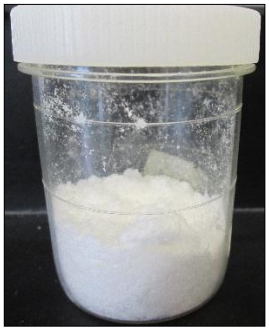


Figure 8: NBPT in Powder Form

The N (n-butyl) Thiophosphoric Triamide, (NBPT) is a chemical based urease inhibitors in white colour powder form (Figure 7). The analysis on 1g of NBPT is conducted by diluting with different concentrations of distilled water to analyse the absorbance at different wavelengths in UV-VIS spectrophotometer. Table 7 showed the dilution ratios. The graph of absorbance (A) against wavelength (nm) on 5 samples of NBPT are obtained using UV- VIS spectrophotometer are attached in Appendices.

Table 7: Dilution Ratios of NBPT

Sample No.	NBPT (g)	Deionized Water (ml)
1	1.0	10
2	1.0	20
3	1.0	30
4	1.0	40
5	1.0	50

3.6 Ammonium Standard

Before performing inhibition studies, the molar absorptivity of ammonium need to be determined in order to calculate the concentration of ammonia gas released when apply with urease inhibitors. In this experiment, urea fertilizer is applied with jack bean urease to stimulate the actual condition of fertilization reaction, ammonia gas, NH_3 will be released due to ammonia volatilisation as showed in equation 4 below:

$$(NH_2)_2CO + H_2O \xrightarrow{urease} 2\,NH_3 + CO_2 \qquad (6)$$

Hence, a standard calibration curve for ammonia gas is conducted by using ammonium chloride and the highest absorbance was found to be 630nm in wavelength range. The absorbance at 630nm is determined and the molar absorptivity of NH_3 is calculated using Beer – Lambert's Law with equation 5:

$$A = \varepsilon bc \qquad (7)$$

A = Absorbance

ε = Molar absorptivity (L mol^{-1} cm^{-1})

b = Path length (cm)

c = Concentration of species (mol L^{-1})

The path length, b value is 1 cm, while the Absorbance is 2.05 and concentration of 1.628 mol L^{-1}. After substituting all the values in equation 5, the molar absorptivity of NH_3 is calculated as 1.259 L mol^{-1} cm^{-1}.

3.7 Inhibition Studies of TS and NBPT

The inhibition studies are conducted prior to compare the performance of TS as a bio based urease inhibitor against NBPT the chemical based urease inhibitor. In this experiment, 50mM urea, 20mM phosphate buffer at pH 2.0 and 2mM EDTA are well mixed into a 25mL standard assay mixture. The phosphate buffer functioned as a medium for controlling the pH value of the solution while EDTA is the nutrients supplier such as copper, zinc and etc. during the experimental time for the urease.

Next, 0.5mg/ml of urease solution and 30mg/ml of the standard garlic extract are mixed at similar volume. 30mg/ml of aliquot with urease-garlic mixture will be transferred into standard assay mixture in 5 minutes intervals for 120 minutes incubation time at temperature of 30°C to maintain the thiols stability.The ammonia concentration in the extract is determined through the enzymatic reaction in UV-VIS spectrophotometer and phenol – hypochlorite method is used to compare with the standard ammonia.

Similarly, this methodology is also applied for NBPT inhibition studies, the only difference is to prepare 0.5mg/ml of urease solution and 30mg/ml of the NBPT solution in equal volume. Then 30mg/ml of aliquot with urease-NBPT mixture will transferred into standard assay mixture in 5 minutes intervals for 120 minutes incubation time.

After transferring 1ml of urease mixture during 5 minutes interval into the standard assay mixture, stirring is done gently to ensure the solution is uniformly mixed. Then it is placed into a cuvette and instantly analyzed the absorbance of mixture in UV-VIS spectrophotometer at 630nm. The graph trends and absorbance values are noted throughout the experiment for both NBPT and TS [7].

3.8 Key Project Milestones

The overall project milestones for Final year Project II is summarized in Figure 8 below:

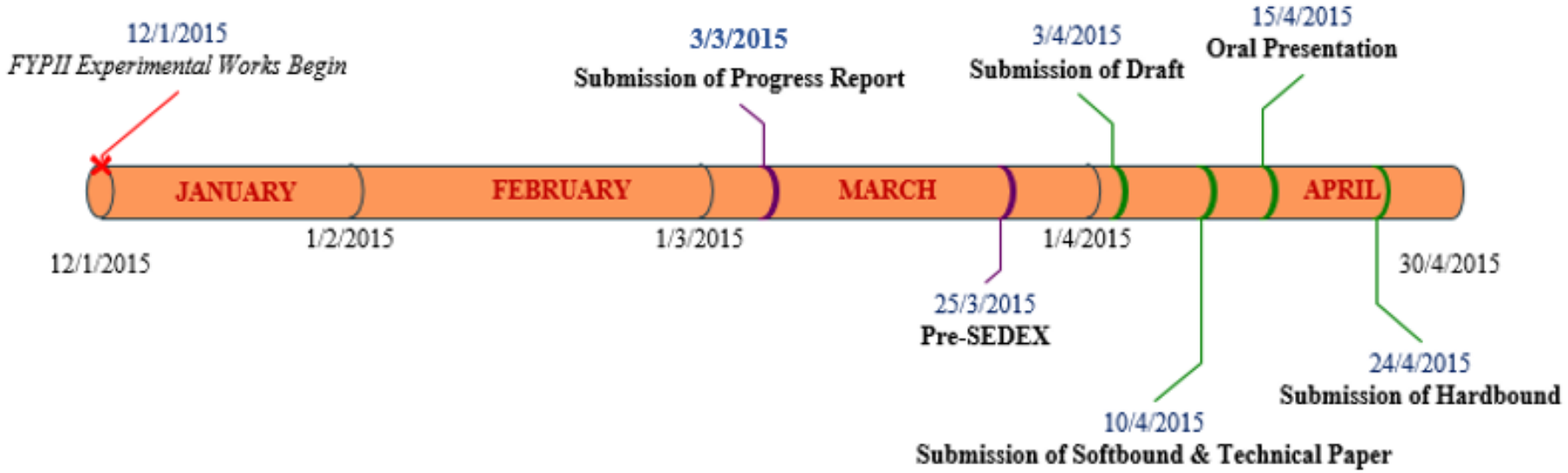


Figure 9: FYPII Key Project Milestones

3.9 Project Timeline

A Gantt-Chart is used to define the project timeline when carrying out FYPII.

No	ACTIVITIES	Week														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	FYPII Experimental Works Begin															
2	Submission of Progress Report															
3	Project Works Continue															
4	Pre-SEDEX															
5	Submission of Draft															
6	Project Analysis and Reporting															
7	Submission of soft bound															
8	Submission of technical paper															
9	Oral Presentation															
10	Submission of Hardbound															

Table 8: FYPII Gantt Chart

3.10 Equipment

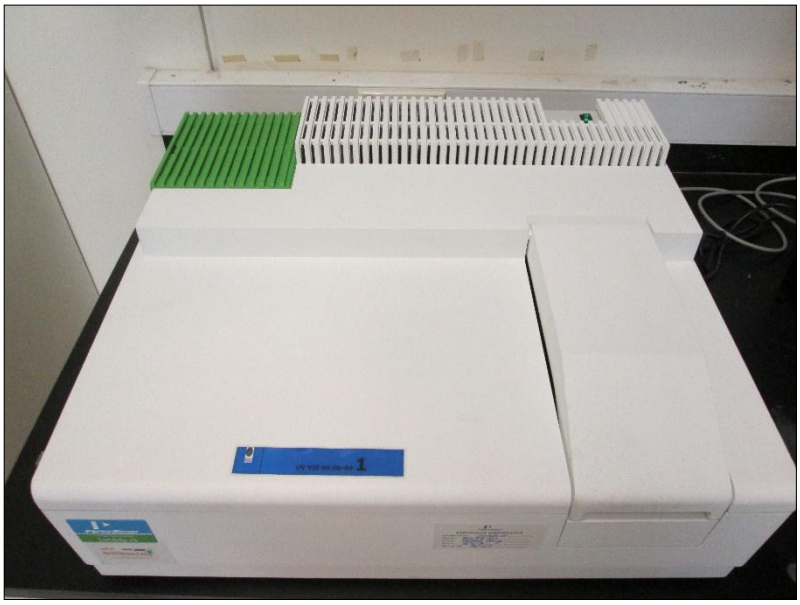


Figure 10: Perkin Elmer UV-VIS Spectrophotometer

Perkin Elmer UV-VIS spectrophotometer is the main instrument used throughout the research study for chlorophyll determination and inhibition studies of TS and NBPT. It can absorb wavelengths of the sample solution and reflect it to be recorded in graphical method. Basically, it operates with the principle of absorbing of photons after passes through a sample solution. In visible spectrophotometry, the absorption or the transmission of a certain substance can be determined by the observed colour. For instance, a solution sample that absorbs light over all visible ranges which transmits none of visible wavelength appears black in theory. This UV-VIS spectrophotometer will be used in the range of 200nm to 700nm [21].

CHAPTER 4: RESULTS AND DISCUSSION

The results obtained in all the experiments are fully discussed in this chapter. For physical changes on the plants' leaves, the images are recorded and mean chlorophyll concentrations are plotted in column charts with standard error bar. Not forgetting the NBPT and TS inhibition studies, calculations are performed according to Beer-Lambert's Law to determine the concentration of ammonia gas released to plot a graph.

4.1 Physical Observations on Plants' Leaves

Water spinach (*Ipomoea aquatic*), spinach (*Spinacia oleracea*) and mustard greens (*Brassica juncea)* plants (Figure 11) are each applied with 180 kg N ha^{-1} urea solution together with 0.012%, 0.062% and 0.125% w/w concentrations of N (n-butyl thiophosphoric triamide) (NBPT) and Thiosulfinates (TS) separately. Control plants with urea solution only are also prepared. Four major physical changes such as chlorosis, necrosis, necrotic leaf margin and leaf - tip scorch are observed on the leaves at day 3, 5 and 7 and recorded accordingly.

Figure 11: (From left) Ipomoea Aquatic, Spinacia Oleracea and Brassica Juncea

The observation data on NBPT application on each plants are tabulated, where, **A** = Urea only, **B** = Urea + 0.012% NBPT, **C** = Urea + 0.062% NBPT, **D =** Urea + 0.125% NBPT, **Y** = Yes, **N** = No

Table 9: Physical Observations on Ipomoea Aquatic with NBPT

Physical Observations on *Ipomoea Aquatic*	**Day**											
	3				**5**				**7**			
	A	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Chlorosis	N	Y	N	N	N	Y	N	N	N	Y	Y	Y
Necrosis	N	N	N	N	N	Y	Y	Y	N	Y	Y	Y
Necrotic leaf margin	N	N	Y	Y	N	N	Y	Y	N	Y	Y	Y
Leaf - tip scorch	N	N	Y	Y	N	Y	Y	Y	N	N	Y	Y

Table 10: Physical Observations on Brassica Juncea with NBPT

Physical Observations on *Brassica Juncea*	**Day**											
	3				**5**				**7**			
	A	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Chlorosis	N	N	N	N	N	Y	Y	Y	N	Y	Y	Y
Necrosis	N	N	Y	N	N	Y	Y	Y	N	Y	Y	Y
Necrotic leaf margin	N	N	Y	N	N	Y	Y	Y	N	Y	Y	Y
Leaf - tip scorch	N	N	Y	N	N	Y	Y	Y	N	Y	Y	Y

Table 11: Physical Observations on Spinacia Oleracea with NBPT

Physical Observations on *Spinacia Oleracea*	**Day**											
	3				**5**				**7**			
	A	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Chlorosis	N	N	Y	Y	N	Y	Y	Y	N	N	Y	Y
Necrosis	N	N	N	N	N	N	N	N	N	N	N	N
Necrotic leaf margin	N	N	N	N	N	N	N	N	N	N	N	N
Leaf - tip scorch	N	Y	Y	N	N	Y	Y	Y	N	Y	N	N

Overall, the affected percentage for each plants applied with NBPT and urea fertilizer are calculated with 52.0% for Ipomoea Aquatic, 58.3% for Brassica Juncea and 27.0% for Spinacia Oleracea. Based on the percentage, the most affected plant is Brassica Juncea whereas Spinacia Oleracea is the least affected.

The observation data on TS application on each plants are tabulated, where,

A = Urea only, **B** = Urea + 0.012% NBPT, **C** = Urea + 0.062% NBPT, **D =** Urea + 0.125% NBPT, **Y** = Yes, **N** = No

Table 12: Physical Observations on Ipomoea Aquatic with TS

Physical Observations on *Ipomoea Aquatic*	**Day**											
	3				**5**				**7**			
	A	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Chlorosis	N	N	N	N	N	N	N	N	N	N	N	N
Necrosis	N	N	N	N	N	N	N	N	N	N	N	N
Necrotic leaf margin	N	N	N	N	N	N	N	N	N	N	N	N
Leaf - tip scorch	N	N	N	N	N	N	N	N	N	N	N	N

Table 13: Physical Observations on Brassica Juncea with TS

Physical Observations on *Brassica Juncea*	**Day**											
	3				**5**				**7**			
	A	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Chlorosis	N	N	N	N	N	N	N	N	N	N	N	N
Necrosis	N	N	N	N	N	N	N	N	N	N	N	N
Necrotic leaf margin	N	N	N	N	N	N	N	N	N	N	N	N
Leaf - tip scorch	N	N	N	N	N	N	N	N	N	N	N	N

Table 14: Physical Observations on Spinacia Oleracea with TS

Physical Observations on *Spinacia Oleracea*	**Day**											
	3				**5**				**7**			
	A	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Chlorosis	N	N	N	N	N	N	N	N	N	N	N	N
Necrosis	N	N	N	N	N	N	N	N	N	N	N	N
Necrotic leaf margin	N	N	N	N	N	N	N	N	N	N	N	N
Leaf - tip scorch	N	N	N	N	N	N	N	N	N	N	N	N

The affected percentage for each plants applied with TS and urea fertilizer are calculated with 0.0% for Ipomoea Aquatic, 0.0% for Brassica Juncea and 0.0% for Spinacia Oleracea. Hence, this showed that TS does not caused any physical changes to the plants applied.

According to the observation results on Table 9, 10 and 11, all of the plants with 3 replicates supplied with 0.012%, 0.062% and 0.125% of NBPT along with urea solution indicated chlorosis, necrosis, leaf-tip scorch and necrotic leaf margin on cotyledon and foliar leaves during 7 days application except for the control plants. The chlorophyll concentration began its measurement at day 3 onwards as there are no significant changes in the first 2 days after applications. Besides, this is also to ensure the urea solution treated with NBPT are fully absorbed by roots into the plant metabolism.

From the data tabulated in Table 9, 10 and 11, the most sensitive plant was found to be Brassica Juncea as majority of the leaves observed to have undergone chlorosis, necrosis, necrotic leaf margin and leaf –tip scorch at day 5 and 7 whereas Spinacia Oleracea is the least likely to show significant physical changes. This may be due to the differences in plant species as Spinacia Oleracea may have portrayed higher resistance against NBPT affecting the urease activity within the leaves compared to the other plants treated with NBPT [2]. The sensitivity of the plants to NBPT treatment can be expressed in descending order as below:

Brassica Juncea > Ipomoea Aquatic > Spinacia Oleracea

Furthermore, the physical changes are mainly due to the excessive accumulation of urea in the leaves after application. Previous studies stated that high concentration of urea accumulated in the plant when treated with more NBPT resulting in strong inhibition of leaf and soil urease which reasoned in yellowing of the leaves at the beginning of experiment. However, in this experiment Ipomoea Aquactic observed to have formation of new leaves after 1 week of NBPT treatment. Most of the affected area will ended up with necrosis where the cell structure in the leaves slowly degraded and detached from the plant stem. [16].

Based on Figure 12 to 14, the physical changes on plant leaves are observed for 7 days when carrying out chlorophyll analysis. The leaves are photographed at day 7 when there are any chlorosis, necrosis, leaf-tip scorch and necrotic leaf margin. The results show that Spinacia Oleracea does not have signs of necrosis and chlorosis observed on leaves. Basically, the symptoms of the leaves are due to ammonium toxicity where excessive amount of urea happened to accumulated in within the leaves tissues led to necrotic leaf margin and leaf tip scorch [2].

Phycsical Changes Observed	**Images of *Spinacia Oleracea* Leaves**
Chlorosis	
Leaf-tip scorch	

Figure 12: Images of Spinacia Olerecea Leaves with NBPT

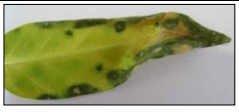
Physical Changes Observed	**Images of *Ipomoea Aquatic* Leaves**
Chlorosis	
Necrosis	
Necrotic Leaf Margin	
Leaf-tip scorch	

Figure 13: Images of Ipomoea Aquatic Leaves with NBPT

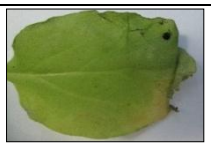
Physical Changes Observed	Images of *Brassica Juncea* Leaves
Chlorosis	
Necrosis	
Necrotic Leaf Margin	
Leaf-tip scorch	

Figure 14: Images of Ipomoea Aquatic Leaves with NBPT

In contrast, based on Table 12, 13 and 14 the plants with 3 replicates treated with 0.012%, 0.062% and 0.125% of TS and urea solution does not have any sign of physical changes. Table 15 showed the plants treated with TS do not have any significant changes. The TS applied with urea fertiliser is a bio based urease inhibitors and it does not cause excessive ammonia accumulation within the plant leaves to occur.

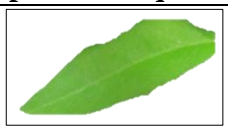
Images of Leaves Treated with TS		
Ipomoea Aquatic	**Brassica Juncea**	**Spinacia Oleracea**
		

Figure 15: Images of (from left) Ipomoea Aquatic, Brassica Juncea and Spinacia Oleracea Leaves with TS

4.2 Chlorophyll Analysis

The graph in Figure 16, 17, 18, 19, 20 and 21 illustrated the mean concentration of chlorophyll against day 3, 5 and 7 for Ipomoea Aquatic, Brassica Juncea and Spinacia Oleracea with NBPT and TS. Generally, it can be observed that the chlorophyll of control plants are much higher than the plants with NBPT treatment and 0.125% NBPT applied with urea gave the lowest chlorophyll contents except in Figure 13, Brassica Juncea treated with 0.012% of NBPT at day 3 has chlorophyll concentration 57.84 mg/mg^3 more than control plant. The cause is related to high absorption rate of urea and NBPT by the plant root at beginning. However, the concentration reduced at following day 5 and 7. This is due to the high accumulation of urea concentration within the leaves resulting in the decreased of chlorophyll concentration [20].

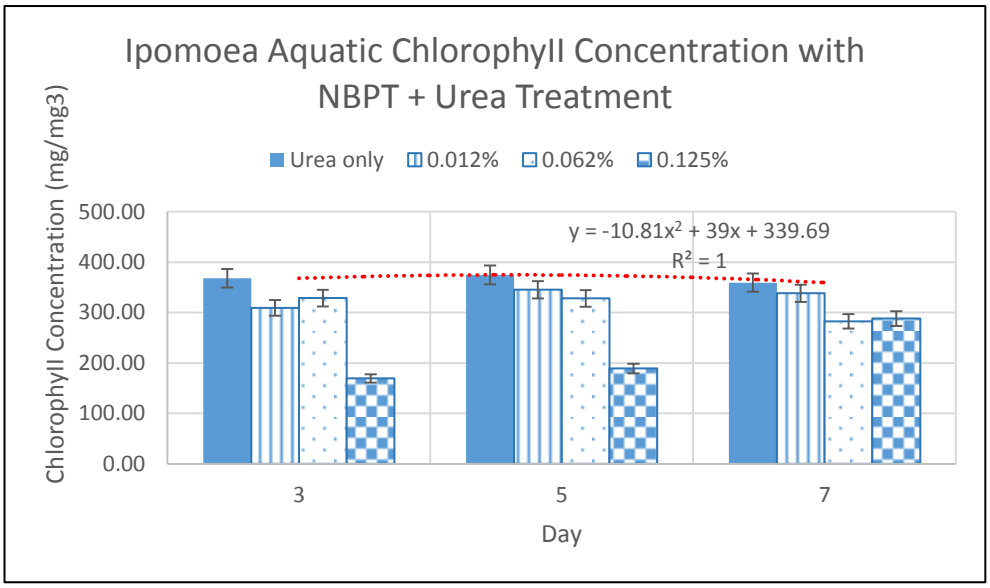


Figure 16: Graph of Ipomoea Aquatic Chlorophyll Concentration with NBPT and Urea Treatment. Data are shown as mean ± Standard Error of 3 replicates.

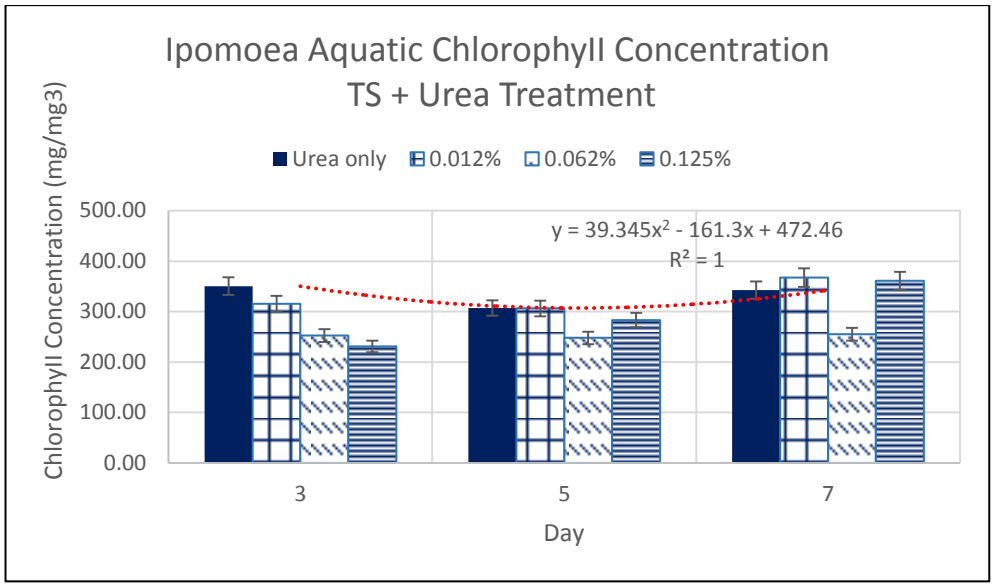


Figure 17: Graph of Ipomoea Aquatic Chlorophyll Concentration with TS and Urea Treatment. Data are shown as mean ± Standard Error of 3 replicates.

Table 15: Mean Chlorophyll Results of Ipomoea Aquatic with 0.012%, 0062% and 0.125% of NBPT / TS with Urea Treatment.

Ipomoea Aquatic								
	(NBPT + Urea w/w %)				**(TS + Urea w/w %)**			
Day	**Control**	**0.012%**	**0.062%**	**0.125%**	**Control**	**0.012%**	**0.062%**	**0.125%**
	Chl a	**Chl a**	**Chl a**	**Chl a**	**Chl a**	**Chl a**	**Chl a**	**Chl a**
3	367.88	309.16	328.99	169.40	350.51	315.70	252.40	230.83
5	374.45	345.40	328.27	189.19	307.25	306.23	247.99	283.23
7	359.40	338.33	282.58	288.08	342.68	367.58	255.26	360.83

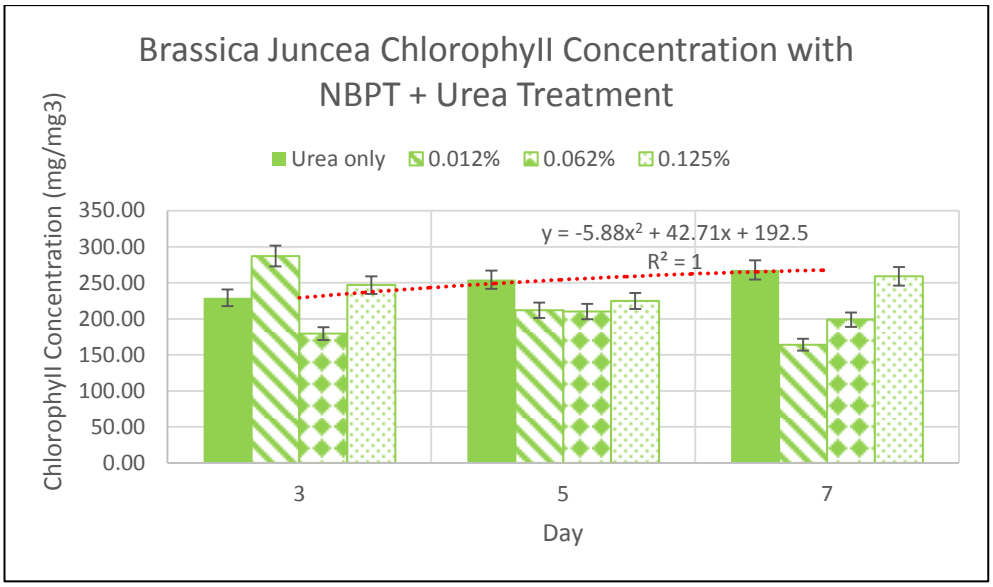


Figure 18: Graph of Brassica Juncea Chlorophyll Concentration with NBPT and Urea Treatment. Data are shown as mean ± Standard Error of 3 replicates.

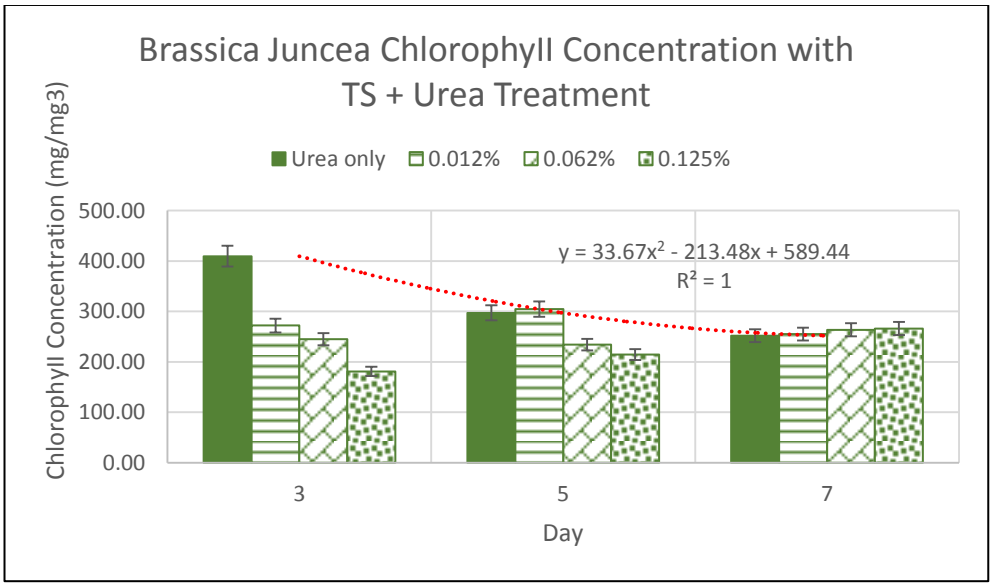


Figure 19: Graph of Brassica Juncea Chlorophyll Concentration with TS and Urea Treatment. Data are shown as mean ± Standard Error of 3 replicates.

Table 16: Mean Chlorophyll Results of Brassica Juncea with 0.012%, 0062% and 0.125% of NBPT / TS with Urea Treatment.

Brassica Juncea								
	(NBPT + Urea w/w %)				(TS + Urea w/w %)			
Day	Control	0.012 %	0.062 %	0.125 %	Control	0.012 %	0.062 %	0.125 %
	Chl a	Chl a	Chl a	Chl a	Chl a	Chl a	Chl a	Chl a
3	229.33	287.17	179.39	246.83	409.63	272.13	245.04	181.16
5	254.40	211.92	210.15	224.76	297.16	304.48	234.15	214.39
7	267.71	164.04	198.96	258.92	252.03	255.26	263.6	266.20

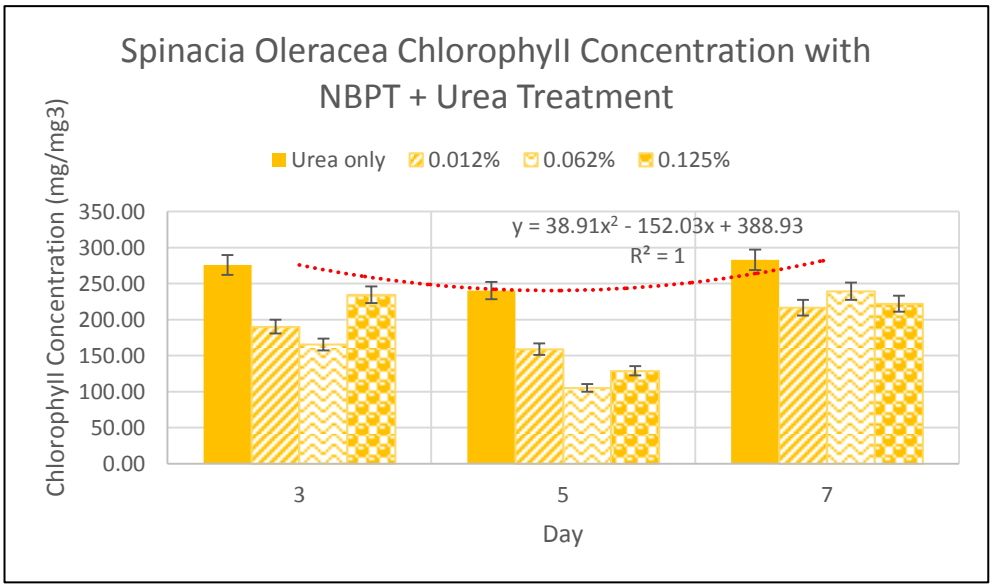


Figure 20: Graph of Spinacia Oleracea Chlorophyll Concentration with NBPT and Urea Treatment. Data are shown as mean ± Standard Error of 3 replicates.

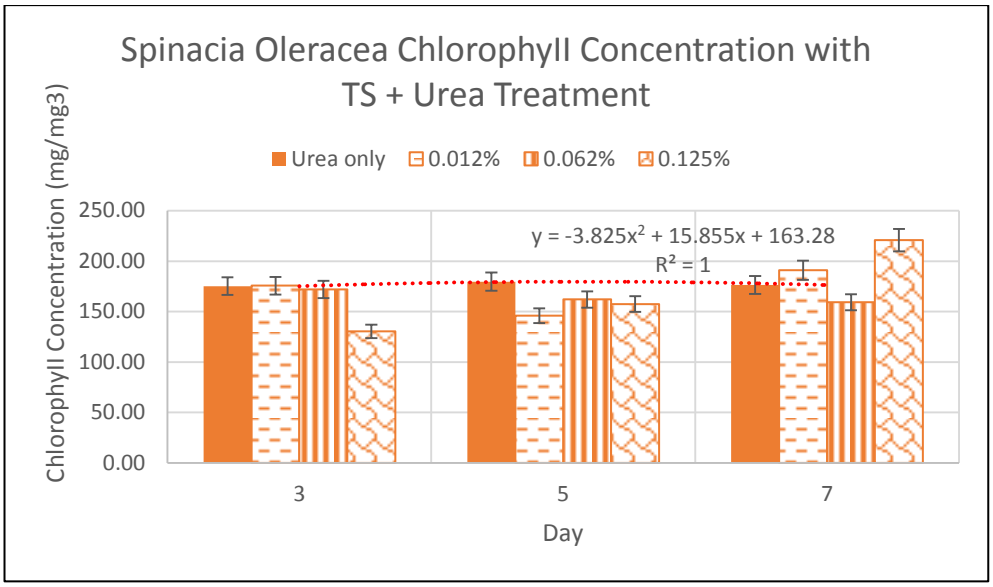


Figure 21: Graph of Spinacia Oleracea Chlorophyll Concentration with TS and Urea Treatment. Data are shown as mean ± Standard Error of 3 replicates.

Table 17: Mean Chlorophyll Results of Ipomoea Aquatic with 0.012%, 0062% and 0.125% of NBPT / TS with Urea Treatment.

Spinacia Oleracea								
	(NBPT + Urea w/w %)				**(TS + Urea w/w %)**			
Day	**Control**	**0.012%**	**0.062%**	**0.125%**	**Control**	**0.012%**	**0.062%**	**0.125%**
	Chl a	**Chl a**	**Chl a**	**Chl a**	**Chl a**	**Chl a**	**Chl a**	**Chl a**
3	275.81	190.31	165.59	234.57	175.31	175.65	171.94	130.42
5	240.51	158.97	105.21	128.87	179.69	145.82	162.12	157.49
7	283.03	216.73	239.56	222.11	176.42	190.96	159.17	220.87

The variations in chlorophyll results are mainly due to the rate of absorption of the plants roots and the availability of NBPT and TS in soil after application. Similarly, the treatment with TS also showed decrease of chlorophyll concentration at day 3 but gradually increase back at day 5 and 7. Moreover, at day 7 most of the chlorophyll concentrations of the plants exceeded the chlorophyll contents of control plants as seen in Figure 12, 13 and 14. This is because treatment with TS did not show any symptoms of ammonium toxicity on leaves unlike NBPT which has been verified as one of the most effective urease inhibitor in previous studies [22].

Furthermore, the consistency of the results obtained for plants treated with TS are higher as the concentration of chlorophyll are not likely to be affected by the treatment . This can be reason that no physical changes are observed on the leaves for TS treatment plants because the chlorophyll contents are less affected.

The analysis on the bar charts are further done by plotting a second order polynomial trendline using Microsoft Excel tool as an estimation to obtain the average chlorophyll concentration when x = 1, 2, 3 and so on. Hence the equations are summarized below.

Table 18: Polynomial Equations of Figure 16, 17, 18, 19, 20 and 21

Figure 16	$y = -10.81x^2 + 39x + 339.69$	(8)	$R^2 = 1$
Figure 17	$y = 39.345x^2 - 161.3x + 472.46$	(9)	$R^2 = 1$
Figure 18	$y = -5.88x^2 + 42.71x + 192.5$	(10)	$R^2 = 1$
Figure 19	$y = 33.67x^2 - 213.48x + 589.44$	(11)	$R^2 = 1$
Figure 20	$y = 38.91x^2 - 152.03x + 388.93$	(12)	$R^2 = 1$
Figure 21	$y = -3.825x^2 + 15.855x + 163.28$	(13)	$R^2 = 1$

In Table 18, each polynomial equation has two unknowns which is x and y. The x value indicates the number of days and y represents the average chlorophyll concentration on that particular day. The equations of Figure 16, 18 and 21 indicated trendline started with a lower value follow by a greater value which reasoned in negative value for x^2. The equations are useful in estimating the mean chlorophyll concentration on any day by substituting x value for results.

On the other hand, R^2 known as the coefficient of determination function as an indicator to show the significant of the results obtained in experimental works and how close it is related to the actual values. It is dimensionless with values ranging from 0 to 1.

4.3 NBPT Solution Analysis

The experimental results are tabulated in Table 19 for 1g of NBPT with 10mL, 20mL, 30mL, 40mL and 50mL of deionized water to analyse the absorbance of NBPT from wavelength 600nm to 700nm. In Appendix, the graphs of absorbance (A) against wavelength (nm) for the 5 samples of NBPT measured using UV- VIS spectrophotometer are attached.

Table 19: NBPT Solution Analysis at Wavelength 600nm to 700nm

Wavelength (nm)	**Absorbance (A)**				
	Volume of Deionized Water(mL)				
	10	**20**	**30**	**40**	**50**
600	0.060	0.090	0.080	0.049	0.050
610	0.070	0.105	0.078	0.075	0.070
620	0.060	0.100	0.070	0.060	0.060
630	-0.010	0.010	0.098	-0.020	0.040
640	0.060	0.040	0.015	0.000	0.000
650	0.050	0.080	0.060	0.049	0.058
660	0.050	0.081	0.060	0.050	0.050
670	0.060	0.082	0.065	0.051	0.052
680	0.070	0.083	0.070	0.052	0.069
690	0.040	0.060	0.085	0.030	0.040
700	0.047	0.060	0.085	0.030	0.040

For this experiment, 0.012g, 0.062g and 0.125g of NBPT are used to dilute with 100mL of urea solution. The relationship of equivalent volume are calculated using equation 8 with the assumption of 0.25mg/mL NBPT concentration in the product:

$$\text{Concentration (mg/ml)} = \frac{Mass}{Volume} \tag{8}$$

Table 20: Calculations on the Equivalent Volume of NBPT

Mass of NBPT (mg)	**Concentration ($\frac{mg}{mL}$)**	**Volume (mL)**
0.012 x 10^3	0.25	48
0.062 x 10^3	0.25	248
0.125 x 10^3	0.25	500

Based on table 17, when pure mass 0.012g. 0.062g and 0.0125g of NBPT are used for crops application, the equivalent volume that need to be diluted is 48mL, 248mL and 500mL in order to have the similar concentration of NBPT in AGROTAIN® product.

4.4 Inhibition Studies of NBPT and TS

After determining the effects of NBPT as chemical based urease inhibitors on plants' physical changes and chlorophyll concentration, the potential of TS in garlic extract as bio based urease inhibitor is analysed through the inhibition study in urea fertilization. Both NBPT and TS inhibition studies are conducted separately to have a vivid comparison by using a UV-VIS spectrophotometer at wavelength 630nm in room temperature and acidic pH 2.0 condition for 2 hours. The absorbance for both are recorded in Table 21.

Table 21: Raw Data of NBPT and TS Absorbance

Sample No.	Time (min)	NBPT Absorbance (A)	TS Absorbance (A)
1.00	5.00	0.2489	0.0230
2.00	10.00	0.2112	0.0268
3.00	15.00	0.2735	0.0266
4.00	20.00	0.3292	0.0282
5.00	25.00	0.3623	0.0179
6.00	30.00	0.3617	0.0194
7.00	35.00	0.3545	0.0200
8.00	40.00	0.3434	0.0232
9.00	45.00	0.2987	0.0237
10.00	50.00	0.2780	0.0230
11.00	55.00	0.2513	0.0243
12.00	60.00	0.2611	0.0236
13.00	65.00	0.2131	0.0251
14.00	70.00	0.1660	0.0258
15.00	75.00	0.1473	0.0269
16.00	80.00	0.1293	0.0285
17.00	85.00	0.1174	0.0297
18.00	90.00	0.1105	0.0240
19.00	95.00	0.0999	0.0260
20.00	100.00	0.0884	0.0253
21.00	105.00	0.0766	0.0264
22.00	110.00	0.0722	0.0269
23.00	115.00	0.0521	0.0269
24.00	120.00	0.0417	0.0269

Table 22: Concentration of Ammonia Gas Released for NBPT and TS Inhibition Studies.

Sample No.	**Time (min)**	**Concentration of NH_3 (mol/L)**	**Concentration of NH_3 with NBPT (mol/L)**	**Concentration of NH_3 with TS (mol/L)**
0.00	0.00	1.6280	1.6280	1.6280
1.00	5.00	1.6280	0.1976	0.1544
2.00	10.00	1.6280	0.1677	0.0183
3.00	15.00	1.6280	0.2171	0.0213
4.00	20.00	1.6280	0.2614	0.0206
5.00	25.00	1.6280	0.2876	0.0142
6.00	30.00	1.6280	0.2872	0.0154
7.00	35.00	1.6280	0.2814	0.0159
8.00	40.00	1.6280	0.2726	0.0184
9.00	45.00	1.6280	0.2371	0.0188
10.00	50.00	1.6280	0.2207	0.0182
11.00	55.00	1.6280	0.1995	0.0193
12.00	60.00	1.6280	0.2073	0.0199
13.00	65.00	1.6280	0.1692	0.0205
14.00	70.00	1.6280	0.1378	0.0214
15.00	75.00	1.6280	0.1169	0.0214
16.00	80.00	1.6280	0.1027	0.0226
17.00	85.00	1.6280	0.0932	0.0236
18.00	90.00	1.6280	0.0877	0.0190
19.00	95.00	1.6280	0.0793	0.0206
20.00	100.00	1.6280	0.0702	0.0201
21.00	105.00	1.6280	0.0608	0.0209
22.00	110.00	1.6280	0.0573	0.0214
23.00	115.00	1.6280	0.0414	0.0214
24.00	120.00	1.6280	0.0331	0.0214

The results calculated for inhibition studies are plotted accordingly in Figure 22. From the graph, the concentration of ammonia gas emitted without urease inhibitors are constant throughout the experiment as jack bean urease in the mixture catalysed the urea fertiliser following its natural course releasing 1.6280 mol/L of ammonia gas.

Besides that, when conducting the experiment significant colour changes can be observed from the standard assay mixture after 1mL of urea-urease mixture is poured into the mixture every 5 minutes interval. The light brown solution will gradually turned into white milky solution followed by a pungent smell of ammonia gas. Hence, respiratory mask is worn when handling the solution. Meanwhile, the introduction of NBPT and TS as urease inhibitors showed distinguished differences in the amount of ammonia gas released compared with the result without urease inhibitors as shown in figure 22.

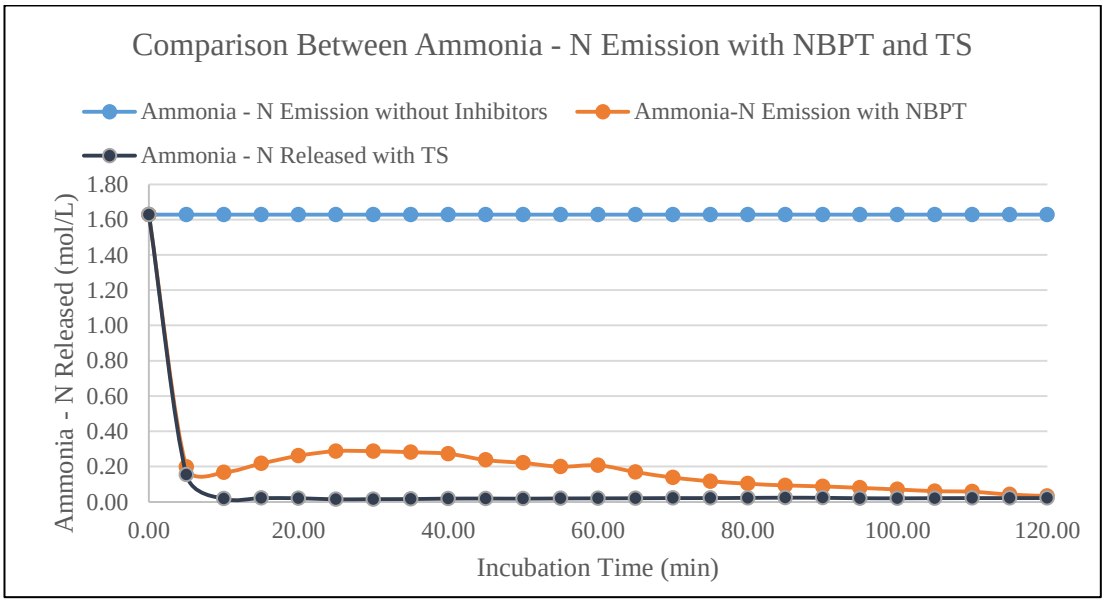


Figure 22: Graph of Comparison between Ammonia-N Emission with NBPT and TS

The scales of graph axes in Figure 14 are further minimise in order to magnify the curves for NBPT and TS to ease the analysing process.

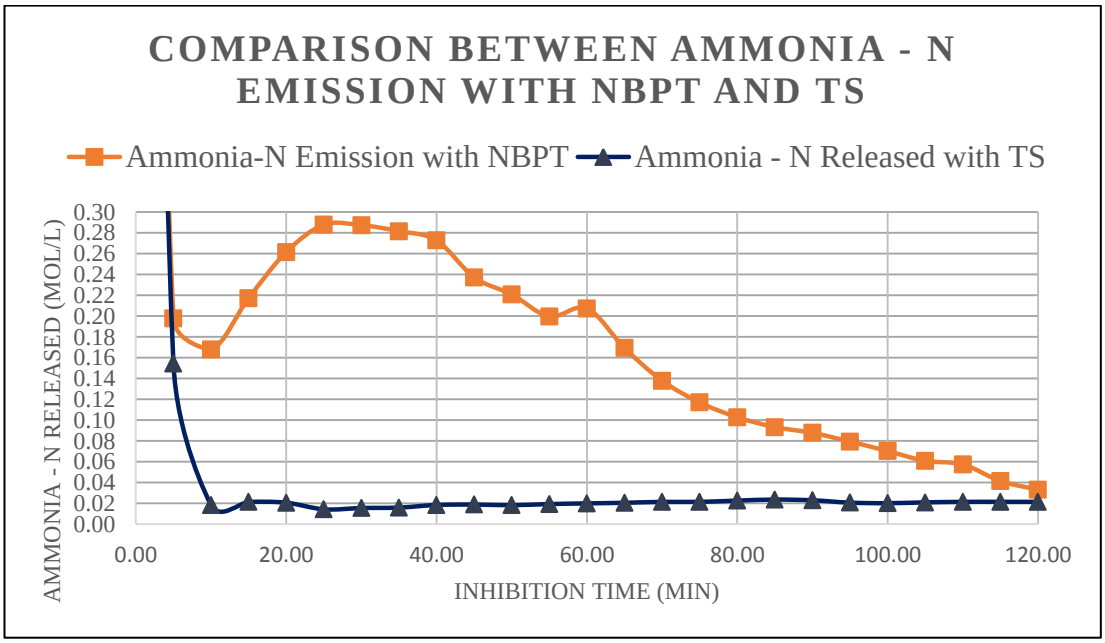


Figure 23: Graph of Comparison between Ammonia-N Emission with NBPT and TS after Magnification

The emission rate of ammonia gas with NBPT is higher at the initial stage ranging from 0.1677 to 0.2876 mol/L in Table 22 for 60 minutes. This is due to a longer time needed for the NBPT compound to oxidise completely into NBPTO, the active form for inhibition reaction to occur. The result is similar with a previous study showing that the ammonia volatilisation rate on field crops are only reduced after 24 hours of NBPT application [24]. After 60 minutes, a descending trend of the curve is observed until 120 minutes. The lowest concentration of ammonia gas recorded is 0.0331 mol/L.

In contrast with NBPT, TS in standard garlic extract showed immediate inhibition properties at jack bean urease, however, the inhibition time only lasted for 20 minutes from the first 20 minutes as shown in Figure 23 and the lowest concentration of ammonia gas released is 0.0142 mol/L (Table 20). After that, the curve for TS started to have a slight increasing trend and the ammonia gas emitted also became greater until it appeared to be constant at 0.0214 mol/L. This indicated that TS compounds in the garlic extract had limitation to perform inhibition on urease activity. Upon a time, the reaction will become saturated as the active site is no longer active to inhibit further.

CHAPTER 5: CONCLUSION AND RECOMMENDATION

Overall, the research done in Final Year Project II is the continuation of Final Year Project I experimental works with relation to the objectives stated in Chapter 1 that is to study the visible changes on plant growth treated with NBPT and TS with urea solution. Besides, this research also aim to compare the inhibition performance of NBPT and TS using 1g/30mL of standard concentration in fertilization condition. In short, the objectives which had successfully achieved are listed below:

- To study the effects of NBPT and TS on plant growth through the presence of chlorophyll.
- To investigate the inhibition studies of urease using bio based and chemical based inhibitors.

It is proven that NBPT does affect the plant growth by promoting ammonium toxicity due to excessive accumulation of urea in plant tissues evident in visible symptoms of chlorosis, necrosis, necrotic leaf margin and leaf-tip scorch on Ipomoea Aquatic and Brassica Juncea leaves except Spinacia Oleracea which showed a higher resistivity to the formation of necrotic leaf margin and necrosis. In contrast, the plants treated with TS does not showed any physical changes.

The mean chlorophyll concentration for NBPT treated plants is higher compared to TS treated plants but the plants treated with NBPT had lower chlorophyll contents than the control plants. For instance, the mean concentration of chlorophyll for Ipomoea Aquatic with 0.125% NBPT + urea solution are 169.40, 189.19 and 288.08 mg/mg^3 compared with the control plants are 367.88, 374.45 and 359.40 mg/mg^3 at day 3, 5 and 7. However, the consistency of chlorophyll results are observed to be higher for TS treated plants as the concentration of TS applied does not cause any toxicity to the plants.

Therefore, both NBPT and TS exibit urease inhibition properties in their own unique ways. NBPT showed more promising results to take the role as urease inhibitor in agricultural field. This is because the inhibition time for NBPT is longer until the extend of having the potential to cause ammonium toxicity eventhough the initial reaction is delayed for 60 minutes. Nevertheless, it can also contribute to the contamination of environment and changes in plant nitrogen metabolism. This can indirectly affect the chlorophyll pigments for photosynthesis process and retard the plant growth.

On the other hand, TS as an organic compound found in garlic extract will not caused any environmental issues as it is non hazardous and biodegradable in nature. In terms of urease inhibitor application, it is only effective as an instantanous urease inhibitor. The experimental results showed that TS can only inhibit approximately 20 minutes when using 1g/30mL of garlic extract. This can be one of the reason why there are not much accumulation of urea in plant tissues as the inhibition is short lived. The supply of TS need to be continous if the application needed for longer period in agriculture. Economically, it is less feasible as more cost need to be invested to purchase the TS inhibitor after it ran out of inhibition properties. Several recommendations that can be done to improve the effectiveness of this research such as the inhibition studies for TS and NBPT can be carried out at constant temperature of 30℃ instead of room temperature that fluctuates due to air conditioning.

For future works include the research on developing Thiosulphinates (TS) instead of using garlic from source of food to commercialize as a bio-based urease inhibitor product; determine the optimum amount TS needed to apply on plants for agricultural field. As a whole, TS has the potential to be an alternative for urease inhibitor, however, more time and research need to be done to develop it as a marketable product.

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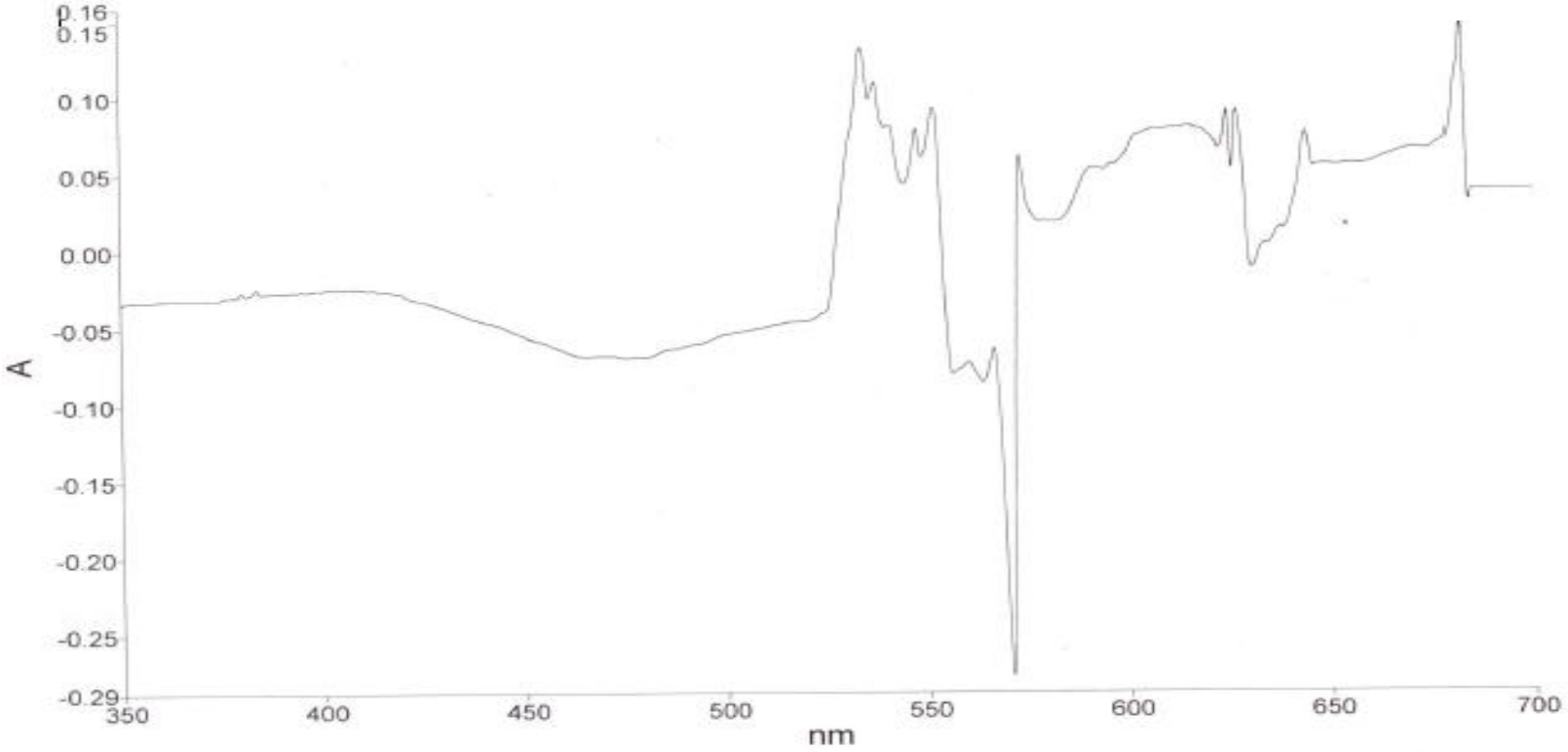
APPENDICES

Appendix 1: Graph of Absorbance (A) against Wavelength (nm) for 1g of NBPT + 10mL of DW



Sample ID	Description	Lambda Max. (nm)	Absorbance at Max.
Sample1	1g NBPT + 10mL DW	682.8	0.1485

PerkinElmer

Printed Student
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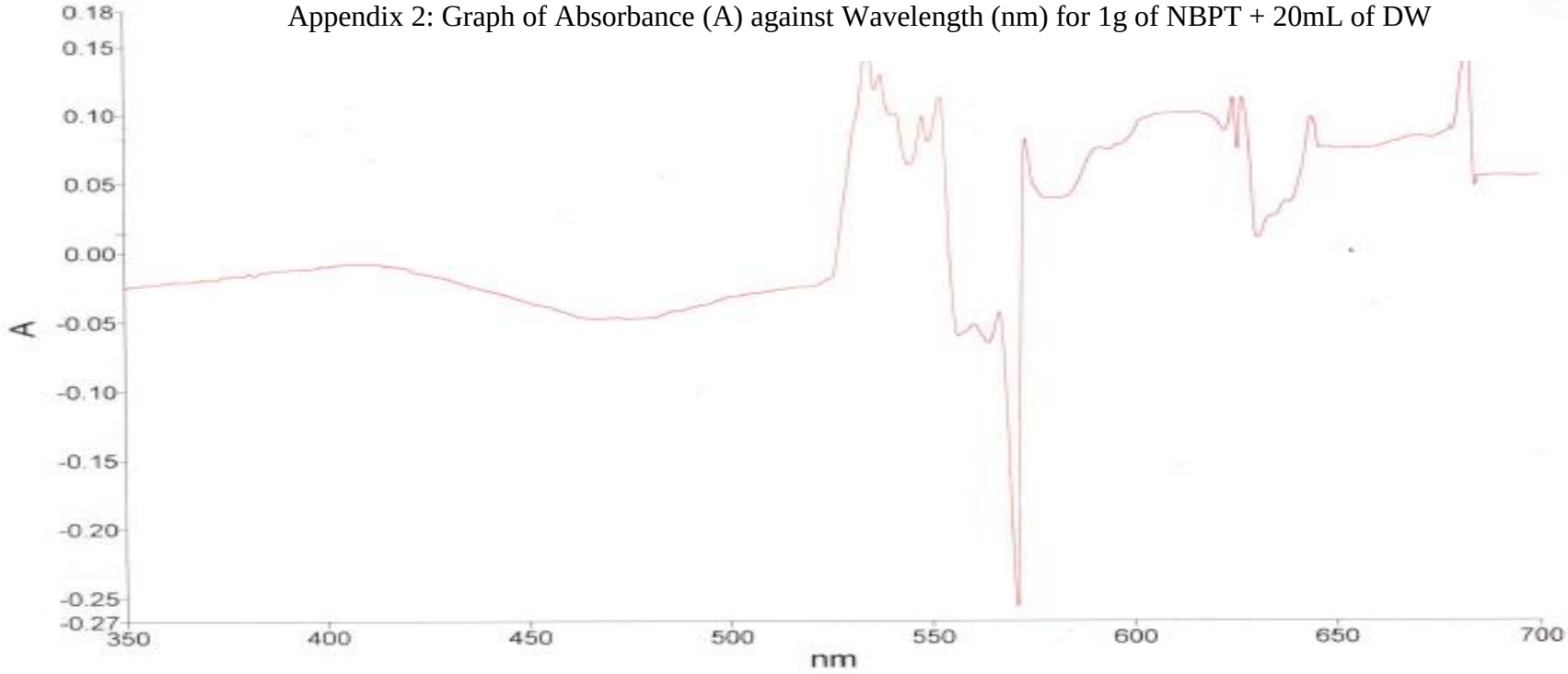
Appendix 2: Graph of Absorbance (A) against Wavelength (nm) for 1g of NBPT + 20mL of DW



Sample ID	Description	Lambda Max. (nm)	Absorbance at Max.
Sample2	1g NBPT + 20mL DW	682.8	0.1653

PerkinElmer

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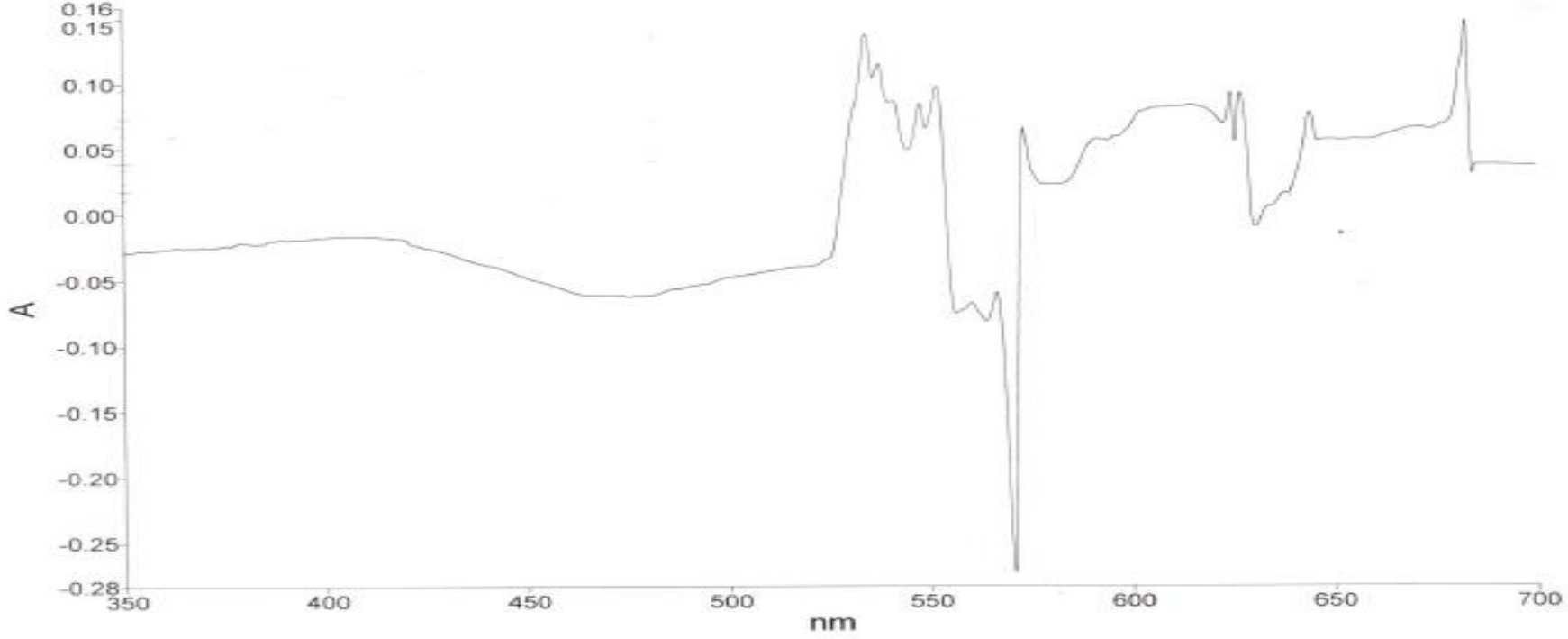
Appendix 3: Graph of Absorbance (A) against Wavelength (nm) for 1g of NBPT + 30mL of DW



Sample ID	Description	Lambda Max. (nm)	Absorbance at Max.
Sample3	1g NBPT + 30mL DW	682.8	0.1481

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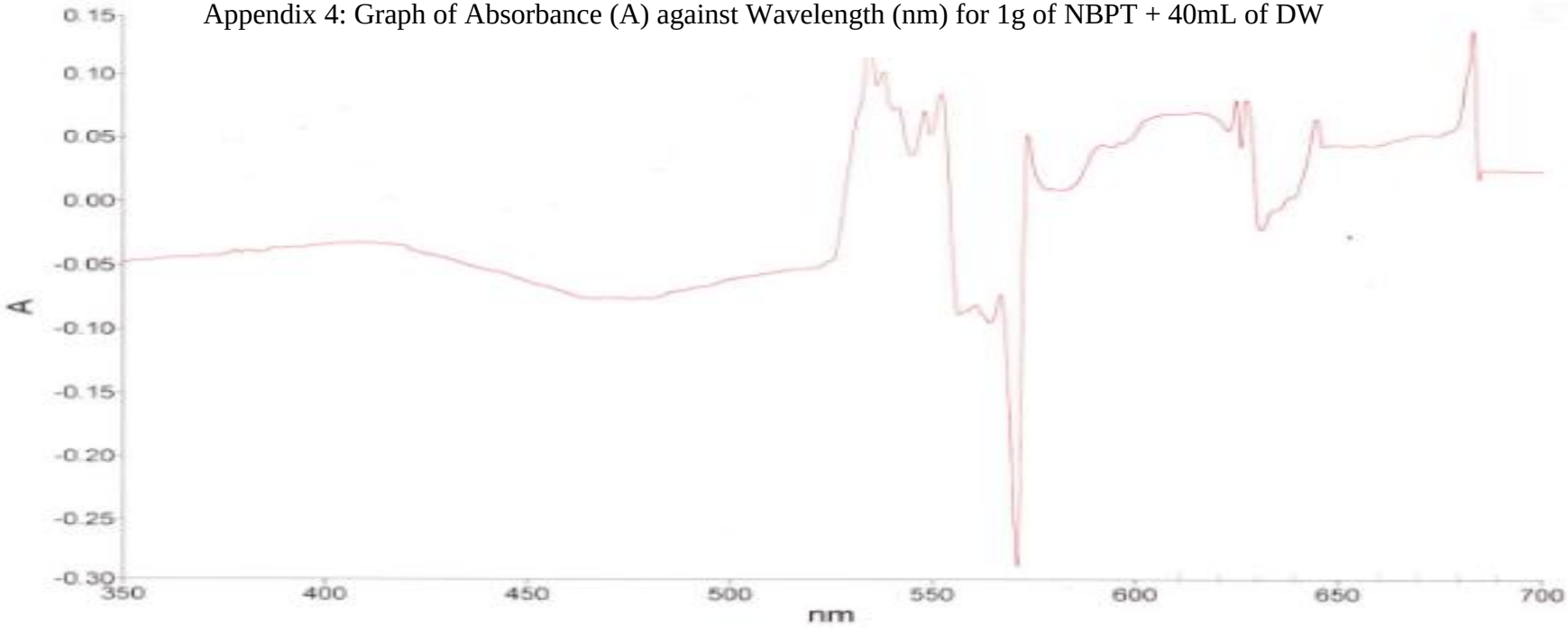
Appendix 4: Graph of Absorbance (A) against Wavelength (nm) for 1g of NBPT + 40mL of DW



Sample ID	Description	Lambda Max. (nm)	Absorbance at Max.
Sample4	1g NBPT + 40mL DW	682.8	0.1354

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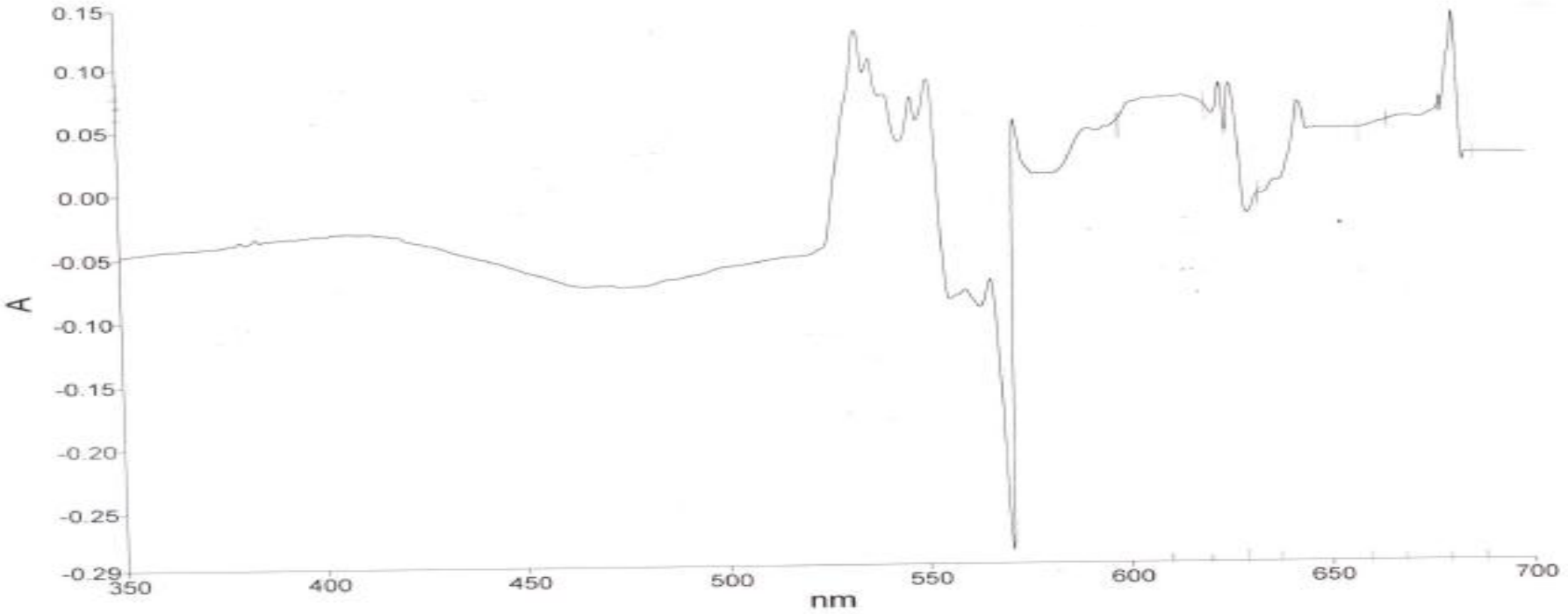
Appendix 5: Graph of Absorbance (A) against Wavelength (nm) for 1g of NBPT + 50mL of DW



Sample ID	Description	Lambda Max. (nm)	Absorbance at Max.
Sample5	1g NBPT + 50mL DW	682.8	0.1363

PerkinElmer

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Print date: 01/21/2015 09:53 Malay Peninsula Standard Time

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