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IJAZAH: DEGREE OF BACHELOR OF AGRICULTURE SCIENCE WITH HONOURS

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IDENTIFICATION AND CHARACTERIZATION OF FUNGI ASSOCIATED DISEASES IN VANILLA CROPS AT KAMPUNG SUNGAI DALING, SANDAKAN

SYAZA SYAZWAN SUHAILI

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIRMENTS FOR THE DEGREE OF BACHELOR OF AGRICULTURE SCIENCE WITH HONOURS

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SCHOOL OF SUSTAINABLE AGRICULTURE
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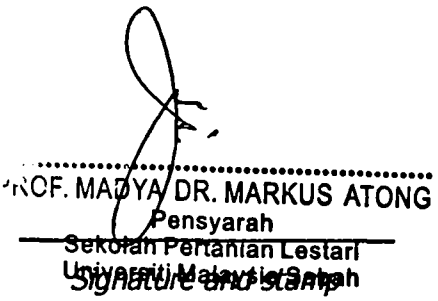

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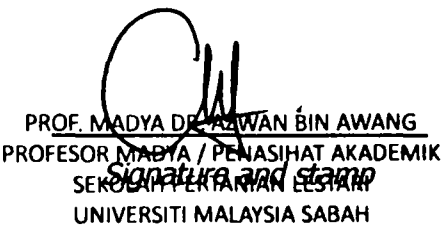

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ABSTRACT

Cultivation of *Vanilla planifolia* in Kampung Sungai Daling, Sandakan were unsuccessful due to disease infestation. Mycological investigation in infected stems, leaves and roots of vanilla, sampled from three sites of vanilla farm located at Kampung Sungai Daling, Sandakan, Sabah was carried out in September 2013. The samples were brought back to School of Sustainable Agriculture (SSA) laboratory, surface sterilized, cut into small pieces with sterilized scalpel blade on sterilized petri dish, placed on PDA and cultured. Pure cultures of resulting fungi were obtained from subcultures of the infected parts. They were identified morphologically and microscopically based on standard procedure. Investigation found that there were *Colletotrichum* sp., *Fusarium* sp., *Mucor* sp. and *Rhizoctonia* sp. with several unidentified species isolated from the vanilla samples, found to be of similar genus infecting other vanilla plantation in several countries.

ABSTRAK

Siasatan mikologi pada batang, daun dan akar vanila yang berjangkit, yang disampel dari tiga tapak ladang vanila yang terletak di Kampung Sungai Daling, Sandakan, Sabah telah dijalankan pada September 2013. Sampel telah dibawa balik ke makmal Sekolah Pertanian Lestari (SPL), disucikan permukaannya, dipotong kecil dengan bilah pisau yang sudah dinyahkuman di atas piring petri yang telah disterilkan, diletakkan pada Agar Kentang Dekstros (PDA) dan dikultur. Kultur tulen diasingkan daripada kulat yang diperolehi daripada subkultur. Kulat telah dikenalpasti secara pemerhatian morfologi dan mikroskopik berdasarkan prosedur standard. Penyiasatan mendapati adanya Colletotrichum sp., Fusarium sp., Mucor sp. dan Rhizoctonia sp. dengan beberapa spesies yang tidak dapat dikenalpasti diasingkan daripada sampel vanila, didapati daripada genus yang sama menjangkiti ladang vanila di beberapa buah negara lain.

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LIST OF SYMBOLS, UNITS AND ABBREVIATION

µm	micrometre
f. sp.	*forma specialis*
g	gramme
km	kilometre
KPD	Korporasi Pembangunan Desa
mL	mililitre
PDA	Potato dextrose agar
RTSB	Rentak Timur Sendirian Berhad
sp.	species
SSA	School of Sustainable Agriculture
UMS	Universiti Malaysia Sabah

CHAPTER 1

INTRODUCTION

1.1 Background of Vanilla in Sabah

Vanilla planifolia, commonly known as vanilla from the family Orchidaceae is a new venture spice crop for this country. In Sabah, the vanilla industry has been embarked by Rural Cooperative Development (KPD) in the year 2007. According to the New Sabah Times e-paper (2007), KPD, at the beginning of the year, had signed a memorandum of understanding with Rentak Timur Sendirian Berhad (RTSB), a company that pioneered the vanilla cultivation in Malaysia, where KPD would act as the implementing agency for the commercial cultivation of vanilla in Sabah. RTSB has a high confidence to the establishment of vanilla industry in Sabah. According to a statement issued by RTSB in Utusan Malaysia (2007), vanilla does not only can be produced in this country, but the industry was put onto a target that will make Malaysia become one of the renown producers for this spice crop practically in 2010.

In Sandakan, vanilla cultivation has started since 2009 (Herman Moinsol, 2013) as a counterpart for the commencement of this valuable spice industry in Sabah. The cultivation project is managed by KPD. KPD had approached the locals, especially those having an estate in Batu 10, Sandakan, to initiate vanilla cultivation through a system called contract farming.

1.1.1 The challenge in growing vanilla in Sabah

Vanilla is a tropical plant that is well adapted to humid tropics. It originates from Mexico and has become the pride of this tropical country. It is a world's delicacy as it became the basis of many beverages namely sweets, ice cream, and soda drinks.

There are only a few countries producing the pods of this tropical orchid, the family that requires special necessities for soil and climate, sensitive to pests and diseases, and because it has little genetic variation in the producing areas due to the propagation method (Verpoorte, 2011) which is by stem cuttings. Other than stem cuttings, seedlings of vanilla plants are also micropropagated, that is by tissue culture.

The vanilla species that is grown in Sabah is *Vanilla planifolia* Andrews (KPD, 2013). Before Malaysia starts venturing in the vanilla industry, the vanilla production in Indonesia has long been established using the spice plant of the same species. Since the ecological conditions of Indonesia and Malaysia are similar, there would be no problem in cultivating vanilla crops in Malaysia (Utusan Malaysia, 2007). The only problem that would not be similar is the pests and diseases attacking the crops. Every region has its own land history of pest infestation. That would be a challenge for Malaysia, especially the regions that are operating vanilla plantation, in the discovery of uncommon pests and diseases and thus, managing the problems caused by them.

V. planifolia Andrews is the species that it provided by KPD to farm operators or participants of the contract farming programme in Sabah. The participants are provided with seedlings made from stem cuttings (KPD, 2013). The length of the cutting influences the maturing time of vanilla plants. The longer the cutting, the shorter the time taken to produce flowers (Bhattacharjee and Shiva, 2008; Hernández Hernández and Lubinsky, 2011). Cuttings are usually planted on site, but can also be stored up to two to ten days, hence can be kept during transportation, (Bhattacharjee and Shiva, 2008).

Contract farming programme was actually introduced and implemented by KPD since year 1991. It is one complete development package for the farm operators or programme participants, with provisions such as farming inputs, consultation service, training, transport, processing and marketing. The programme participants only needs to prepare a land or a cultivation area for vanilla (Esnin Satur, 2013). The participants work for themselves and they are the farm operator themselves. When the harvests are ready, KPD will buy the product back from the farmers. KPD officer in charge will make periodical visits to the farm to check on the farm status and progress.

1.1.2 Identification of plant diseases

Physical damages seen on vanilla might indicate the symptoms for a specific disease and may be caused by pest and mechanical damage. Vanilla is usually damaged at the stem, leaf, root and pod following a disease infection. During field observation, there are plenty of diseases with symptoms witnessed on site. There were mostly rots on the leaves and stems, roots were mostly dried and unlively. There are actually a few more common diseases of vanilla but not seen on site of Kampung Sungai Daling vanilla plantation. Vanilla bean rot, immature bean shedding and shoot tip rot are some other diseases of vanilla, but vanilla crops at Kampung Sungai Daling have not yet produce any flowers, therefore they cannot produce pods.

To determine what is the causal agent for the disease infecting the vanilla plant, Koch's postulate will be carried out. Koch's postulate became the standard procedure for proving that a disease is caused by a bacterium or any other kind of pathogen (Agrios, 2005).

To go through Koch's postulate, the signs and symptoms must first be observed from the infected plant parts. Referring to Agrios (2005), when the signs and symptoms are determined to be harmful towards the plant, the potential pathogen causing it is isolated in a pure culture. After a pure culture is obtained, the pure culture is inoculated into a healthy plant (host) tissue. Observation is made to see if the pure culture causes the same signs and and symptoms to the inoculated tissue. Observation may take weeks to obtain the results. Lastly, the pathogen from the

diseased (inoculated) tissue is re-isolated. If the same pathogen is obtained from the diseased (inoculated) tissue, then that is the pathogen causing the damage on the vanilla plant.

Bhai and Dhanesh (2008) also had conducted a study by applying Koch's postulate to determine fungi causing diseases in Kerala, India. Their research were comprised of survey, identifying fungal cultures and morphological characteristics in the laboratory and inoculation of fungi to rooted vanilla cuttings in order to determine the pathogenicity of fungi. Fungal cultures were kept in repository for future reference.

1.2 Justification

Several vanilla plant diseases had been identified, published in journals but not from local findings. Pathogens that attacked the local vanilla crops may be different from the pathogens that has been attacking vanilla plantations in other countries. This research is to be carried out in order to identify fungal pathogens attacking vanilla crops here, especially in Sandakan.

Rural Cooperative Development (KPD) had reported that there are occurrences of certain diseases attacking the vanilla crops grown by their contract farm participants in several plantations at Batu 10, Sandakan. School of Sustainable Agriculture (SSA) then takes up the challenge to do a research on the case to identify the source of the problems in the vanilla plantation. This case gives an opportunity to both SSA and KPD to build up an amenable tie that benefits each other, in terms of agricultural research.

The outcome of this research will certainly benefits other researchers, vanilla plantation operators and managers, whether in Sabah or all over Malaysia, to ascertain the fungal pathogens that have been affecting the production of vanilla, thus, seeking its cure and might also to eliminate the source of that disease.

1.3 Research Objective

This research was carried out to identify the fungi that have been infecting the vanilla crops at Kampung Sungai Daling, Sandakan.

1.4 Problem Statement

This research focuses on one problem statement;

Is the fungus isolated from diseased vanilla plants sampled from Kampung Sungai Daling similar to the fungal pathogen found in several reported places of occurrence?

CHAPTER 2

LITERATURE REVIEW

2.1 Vanilla

People have known vanilla for its flavour and aroma, mostly found in food, beverages, and also fragrances. Not many have known that vanilla actually came from the Orchid family; Orchidaceae.

Cameron (2011) stated in the book, Vanilla: Medical and Aromatic Plant – Industrial Profiles, that vanilla and its relatives are the surviving members of an ancient descendants of flowering plants and some of the relatives are already threatened with extinction. Until the end of the twentieth century, the vanilloid orchids had been difficult to classify into tribes of the Orchid family. Orchids share the presence of a fully bent, single, fertile anther with various advanced orchid lineage. Considering that the single fertile anther at the apex of vanilla flower's column to have risen by way of a different evolutionary process than that of nearly all other orchids, and also the fact that they produce whether flavourless capsule or aromatic fruit, vanilla are now classified within their very own and unique subfamily, Vanilloideae.

Figure 2.1 Vanilla vine
Source : Bing images

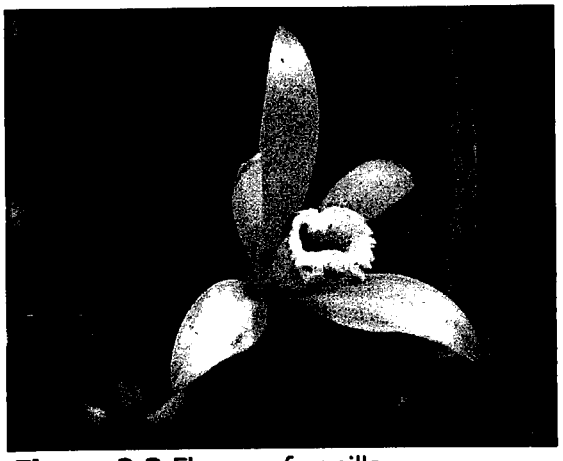

Figure 2.2 Flower of vanilla
Source : Bing images

2.1.1 Orchid taxonomy

All plants are put under the domain Eukarya because they are comprised of cells with nuclei, and other membrane bound organelles. Having chloroplasts that work the photosyntesis process in plants, vanilla are put under kingdom Plantae. The capability of vanilla in producing flowers classifies it into angiosperms. Angiosperms are included in the division Magnoliophyta where flowering plants within this division have advanced venation patterns in leaves and a true flower. Then, vanilla is classified into Liliopsida in which monocotyledonous plants are accommodated since the plant only possess one cotyledon in their seed, as the name suggest. The nature of vanilla plant that lives in association with fungus (mycotrophic) puts it in the order Orchidales, producing many tiny seeds, but lacks endosperm. As mentioned earlier, vanilla came from the family Orchidaceae for it is terrestrial and epiphytic herb, mainly tropical. This order then brought vanilla into the genus *Vanilla* (USDA, 2013). The summary for this taxonomy classification order is concluded as in Figure 2.1.

Kingdom	Plantae – Plants
Subkingdom	Tracheobionta – Vascular plants
Superdivision	Spermatophyta – Seed plants
Division	Manoliophyta – Flowering plants
Class	Liliopsida – Monocotyledons
Subclass	Liliidae
Order	Orchidales
Family	Orchidaceae – Orchid family
Genus	*Vanilla* Mill. - vanilla

Figure 2.3 Classification of vanilla according to its taxa
Source : USDA, 2013.

2.1.2 Agronomy information

Vanilla are unusual plants, some species having large alternate leathery leaves, while the viney stems in some other species are essentially leafless (Bhattacharjee and Shiva, 2008). The flowers would not last long, some shed after a few hours of blooming. In order for beans to be produced, vanilla have to be assisted in pollination as it cannot self-pollinate because of the presence of rostellum that block the way between the anther and stigma.

The genus has been the object of many botanical and technical studies in connection with the production of vanilla (Bhattacharjee and Shiva, 2008). The current worldwide checklist of all vanilla species today recognizes 204 species of vanilla (Goaverts *et al.*, 2013), given some accepted name and some are non-accepted.

According to Cameron (2011) in terms of classification of species within the genus vanilla, these were formally placed into one of two possible sections by Rolfe (1896). However, the section is not considered monophyletic because Rolfe's classification is not molecularly demonstrated. Instead, the classification is only based on the vegetative morphology derived by convergent evolution.

The first vanilla section is the Aphyllae where all the leafless species in the genus is accommodated. For the remaining species that is not classified in section Aphyllae, Rolfe created section Foliosae for leafy species of vanilla. There are also subsection created after vanilla section Foliosae, that are Lamellosae and Papillosae. Lamellosae was named so because species within this subsection are characterized by flowers with flattened scale-like appendages (lamellae) and the ornamentation of labella is always fused to the column along its margin to form a floral tube. Vanilla species under subsection Papillosae on the other side have their leaves and flowers usually with thick trichomes position in the center of the labellum, but without lamellate scales (Cameron, 2011).

The vanilloid orchids are a tremendously diverse group of flowering plants, but most are endangered because of deforestation and land development. The three commerciallized vanilla are *V. pompona*, *V. tahitensis* and *V. planifolia*. The "Tahitian Vanilla", *V. tahitensis* was said to be a primary hybrid between Neotropical *V. odorata* and *V. planifolia* (Cameron, 2011).

Among the three species of vanilla (*V. planifolia*, *V. pompona*, *V. tahitensis*) that are commercially cultivated, *V. planifolia* is the species that is widely grown for its flavour and aroma. Cameron (2011) also mention that this species has been the focus of most research regarding vanilla plants. It is important to realize and appreciate that this is the only one species of a lineage that has become adapted to a variety of habitats, lives in greater or lesser partnership with fungi, exhibits a variety of growth habits, relies on different pollinators, and develops flowers of diverse form. In other words *V. planifolia* may be the only orchid species of significant agricultural value (out of more than 25,000 naturally occuring species).

2.1.3 Economic importance

Vanilla is one of the floriculture product, produce the only edible fruit of the orchid family and is the most labour-intensive agricultural product in the world (Naturland, 2000). Vanilla flavour is well-known and appreciated in the world as the best and widely used in the food industry (Bhattacharjee and Shiva, 2008). The compound

vanilin ($C_8H_8O_3$) is what gives vanilla a place in the spice market all around the world.

In world markets, there are artificial seeds of vanilla (also known as synthetic seeds) that are used to substitute pure vanilla seeds, due to the high price of natural vanilla. The price of the synthetic vanillin produced by this artificial seeds, which is less than $15 per kilo, is so much lower than the price of vanillin extracted from vanilla pods, which is between $1,200 to $4,000 per kilo (Walton *et al.*, 2003). Walton *et al.* (2003) also stated that synthetic vanillin is used in both food and non-food applications, in fragrances and as a flavouring in pharmaceutical preparations. However, Naturland (2000) had drawn a line in the usage of synthetic vanillin, asserting a statement that synthetic vanillin is not allowed to be used in organic food stuff. Customers, likewise, prefer natural vanillin because the aroma is more superior compared to the synthetic counterpart (Xia-Hong He, 2007).

In Malaysia, vanilla cultivation was first undertaken by Rentak Timur Sendirian Berhad (RTSB). The business company's basis are management in agriculture and biotechnological activities and they had started their research and development programme on vanilla since 2003. Their main objective is to make Malaysia as one of the leading vanilla producer in the world (Khuzairi, 2007).

In the year 2007 also, RTSB had entered vanilla business in Sabah, venturing together with the KPD agency. In a news report provided by Bernama (2007), Datuk Abdul Rahim Ismail, the Minister of Sabah Ministry of Agriculture and Food Industries, said the vanilla planting project was highly welcomed in Sabah as it would be part of the government efforts to diversify the economic activities in Sabah in order to reduce poverty among people living in the rural areas of the state.

2.2 *Vanilla planifolia* Andrews

Cultivated vanilla, *Vanilla planifolia* Andrews is naturally distributed in Mexico and Central America, but now cultivated in other parts of the tropics too, it is now considered the aroma of the planet (Divakaran *et al.*, 2006). This species is the one cultivated in Sabah and many more vanilla producers in Malaysia because of suitability

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