



UNIVERSITI PUTRA MALAYSIA

**CHARACTERISATION OF *SYNCHYTRIUM PSOPHOCARPI* (RAC.)
BAUMANN AND ITS PATHOGENICITY ON WINGED BEAN IN
PENINSULAR MALAYSIA**

**ABDOLLAH KARAMI
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ABDOLLAH KARAMI

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
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Science**

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DEDICATION

*Specially dedicated to,
My dear supervisor, my beloved Father, Mother and my dear wife
for their invaluable love, understanding, tolerance, sacrifice, moral
support*



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in
Fulfilment of the requirement for the Degree of Master of Science

**CHARACTERISATION OF *SYNCHYTRIUM PSOPHOCARPI* (RAC.)
BAUMANN AND ITS PATHOGENICITY ON WINGED BEAN IN
PENINSULAR MALAYSIA**

By

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Winged bean (*Psophocarpus tetranologobus*) is an important vegetable crop grown in various tropical climates around South East Asia and Oceania. Its edible parts include leaves and bean pods. An important disease damaging to winged bean is false rust or orange gall caused by *Synchytrium psophocarpi* (Rac.) Baumann. It causes the appearance of bright-orange pustules along the veins of young leaves, stems, pods, and sepals of winged bean flowers. It also reduces pod production and seed yield which is more prevalent during rainy season and high moisture. Thus far, there has been no literature documenting the incidences of orange gall and the causal organism on winged bean in Malaysia. The main objective of this study were to record the local distribution of the disease and to characterized of the causal organism. Evaluation of pathogenicity on winged bean and various other



leguminous plants was also conducted. Fungi occurring on diseased winged bean leaves, pods and stems collected from the states of Selangor and Johor showed typical symptoms. Samples were examined using light and scanning electron microscope for the characterization of sporangia and zoospores. The sporangia have thin walls that are clear and colorless. At higher magnification (1000x), these sporangia appear to contain yellow or orange granules that can be very dense. The average diameter of each sporangium is about 28.64µm by 40.77µm, implying the variation in the sizes. They were generally spherical than ovoid in shape. This diameter is in accordance with previous reports where the average was about 41.20µm.

The average number of zoospores were counted using a haemocytometer. 4.88×10^6 zoospores per mL. of *S. psophocarp*i zoospores were inoculated onto healthy winged bean plants and incubated at different temperatures and humidity's. Fungal growth was measured by counting the amount of zoospores per mL formed. It was found that the optimal temperature for sporangia formation was 29°C and the best humidity was 100%. Both sporangia and zoospores matched with those previously described in literature. Zoospores were found to germinate at about 1 hour and 40 minutes after suspension in water. The mean zoospore head size was 2.46µm (length) x 1.76 µm (width) and the average length of the flagellum was 10.87µm. Zoospore head shape and flagellum length were different from previous reports, probably because of organic solvent treatment during processing. The pathogenicity was tested on winged bean seedlings in the shade house by the mist chamber method and detached leaf inoculations

method on a Petri dish. Symptoms of orange gall were only seen 8 days after inoculation with the maximum number of disease incidences and severity observed on day 18 after inoculation. The host range test was subsequently evaluated by both methods to assess pathogenicity on five leguminous plants. Four varieties of *Phaseolus vulgaris* (black bean, white bean, green bean and red bean) and soybean (*Glycine max*) were tested for pathogenicity. White bean plants developed symptoms at 20 days after inoculation whereas green bean developed after 25 days. The rest were unaffected. It can therefore concluded that the causal agent for orange gall in Malaysia is the fungi *Synchytrium* whose morphology and physiology matches those of previous reports. In this study, its pathogenicity to other leguminose plants includes members of the genus *Phaseolus* were confirmed.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi untuk Ijazah Master Sains.

**PENCIRIAN DAN KEPATOGENAN *SYNCHYTRIUM PSOPHOCARPI*
(RAC).BAUMANN PADA KACANG BOTOL DI SEMENANJUNG
MALAYSIA**

Oleh

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Kacang botol (*Psophocarpis tetranologobus*) adalah tanaman sayuran yang penting di kawasan tropika di sekitar Asia Tenggara dan Oceania. Bahagian yang boleh dimakan termasuk daun dan buah kacang. Salah satu penyakit penting yang merosakkan kacang botol adalah karat palsu atau puru jingga yang disebabkan oleh *Synchytrium psophocarpi* (Rac) Baumann. Ia menyebabkan kemunculan benjolan berwarna jingga terang pada bahagian urat daun dan batang muda, buah dan kelopak bunga kacang botol. Ia juga menyebabkan pengurangan pengeluaran kacang dan hasil biji yang serius. dan lebih ketara dalam musim hujan dan keadaan kelembapan yang tinggi. Sehingga kini, belum ada penulisan yang mencatatkan kejadian penyakit dan organisma penyebab pada kacang botol di Malaysia. Objektif utama kajian ini adalah untuk mencatat taburan tempatan penyakit dan ciri morfologi organisma penyebab. Penilaian kepatogenan pada kacang botol dan beberapa tanaman kekacang yang lain telah dilakukan. Kulat daun, kacang

dan batang kacang botol yang berpenyakit yang menunjukkan simptom biasa telah dikutip dari negeri Selangor dan Johor. Sampel telah di periksa dengan mikroskop cahaya dan imbasan elektron untuk pencirian sporangia dan zoospora. Sporangia mempunyai dinding nipis yang jelas dan tanpa warna. Pada pembesaran tinggi (1000x), ia kelihatan mengandungi butiran kuning atau jingga yang kelihatan agak tebal. Purata garis pusat setiap sporangia berukuran antara 28.64 μ m hingga 40.77 μ m, yang menunjukkan variasi saiz kerana umumnya mereka lebih kebulatan dari bujur dari segi bentuk. Garis pusat ini bersamaan dergan penulisan sebelumnya dengan catatan purata kira-kira 41.20 μ m.

Purata bilangan zoospora telah dikira dengan hemositomiter dan dianggarkan pada 4.88 x 10⁶ zoospora per mL. Zoospora *S. psophocarpi*. telah di inokulasi pada pokok kacang botol yang sihat dan didedahkan kepada suhu dan kelembapan yang berbeza. Tumbesaran kulat telah diukur dengan mengira bilangan zoospora per mL yang terbentuk. Suhu optimum untuk pengeluaran zoospora ialah pada 29°C dan kelembapan bandingan terbaik 100%. Kedua-dua sporangia dan zoospora yang dilihat adalah berpadanan dengan yang telah dicatat dalam penulisan terdahulu. Zoospora dapati bercambah selepas 1 jam dan 40minit diampai dalam air. Purata saiz kepala zoospora berukuran 2.46 μ m (panjang) x 176 μ m(lebar), dan purata panjang flagella adalah 10.87 μ m. Bentuk kepala zoospora dan panjang flagella adalah berlainan dari laporan terdahulu mungkin kerana rawatan larutan organan semasa pemerosesan. Kepatogenan telah diuji pada anak benih kacang yang disimpan dalam rumah teduh dengan kaedah kebuk

berwap dan inokulasi daun terpotong dalam piring Petri. Simptom karat palsu telah dilihat hanya lapan hari selepas inokulasi dengan kejadian penyakit yang tertinggi dilihat pada 18 hari selepas inokulasi. Ujian julat perumah telah seterusnya dimyujikan dengan kedua-dua kaedah untuk menilai kepatogenan pada lima tanaman kekacang. Lima tanaman perumah telah diuji iaitu empat varieti *Phaseolus vulgaris* (kacang hitam, kacang putih, kacang hijau dan kacang merah) dan kacang soya (*Glycine max*). Kacang putih menunjukkan simptom 20 hari selepas inokulasi manakala kacang hijau menunjukkan simptom sedikit lewat iaitu 25 hari selepas inokulasi. Semua tanaman kekacang yang lain tidak dijangkiti. Oleh itu rumusan di buat bahawa organisma penyebab penyakit karat palsu kacang botol di Malaysia adalah disahkan sebagai kulat *Synchytrium* dengan bentuk don fisiologi yang berpadanan dengan penulisan sebelumnya. Dalam kajian ini, kepatogenannya telah dapat disah kepada tanaman kekacang lain termasuklah dalam genus *Phaseolus* iaitu kacang hijau dan kacang putih.

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This thesis is submitted to the Senate of University Putra Malaysia and has been accepted as fulfilment of requirement for degree of Master of Science the members of the Supervisory Committee were as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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LIST OF ABBREVIATION

ANOVA	Analysis of Variance
°C	Degree Celsius
cfu	Colony forming unit
cm	Centimeter
dH ₂ O	Deionized sterile distilled water or Denatured water
e.g.	Exempli gratia (for example)
et al.	Et alia
etc.	Et cetera
gL ⁻¹	Gram per liter
LSD test	least significant different test
μL	Microliter
M	Molar; Molarity
mM	Millimolar
μM	Micromolar
mgL ⁻¹	Milligram per liter
NaOH	Sodium hydroxide
<i>p</i> =0.05	Probability at 95%
pH	Potential Hydrogen or –Log (H)
SD	Standard deviation
ZnSO ₄ 7H ₂ O	Zinc sulfate 7-water
%	Percent





CHAPTER 1

INTRODUCTION

1.1 Background

Psophocarpus tetragonolobus or commonly known as winged bean belongs to the family of leguminosae. The origin of the plant is unknown whether indigenous to East Africa or Southeast Asia (Venketeswaran *et al.*, 1990). However, latest information supported that the origin of winged bean is indigenous to Africa (Duke, 2007).

In Southeast Asia such as Myanmar and Papua New Guinea as well as a few islands of the Pacific region, the winged bean is developed as a meadow crop. The plant has economic value from almost all plant parts containing unpoisonous and rich protein sources especially in the green pods and tubers. Meanwhile, young leaves can be used as vegetables, and the flowers can be auxiliary to salads. The leaves are cooked and eaten like spinach and rich in vitamin A and probably could help to fulfill vitamin A deficiency causing ten thousands of children blind every year in tropical countries region (Stephenson, 1979). Whereas, the flowers can make a sweet garnish and texture like mushrooms when steamed or fried. It has potential to capture various interests internationally (Richard *et al.*, 1981).



According to Venketeswaran *et al* (1990), the dehydrated seeds were similar to soybean and could be used for extracting oil, animal feed and creating milk for traditional Southeast Asian foods such as tempe, tofu and miso. The flour which was extracted from winged bean could be used as a protein additive in bread making. Some studies reported that the composition and nutritional value between winged bean and soybean were similar in terms of proportions of protein, oil, minerals, vitamins, essential amino acids and other constituents and both had high digestibility (Jaffe and Korte, 1976).

Currently, there are populations in the tropical areas attempting to replace soybean with winged bean as plant sources of protein. Nevertheless, soybean is produced in the temperate countries and is still the protein crop leader. Soybean widespread programs had been launched to be accustomed in the tropical region but the production was not achievable in terms of economic returns (Duke, 2007).

Like other legume crops, the winged bean is able to change nitrogen gas from the air into forms usable by plants. This is actually executed by soil bacteria belonging to the genus *Rhizobium*. The bacteria inhabit swellings (nodules) on the root surface. Within these nodules the rhizobia multiply and thrive. They absorb air from the soil and fix the nitrogen. The plant, in turn, absorbs much of the nitrogen produced, which it transfixes to protein, some vitamins, and other nitrogen-containing compounds.

Various diseases and insect pests are known to limit winged bean yield. The most widespread and damaging disease is false rust or orange gall caused by the obligate fungal parasite *Synchytrium psophocarp* (Rac.) Baumann. The fungus is an obligate biotroph and has no resting spore and is holocarpic (De Vera-Chaston, 1977). The sporangia can be dispersed by wind, insects and other natural agents. However, the evidence in support of these modes of dispersal is limited and suggests the need for study on the liberation and dispersal of the sporangia.

Infection of winged bean by *S. psophocarp* had been reported by inoculating with sporangia (Gaumann, 1927) or zoospores (Alicbusan, 1965; De Vera-Chaston, 1973; De Vera-Chaston, 1977). The development of the pathogen in the host had been investigated by De Vera-Chaston (1977) but there have been few investigations into the factors affecting infectivity by the pathogen. Alicbusan (1965) observed that young leaves were very susceptible to infection, the lower surface being heavily infected along the veins. False rust or orange gall disease attacked a number of South East Asian countries such as Papua New Guinea, Philippines, Indonesia, and Malaysia where traditionally winged bean is grown (Anonymous, 1980b) and it is considered as one of the major diseases of this crop in the Philippines (Reinking, 1918; 1919), Papua New Guinea (Price, 1980) and Indonesia (Thompson and Haryono, 1979).

The symptoms of false rust or orange gall can be identified by the appearance of bright-orange pustules along the veins of young leaves and on

stems, pods, and sepals of flowers. Infection leads to hyperplasia and galling, with abnormal branching at the nodes. However, the fungus is known to attack only *P. tetragonolobus*, while *Psophocarpus scandens* is immune. (National Research Council, 1981).

Synchytrium spp is the only member of the Chytridiomycota that is known in Malaysia. It was recorded as a pathogen (obligate parasite) of winged bean by Singh (1980). Currently, there is no published literature of this or other related species in Malaysia. Therefore, there is a very serious lack of knowledge on the taxonomic characterization and pathogenicity of the local fungus. Therefore, there is an important need for local information on this fungus to be documented. This research describes the characterization and pathogenicity of *Synchytrium* spp on winged bean.

1.2 Objectives of study

- (i) To study the distribution of *Synchytrium* and false rust disease on winged bean in selected areas of Peninsular Malaysia.
- (ii) To examine the morphometric of *Synchytrium psophocarpi* and knowledge on its pathogenicity.
- (iii) To determine the host range among leguminous plants.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Botany of Winged Bean

The winged bean's origin remains in dispute whether indigenous to East Africa or Southeast Asia. There are at least four sites that have been recognized as the origin of possibilities such as Papua New Guinea, Mauritius, Madagascar, and India (Venketeswaran *et al.*, 1990). It belongs to the family of leguminosae (Figure 2.1). Nine species have been identified within the genus. Nevertheless, only two species (*P. tetragonolobus* and *P. palustris*) can be processed into food. Both being indigenous to Africa (Duke, 2007).

The greatest diversity centers of the species are Papua New Guinea and Indonesia. Currently the number of varieties has increased and discovered in Thailand and Bangladesh. The winged bean was cultivated for continuous generations in the humid tropics of South and Southeast Asia including India, Sri Lanka, Bangladesh, Myanmar, Malaysia, Thailand, Vietnam, Laos, Cambodia, Philippines, Indonesia, and Papua New Guinea. It can be grown in the wet zone as well as in the dry zone in regions up to an elevation of 1000 above on the sea level. It grows in abundance in hot (21-31 °C), humid equatorial countries in Asia. It is a climbing, herbaceous perennial plant similar to a pole bean and its length could reach approximately 4 m if the