POTENTIAL OF SHRIMP-BASED FERMENTED FOOD ON CHILLI (*Capsicum annum* L.) GROWTH AND METABOLITE CONTENT

By

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The utilisation of indigenous microorganisms (IMO) from Malaysian fermented foods as biofertilisers for *Capsicum annum* L. (chilli) plants presents a novel approach at the intersection of food science and agricultural sustainability. Fermented foods are rich in safe microbial diversity, traditionally used by local communities but underexplored in scientific research. Despite local practices, reports on using IMO from Malaysian fermented foods to enhance chilli growth are limited. This study investigated the mechanism of IMO from fermented *belacan* (shrimp paste) in promoting chilli plant growth. *Belacan* was supplemented with different ratios of molasses (3:5, 1:1, and 5:3) to cultivate potential bacteria, which were isolated and identified using 16S rRNA sequencing. Two strains, *Bacillus velezensis* and *Lysinibacillus fusiformis*, exhibited biofertilising abilities, including antifungal, auxin production and phosphate solubilisation. These IMO and *belacan* were applied to chilli plants in a completely randomised design. The treated plants showed significant improvements in growth parameters such as shoot length, leaf dimensions, and chlorophyll content compared to negative and positive controls. Gas chromatography analysis revealed changes in the

metabolite of the chilli fruits due to IMO treatment. The study demonstrates that IMO from Malaysian fermented foods can enhance chilli plant growth, yield, and nutritional value.

Keywords: Fermented foods, biofertiliser, belacan, *Lysinibacillus fusiformis*, *Bacillus velezensis*

SDG: GOAL 12: Responsible consumption and production

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

POTENSI SUBSTRAT MAKANAN BERFERMENTASI (*Belacan*) BERASASKAN UDANG DALAM MENINGKATKAN PERTUMBUHAN DAN KANDUNGAN METABOLIT DALAM *Capsicum annum* L. (CILI)

Oleh

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Penggunaan mikroorganisma asli (IMO) daripada makanan yang diperam di Malaysia sebagai bio-baja untuk tanaman *Capsicum annum* L. (cili) merupakan pendekatan baharu yang menggabungkan sains makanan dengan kemampanan pertanian. Makanan yang diperam kaya dengan kepelbagaian mikrob yang selamat, yang digunakan secara tradisional oleh komuniti tempatan tetapi kurang diterokai dalam penyelidikan saintifik. Walaupun amalan tempatan wujud, laporan mengenai penggunaan IMO daripada makanan yang diperam di Malaysia untuk meningkatkan pertumbuhan cili adalah terhad. Kajian ini menyiasat mekanisme IMO daripada *belacan* yang diperam dalam mempromosikan pertumbuhan tanaman cili. *Belacan* dicampur dengan molasses dalam nisbah yang berbeza (3:5, 1:1, dan 5:3) untuk menanam bakteria berpotensi, yang kemudiannya diasingkan dan dikenal pasti menggunakan penjujukan 16S rRNA. Dua strain, *Bacillus velezensis* dan *Lysinibacillus fusiformis*, menunjukkan keupayaan bio-baja, termasuk antikulat, pengeluaran auksin dan keupayaan melarutkan fosfat. IMO ini diaplikasikan pada tanaman cili dalam rekabentuk rawak sepenuhnya. Tumbuhan yang dirawat menunjukkan peningkatan ketara dalam parameter pertumbuhan seperti panjang pucuk, dimensi daun, dan kandungan klorofil berbanding kawalan. Analisis kromatografi gas mendedahkan perubahan dalam komposisi metabolit buah cili akibat rawatan IMO. Kajian ini menunjukkan bahawa IMO daripada makanan yang diperam di Malaysia boleh meningkatkan pertumbuhan, hasil, dan nilai pemakanan tanaman cili.

Kata Kunci: Makanan terfermentasi, bio-baja, *belacan*, *Lysinibacillus fusiformis*, *Bacillus velezensis*

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
FAO	Food and Agriculture Organization of the United Nations
pH	Potential Hydrogen
UPM	Universiti Putra Malaysia
PCR	Polymerase Chain Reaction
DNA	Deoxyribonucleic Acid
DOA	Department of Agriculture
rRNA	Ribosomal Ribonucleic Acid
EDTA	Disodium Ethylene Diamine Tetraacetate
Min	Minute
LSD	Least Significant Difference
Sec	Second
Fe Cl3	Iron (III) chloride
HCLO4	Perchloric acid
IAA	Indole Acetic Acid
SDS	Sodium Dodecyl Sulfate
SDW	Sterile Distilled Water
CTAB	Hexadecyltrimethyl-Ammonium Bromide
UV	Ultra Violet
NCBI	National Center for Biotechnology Information
V	Volts
rpm	Revolutions Per Minute
Tris	Tris (hydroxymethyl) aminomethane
R2	*Bacillus velezensis*
R1	*Lysinibacillus fusiformis*

%	Percent
FW	Food Easte
EM	Eco-Friendly Microblal /Effective Microorganisms
B1	15 % fermented food (*belacan*) and 25% molasses (3:5)
B2	20% of fermented food (*belacan*) and molasses (1:1)
B3	25% of fermented food (*belacan*) and 15 % of molasses (5:3)
BB	Beneficial to Human Beings
HR	Health Risk exists
AFP	Aroma of Flavour Promoter
MC	Microbial Control
NA	Nutrient Agar
±	Plus–minus
ML	Millilitre
NB	Nutrition Broth
:	Ratio
MRS	De Man – Rogosa – Sharpe
v/v	Volume per volume
°C	Degree celsius
µL	Microliter
µm	Micrometer
bp	Base pair
RCBD	Randomized Complete Block Design
PC	Positive Control
+NPK	Treatments with chemical fertiliser
-NPK	Treatments without chemical fertiliser
CCD	Coconut Coir Dusit
T	Treatment

cm^2	Square Centimetre
FD	Dirst Dilution of *belacan* treatment
S	Stock of *belacan* treatment
NC	Negative Control
PGPB	Plant-Growth Promoting Bacteria
g	Gram
mg	Milligram
µg	Microgram
kb	Kilobase
L	Litre
M	Molar
CFU	Colony Forming Units
BLAST	Basic Local Alignment Search Tool
W/V	Weight/Volume percentage concentration
w/w	Weight per weight
IMO	Indigenous Microorganisms
N	Nitrogen
P	Phosphorus
K	Potassium
IMP	Disodium 5′-innosinate
GMP	Disodium 5′-guanylate
EAA	Essential amino acids
PUFA	Polyunsaturated fatty acids
MUFA	Monounsaturated fatty acids
MeOX	Methoxyamine hydrochloride
MSTFA	N-methyl trimethylsilyl trifluoroacetamide

SFA	Saturated fatty acids
amino N	Amino acids
NH3-N	Ammonia nitrogen
Kg	Kilogram
MCO	Movement Control Order
BFF	Biofertiliser Fermented Food
LAB	Lactic Acid Bacteria
TBE	Tris Borate EDTA

CHAPTER 1

INTRODUCTION

1.1 Background

Fermentation is a food preservation and transformation technology that uses the growth and metabolic activities of microorganisms. In addition to enhancing the taste and nutritional value of food, fermentation can also improve food safety by inhibiting the growth of harmful bacteria and pathogens (Gaggia et al., 2011). During fermentation, microorganisms such as yeasts, bacteria, and molds break down complex organic compounds in the food materials into simpler molecules, resulting in a range of chemical and physical changes in the food (Cempaka et al., 2021). In Malaysia, fermented food is an integral part of Malaysian cuisine and culture such as *belacan*, *cincalok*, *budu*, and *pekasam*. *Belacan* is a type of fermented shrimp, commonly in solid form, that is commonly used in Nusantara cooking. Curiously, these fermented foods were touted to also contributed to the enhancement of plant growth by promoting plant growth, enhancing nutrient uptake, and increasing tolerance to environmental stresses (Huda, 2012a; Khudair et al., 2023). These properties are mainly contributed by the presence of indigenous microorganisms (IMO). IMO is known to possess bacteriocins (inhibit plant pathogens), increase solubilization of plant macronutrients such as Nitrogen (N), Phosphorus (P), and Potassium (K), and produce phytohormones, such as indole acetic acid (IAA) or auxin (Lamont et al., 2017). Currently, the local practice often utilise these fermented food substrates on important agricultural crop, such as chilli (*Capsicum* spp.) (Khudair et al., 2023; Mohd Zaini et al., 2022). Chilli is known as the second most popular vegetable crop in Malaysia, it is largely cultivated for its fruits and occupies a significant amount of agricultural land.

1.2 Problem Statement

Despite the widespread reliance on chemical fertilizers in Malaysian chili cultivation, which has led to environmental concerns and the development of resistant pathogens, there is limited research on eco-friendly alternatives such as indigenous microorganisms (IMO) from Malaysian fermented foods. While local communities have traditionally used fermented foods rich in microbial diversity, their application as biofertilizers in agricultural practices remains underexplored. Specifically, the potential of IMO from fermented belacan to enhance the growth, yield, and nutritional value of Capsicum annum L. (chili) plants has not been thoroughly investigated. This study addresses this gap by characterizing the biofertilising properties of bacteria isolated from belacan and evaluating their impact on chili plant growth and fruit quality. The findings provide new insights into sustainable agricultural practices that leverage the unique microbial resources of traditional fermented foods.

1.3 Research Questions

1. How do indigenous microorganisms (IMO) isolated from optimal fermented *belacan* influence the growth and development of *Capsicum annum* L. (chili) plants?
2. How does the application of *belacan* biofertiliser affect the nutritional composition and chemical profile of chili fruits?

1.4 Hypotheses

1. *Belacan* contains various beneficial Indigenous microorganisms (IMO) that can be enriched using optimal carbon sources and exhibit strong biofertilising properties

2. The application of *belacan* and IMO from fermented *belacan* leads to significant changes in the nutritional composition and chemical profile of chili fruits, improving their overall quality and significantly enhancing the growth parameters (e.g., shoot length, leaf dimensions, chlorophyll content) of *Capsicum annum* L. (chili) plants.

1.5 Aims

1. To investigate and determine the optimal enrichment process for *belacan* and to isolate and characterize the biofertilising properties of IMOs isolated from *belacan*.
2. To evaluate the impact of fermented *belacan* and IMO treatment on the nutritional composition, growth, and metabolite profile of chili and its fruits.

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