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Preliminary Observation on Soil Fertility and Yied of Several Crops Plant by Paper Chromatography as an Approach to Asses their Semi Quantitative Status

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Abstract: Different types of plant pigments affect how plants perform photosynthesis. The method uses chlorophyll, a pigment that transforms solar energy into chemical energy. This study employed a variety of croton leaf types—green, yellow, and red—as well as other materials like alcohol, filter paper, mortar, and other auxiliary equipment to determine the kinds of pigments found in leaves. Chlorophyll-*a*, chlorophyll-*b*, anthocyanin, and xanthophyll pigments are typically found in the many types of croton leaf that have been discovered. These pigments can be qualitatively evaluated by examining the colour of the pigments that break down on filter paper. Four types of crop plants were observed using paper chromatography: the Yellow Coconut variety, the Snake Fruit (*Salaca edulis*) of "Pondoh" variety, the Mango (*Mangifera indica*) "Harum Manis" variety, and the Sweet Potato (*Ipomoea batatas*) or Anthocyanin Sweet potato variety. Retention Factor (RF) values are employed as an indicator to evaluate each plant's quantitative yield as a means of measuring the qualitative parameter. The RF of Yellow Coconut variety was 0.94 (high fertile), the RF of Snake Fruit of "Pondoh" variety was 0.92 (high fertile), the RF of Mango Harum Manis variety was 0.96 (high fertile), and the RF of Anthocyanin Swet potato variety was 0.99 (high fertile). This report is in the semi-quantitative parameter, but it still has to be converted to the complete quantitative value.

Keywords: *Coconut, Mango "Harum Manis", Paper Chromatography, Snake Fruit "Pondoh", Sweet Potato.*

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I. INTRODUCTION

A method to separate the colour pigment components of some plants called paper chromatography. This method was developed by a German Chemistry scientist Christian Friedrich Schonbein in 1865 (Simon, and Thomas, 1949; Sherma, and Zweig, 1971). Paper chromatography is separation technique that separates the pigment molecules mostly according to their size (Dubey, and Firdosi, 2024; Kaja, et al., 2024). Chlorophyll, the primary pigment molecule found in green leaves, is responsible for the plant's photosynthesis. Other plant leaf pigments, such as garden croton or variegated croton, can be utilized as a pigment source (*Codiaeum variegatum* L.) that have several colours of plant leaves such as green, yellow, red, brown, orange and so on (Li, and Gilbert, 2008). Additionally, other pigments like anthocyanins and carotenoids are present.

In several plants such as Coconut (*Coconut nucifera*), Snake Fruit (*Salaca edulis*), Mango (*Mangifera indica*), and Sweet Potato (*Ipomoea batatas*) are the popular and important plants (Figure-1) in whole areas of the Indonesian islands and widely planted in elsewhere (Supriyadi, et al., 2002). They have the leaves that vary in colour with green that used more light in making carbohydrates during photosynthesis. Solar-induced chlorophyll fluorescence (SIF), a direct byproduct of photosynthesis, provides a physiologically meaningful indicator of vegetation functioning (Otitoju, and Onwurah, 2010; Yuhan, et al., 2026). Those plants are green crops during the whole years.

It is present in plant cells in organelles known as chloroplasts, which must be torn open to expose their pigment molecules. The quantity and kinds of pigment molecules alter when leaves change colour during certain seasons, such as the fall in temperate zone areas. A wonderful scientific exercise that allows you to view these various hues is leaf chromatography. The various pigments will give the leaves their various hues. Since most plants have many pigment

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molecules, it is possible to experiment with a variety of leaf species to observe the vast array of colour variations.

Plant pigments are coloured organic substances derived from plants. Taiz and Zeiger, (2010) have stated that the pigments absorb visible radiation is between 389 nm (violet) and 760 nm (red). They give colour to stem, leaves, flowers, and fruits. There also regulate processes like photosynthesis, growth and development. Plants produce various forms of pigments based on origin, function, water solubility, and plant pigments (Block, et al., 1955; Ford, et al., 2021).

Four categories are used to group the colours plant, namely: Chlorophylls (green), Carotenoid (yellow, orange-red), Anthocyanins (red to blue, depending on pH) and Beta alanine (red or yellow). Chlorophyll is a green photosynthetic pigment that included the chlorophyll *a* and chlorophyll *b*. They can be observed in the plant's chloroplasts. They are water-repellent due to the phytol side chains. Their structure is similar to that of haemoglobin, however the core metal in them is magnesium rather than iron. Carotenoids show as yellow-orange pigments. They are also incredibly long pigments that repel water. They are found in plants' chromoplasts or plastids. Plant cell vacuoles contain anthocyanins, which are red pigments. Water-soluble pigments are called anthocyanins. They give the fruit, leaves, and flowers a pink-red hue. In plants, beta alanine is a water-soluble pigment produced from tyrosine. The two pigments that fall into this category are betaxanthin (yellow orange) and betacyanin (red violet). They are found in plant cells' vacuoles (Celik, et al., 2021; Chaudhry, et al., 2024).

For example, to facilitate the extraction of the pigments into alcohol, the macerated leaves are submerged in a small amount of alcohol as a solvent. Additionally, the plant debris is softened by the use of hot water. A piece of paper's end is dipped into the alcohol, water, and pigment mixture. While alcohol moves up the paper via capillary action and pushes the pigment molecule toward it, the other end stands straight up and graphite pulls on the molecule. The selection of paper is crucial because few pigment molecules will be small enough to fit through the maize cellulase fibrous to move upward if the fibrous mesh is very dense (Christopher, et al., 2014; Payne, et al., 2024;).

All of the pigment molecules can readily go up the paper if the mesh is too open, making it challenging to separate them. Additionally, some pigments may dissolve better in water than in alcohol. A molecule passes through the paper if it is extremely soluble in alcohol. The liquid may contain an insoluble molecule. Sample purity is tested using this method; a pure solution should only show one band. Additionally, it is employed for fraction isolation and purity. The various bands may be separated and the pigments recovered once the chromatogram has developed.

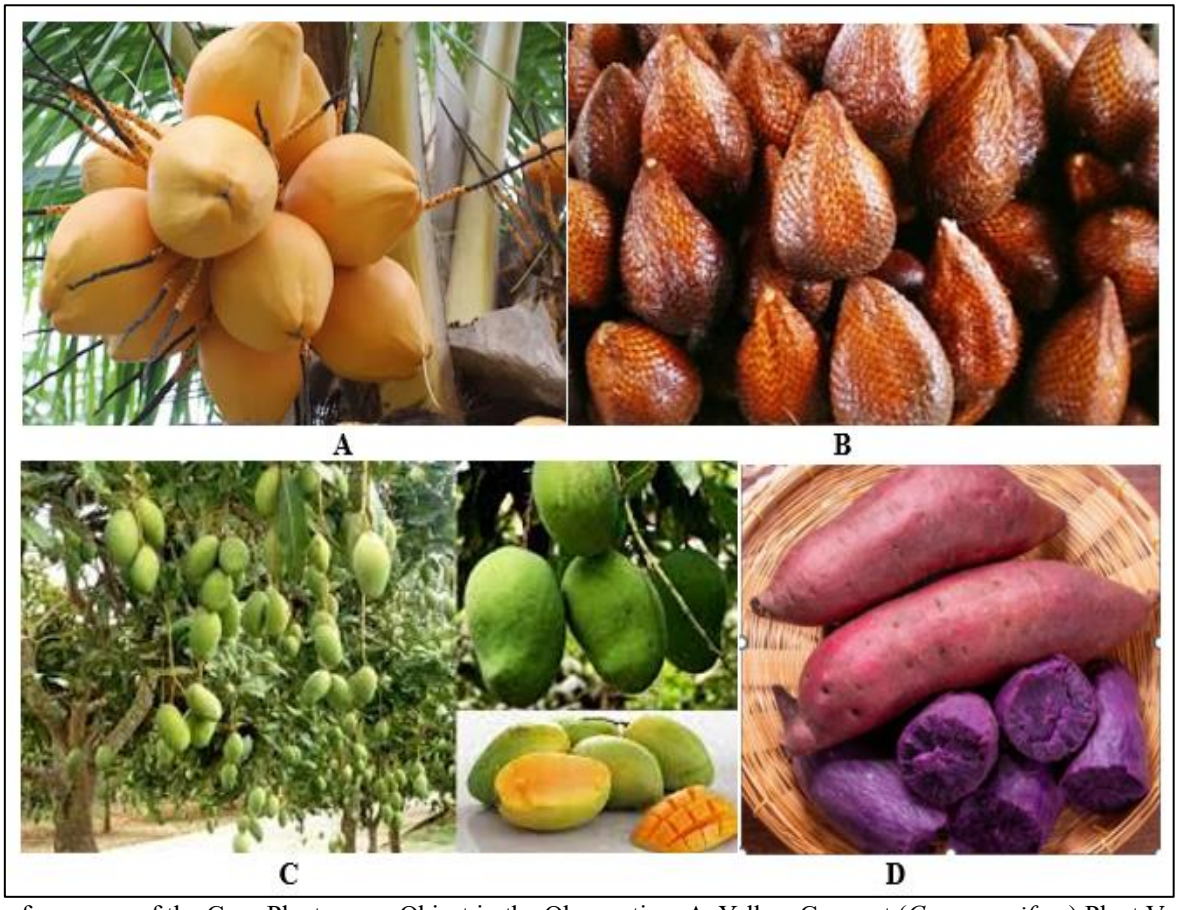

Fig 1 Performances of the Crop Plants as an Object in the Observation: A. Yellow Coconut (*Cocos nucifera*) Plant Variety, B. Snake Fruit (*Salaca edulis*) "Pondoh" Variety C. Mango (*Mangifera indica*) "Harum Manis" Variety, and D. Anthocyanin Sweet-Potato (*Ipomoea batatas*) Variety.

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The possibilities of using chromatography analysis in qualitative value through retention or retardation factor (RF) value to soil traits and plant yield will be covered in this paper. The ratio of the distance travelled by a coloured band and to the solvent distance travelled by the solvent front. Additionally, to comprehend the connection between the RF value and crop plant condition growth are may be established. Soil health plays a crucial role in crop production both in quantity and quality, highlighting the importance method for preserving soil quality and to ensure global food security (Chaudhry et al., 2024).

As measures of soil healthy in qualitative, semi-quantitative, and quantitative field analysis are based on soil nutrient availability, pH, organic matter and total Nitrogen (Celik, et al., 2021). These measurements were determined the soil healthy in common term after measured in quantitatively which could be analyses through special variable of the variant parameter. Unfortunately, these variables were not measured by quantitative data, but it is measured by RF value according to plants performances during the field growing.

Relationships between fertility of the soil and its physical performances are not differentiates except the black colour commonly in general term, but on the colour performances according to the chromatogram expression are not available. There is no information available the colour of soil was explained by paper chromatography. However, the fertility status of the soil was identified by general health of the soil by organic content, microbial composition and activity, and pH and salinity. The higher soil organic content and microbial activity have significantly greater in outer and middle zone bread development of radial features and more colour intensity than soil with carbon content and microbial activity and soil poorer quality would produce chromatograms with stronger concentric features (Ford, et al., 2021).

II. MATERIALS AND METHODS

➢ *Plant Materials, Chemical and Laboratory Equipment*

Four types of crop plants were utilized as the material of the experiment, namely: (1) Anthocyanin Sweet potato (*Ipomoea batatas*) local variety, (2) Mango (*Mangifera indica*) "Harum Manis" variety, (3) Yellow Coconut (*Cocos nucifera*) variety, and (4) Snake Fruit (*Salaca edulis*) "Pondoh" variety (Figure 1). The plant's leaf was isolated in a fresh condition, with the substance remaining intact. In the field, all four plant materials were growing upright. The chemical used was 75% ethanol and the laboratory equipment used were paper chromatography (Watman No.1), paper clips, pencil, ruler, scissors, calculator, and containers. All procedures were employed according to Mansi, et al., (2018) and Reshma, et al., (2024), those procedures are as follows.

➢ *Experimental Procedures*

- *Sample Preparation*

Take a strip of chromatography paper approximately 18 cm long which has it one end as blunt end other as pointed. With the help of pencil draw a line of 2 cm from pointed end. Attach a cork with push pin by bending the blunt end of the paper. Adjust length of paper so that it barely touches the end of test tube without any banding. Place a ruler cover the leaf so that the ruler covers the line made by pencil from pointed end. Allow the line to dry, and repeat the process until the pencil line is completely covered by the narrow green band.

- *Extraction of Pigment*

Measure the distance from the pencil line to the solvent from and also the colour and take a record of it. Cut each band of colours apart carefully and trim off the excess paper. It should include all the pigment for each band. Label each test tube for each pigment. Cut each band of colour into pieces small enough to fit into 20 – 30 ml test tube. Insert the paper pieces into 20 – 30 ml test tube and in the appropriate test tubes. Add 5 ml of isopropyl alcohol to each test tube and seal with small piece of plastic wrap. Allow the samples to stand overnight.

- *Separation of Pigments*

Use a 6 ml syringe to dispend 5 ml of chromatography solvent in test tube. Carefully lower the strip in the test tube and secure the cork in top. The solvent should touch the pointed end but not the green line. Be careful not to slosh the solvent so the test tube must remain undisturbed. Observe the solvent movement and the separation band when the pigment has separated into different bands. Lift the cork with paper attached from the test tube, and remove the cork from it and allow the paper to dry completely.

- *Extraction of Pigment*

Each of the various plant leaves should be nearly similar in size before being divided into test tubes. Each variety of plant should have a minimum of three test tubes with labels. Determine the colour and the distance between the pencil line and the solvent front. Give it a moment. Carefully cut out each band, making sure to incorporate all of the colour, then snip off any extra paper. One test tube should be labelled for each pigment. Cut each colour band into pieces that are tiny enough to fit in a test tube that holds 20 to 30 milli litters. Place the pieces of paper into a test tube that holds 20 to 30 milli litters. Place the pieces of paper in the corresponding test tubes. Add each test tube with 5 ml of isopropyl alcohol, then cover with small pieces of plastic wrap, letting the samples stand overnight.

- *Calculation the Retention or Retardation Factor (RF) Value*

The retardation or retention factor of each component's migration is quantified using the RF, which is determined using the formula Dubey and Firdosi, (2024).

$$RF = \frac{Distance\ traveled\ by\ the\ solute\ from\ origin\ line}{Distance\ traveled\ by\ the\ solvent\ from\ origin\ line}$$

The value of RF is higher when the performance of good in the plant growth, it means that the plant and soil are the high fertile growth condition, the value of RF is medium when the performance of medium in the plant growth, it means that the plant is the medium fertile condition, and the

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value of RF is lowest when the performance of low in the plant growth it means that the plant has the lowest fertile of the plant growth.

➢ *Statistical Analysis*

The fertile status of both parameters was only compared to the RF value that was used to evaluate the soil's fertility and the plant development in semi-quantitative growth. RF measurement values on variables is an indicator of fertility, either in the soil or in the growth performances of plants. Only the chromatogram data represents the RF value of plants studied were gathered and used as a variable to get summary on healthy of both plant and soil conditions. Meanwhile, the variable soil healthy and its fertility were not recorded due to the experiment procedures included.

III. RESULTS AND DISCUSSION

A. Results

Because the experiment was conducted at the same time and the paper chromatograph was used on the same paper, the solvent line is at the same location. As shown in Figure 2, the pencil line onto the zero point, and the solvent line (maximum line) was 49 mm for all crop plants.

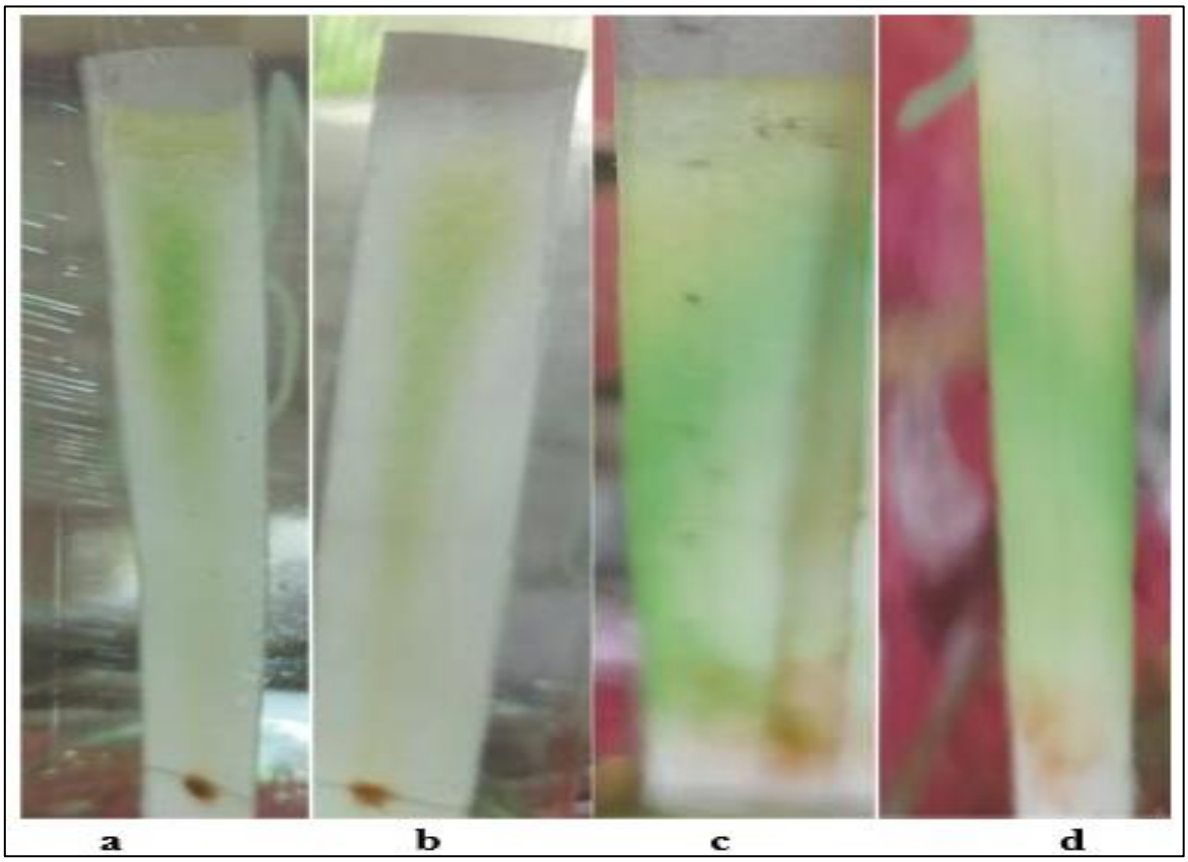

Fig 2 Chromatogram Performances of Four Crop Plants Observed.

- Note: *a* is a mango "harum manis" variety, *b* is an anthocyanin sweet potato variety, *c* is a snake fruit "pondoh" variety, and *d* is a yellow coconut variety.

The solvent or maximum line was achieved at 49 mm for all crop plants. In the meantime, all of the plants' pencil lines, or lines below, are in the zero point. The chromatography system only contains the two spots that are common to each plant, even if there is only one spot. For instance, the blue colour (chlorophyll *a* and chlorophyll *b*) is made up of both blue and yellow blue. While the two additional colours (carotene and xanthophylls) were lacking from this observation, the colours blue and yellow were present (Reiss, 1994; Carlos, et al., 2020). The data experiment's results were collected and analysed, as Table 1 illustrates.

Table 1 Data Measurement and Analysis of Paper Chromatography of Several RF Values of Crop Plants

Item	**Anthocyanin Sweet Potato**	**Yellow Coconut**	**Mango *Harum Manis***	***Salaca edulis* *Pondoh***
Solvent (*mm*)	49	49	49	49
Spot$_1$ (*mm*)	44	46	47	45
Spot$_2$ (*mm*)	28	38	27	31
RF$_1$ (Retention Factor) RF$_2$	44/49 = 0,99	46/49 = 0,94	47/49 = 0,96	45/49 =0,92
	28/49 = 0,57	38/49 = 0,77	27/49 = 0,55	31/49 = 0,63

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- Note**:** $Spot_1$ is the blue color, and $Spot_2$ is the yellow color.

The RF_1 is the retention factor of the blue color, and the RF_2 is the retention factor of the yellow color.

Table 1 reveals that there are only two areas with distinct colour presence. The RF values of each plant varied, ranging from a smaller value (0.55) to a bigger value (0.99). Only the sweet potato is an annual plant; the other three plants (Coconut, Snake Fruit, and Mango plants) were perennial groups. Every plant exhibits a variety of leaf colours, ranging from dark blue to lighter blue. The orange (*carotene*) and light black (*pheophytin*) hues were absent from this paper's chromatography system due to a malfunction during the laboratory work process.

B. Discussion

The smallest pigment molecule is the ones that travelled the greatest distance and the largest molecule is the ones that travelled the least distance (Payne et al., 2024; Roberta, et al., 2024). It was noted that not every colour would appear as it indicated in the earlier studies (Figure 2). According to Pokorny, Kalinova, and Dysseler, (1995) and Tarrago-Celada and Novell (2019)**,** are the three broad classes of plant pigments, i.e.: porphyrins, carotenoids, and flavonoids. The main porphyrin is chlorophyll molecules. There are actually multiple forms of chlorophyll, but you can recognize them because they are green. Carotenoids include *carotene* (yellow or orange), *lycopene* (orange or red), and *xanthophyll* (yellow). *Flavonoids* include flavone and both *flavanol* (yellow) and *anthocyanin* (red, purple, or even blue). These chlorophylls, carotenoids, and xanthophyll pigments may be used in determination and become the standard for the plant health and yield estimation based on plant growth performances (Kromdijk, et al., 2016; Seid, 2022; Matala, and Sanginengi, 2024;). They lack the details regarding this occurrence, but the phenomenon might serve as a theory that some researchers will need to test in the future. Information about the issue has not yet been published in any journals, but we anticipate that in the near future, there will be more concerns (Andres, et al., 2023).

Relationship between chlorophyll content and yield of the plant are determined by RF value of the chlorophyll content (quantitative) and the RF value of the soil characteristics which is compound is it (quantitative) if is measured. In this observation, there is no values that gathered to the soil healthy as components such as: pH, Organic matter (OM), and total Nitrogen (Chaudhry, et al., 2024). OM and soil pH are critical indicators of soil fertility as they significantly impact various soil function both in soil fertility and availability of essential nutrients for plant growth and development (Estrada-Herrera, et al., 2017; Nguemezi, et al., 2020).

The availability of certain key nutrients such as N, P, and K is a vital nutrient for the growth and development of plants. Then, the Ca and Mg also play an important role in soil fertility as they increase the availability of the essential nutrients, regulate soil pH, improve soil structure and contribute to overall soil fertility (Norfaziro, et al., 2015; Tarrago-Celada and Novel, 2019).

Suaib Suaib (2025) on the National Seminar of the "Pertanian Pesisir (SENATASI)" at the Agronomy Section of Agriculture Faculty of Bengkulu University stated that: (1) the advantages and the weakness of the solvent usages are the pigments name that contained in the solvent, (2) the advantages and the weakness of value of RF contained in the solvent is could be correlated event there were not analysed, and (3) the relationship between pigment status and the RF value to the soil and plant growth conditions is fertile by qualitative of plant growth event without statistical test). This observation signifies the first time that the correlation between the fertile condition and the RF value has been reported and opportunities have been opened up, considering that the RF value is quantitative and the values for soil condition and plant growth state are qualitative.

Only two of the four colours in the RF value were present overall, according to the observation result. Not all of the four colours present in the eluent are being fully performed by the chromatography process. It is a concern since all of the plants that were observed might not have been able to display the RF levels of two remaining (Carotene and Xanthophyll) colours during the earlier observations. Additionally, the research was nominated in the field of chemistry, namely pure chemistry, whereas other chemical applications, such as agricultural chemistry, were not used.

IV. CONCLUSION

According to the observation's result, the *Anthocyanin* sweet potato's RF of blue colour was 0.99 and its RF of yellow colour was 0.57, indicating that the soil and plant growth fertility are in a fertile status. The blue colour was 0.94 and the yellow colour was 0.77 at the Yellow Coconut, indicating that the soil condition and plant growth fertility are in the fertile status. The blue colour was 0.96 and the yellow colour was 0.55 at the Mango "Harum Manis", indicating that the soil condition and plant growth fertility are in the fertile status, and the blue colour was 0.92 and the yellow colour was 0.63 at the *Salaca edulis* "pondoh" variety, indicated that the soil condition and plant growth fertility are in the fertile status. All the conclusion above were determined based on data gathered in chromatogram analyses and physical performances of quantitatively plants by RF values.

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