

UNIVERSITI PUTRA MALAYSIA

THE EFFECT OF *PSEUDOMONAS SPP*. AND UPMB10 AND DIFFERENT RATE OF UREA FERTILIZER ON PROTEIN CONTENT AND GROWTH OF LEAF MUSTARD

NOORSURAYA HANI SHAHABUDDIN

FP 2015 163

THE EFFECT OF PSEUDOMONAS SPP. AND UPMB10 AND DIFFERENT RATE

OF UREA FERTILIZER ON PROTEIN CONTENT AND GROWTH OF LEAF

MUSTARD.



NOORSURAYA HANI BINTI SHAHABUDDIN

A project report submitted to Faculty of Agriculture, Universiti Putra Malaysia, in fulfillment of the requirement of PRT4999 (Final Year Project) for the award of Degree of Bachelor of Agricultural Science

Faculty of Agriculture

Universiti Putra Malaysia

2014/2015

ENDORSEMENT

This project paper entitled " **The effect of** *Pseudomonas spp.* and **UPMB10** and **different rate of Urea fertilizer on protein content and growth of leaf mustard** " is prepared by Noorsuraya Hani binti Shahabuddin and submitted to Faculty of Agriculture in fulfillment of the requirement of PRT4999 (Final Year Project) for the award of Degree of Bachelor of Agricultural Science.

Student's name:

Student's signature:

NOORSURAYA HANI BINTI SHAHABUDDIN

Certified by:

.....

(ASSOC. PROF DR. HALIMI BIN MOHD SAUD)

Project supervisor,

Department of Agriculture Technology,

Faculty of Agriculture,

Universiti Putra Malaysia.

Date :



Shahabuddin bin Mohd Kasim & Rozitah binti Muhammad Ali

Strong support - Encouragement - Constant Love

ACKNOWLEDGEMENT

Assalamualaikum W.B.T.,

In the name of Allah S.W.T., The Most Gracious, and Merciful. Thanks to Allah for His grace that I have completed my final year project succesfully.

Firstly, I would like to express my special appreciation to my supervisor, Assoc. Prof. Dr. Halimi bin Mohd Saud for his tremendous guidance, help, and advice for me throughout this project.

Also a special thanks to all the lecturers and staffs of Department of Agriculture Technology, and Department of Crop Science for their sincerity in helping me and guiding me throughout this project. Not to forget thank you to all my friends who are helping me directly or indirectly and for their moral support to me.

Lastly, a very special thanks to my family, especially my parents, Shahabuddin bin Mohd Kasim and Rozitah binti Muhammad Ali and also a special thanks to my fiance, Nashrullah bin Norisham, thank you for supporting and encouraging me throughout this journey and experience as a student. Your prayers are everything for me.

Thank you. Wasalam.

TABLE OF CONTENTS

| | LIST | | PAGE | |
|--|-------------------------------|--------------------------------------|-------|--|
| | | | | |
| | ACKN | NOWLEDGEMENT | i | |
| | TABL | E OF CONTENTS | ii | |
| | LIST (| OF TABLES | iii | |
| | LIST OF PLATES | | | |
| | LIST (| OF APPENDICES | V | |
| | LIST OF FIGURES | | | |
| | vii | | | |
| | viii | | | |
| | | | | |
| | CHAI | PTER 1 : INTRODUCTION | 1 - 3 | |
| | CHAPTER 2 : LITERATURE REVIEW | | | |
| | | | | |
| | 2.1 | Green mustard (Brassica spp.) | 4 | |
| | | 2.1.1 Taxonomy and Morphological | 5 | |
| | 2.2 | Biofertilizer | 6 | |
| | 2.3 | Nitrogen | 7 | |
| | | 2.3.1 Nitrogen fixation | 8 | |
| | | 2.3.2 Nitrogen fertilizer | 9 | |
| | | 2.3.3 Nitrogen-fixing Bacteria | 10 | |
| | 2.4 | Plant growth promoting rhizobacteria | 11 | |

CHAPTER 3 : MATERIALS AND METHODS

| | 3.1 | Location | 13 |
|--|--------|---|----|
| | 3.2 | Bacterial Inoculum | 13 |
| | 3.3 | Preparation of Bacterial Inoculum | 13 |
| | 3.4 | Inoculation method | 14 |
| | 3.5 | Media Preparation | 14 |
| | 3.6 | Seedling preparation | 14 |
| | 3.7 | Transplanting | 14 |
| | 3.8 | Experimental design and treatments | 15 |
| | 3.9 | Maintenance of growth | 16 |
| | 3.10 | Harvesting | 16 |
| | 3.11 | Parameters | |
| | | 3.11.1 Total protein content | 17 |
| | | 3.12.2 Plant fresh weight | 17 |
| | | 3.12.3 Plant dry weight | 17 |
| | | 3.12.4 Height of the plants | 18 |
| | | 3.12.5 Chlorophyll content | 18 |
| | | 3.12.6 Nutrient content analysis (N, P and K) | 18 |
| | 3.12 S | tatistical analysis | 19 |

CHAPTER 4 : RESULTS AND DISCUSSION

4.1 Fresh weight

| | 4.1.1 Leaf | 20 | |
|------------------------|--------------------------|----|--|
| | 4.1.2 Stem | 21 | |
| | 4.1.3 Root | 23 | |
| 4.2 | Dry weight 4.2.1 Leaf | 24 | |
| | 4.2.2 Stem | 26 | |
| | 4.2.3 Root | 27 | |
| 4.3 | Chlorophyll content | 29 | |
| 4.4 | Plant height | 31 | |
| 4.5 | Nitrogen (N) content | 33 | |
| 4.6 | Phosphorus (P) content | 36 | |
| 4.7 | Potassium (K) content | 39 | |
| 4.8 | Total protein content | 42 | |
| | | | |
| CHAPTER 5 : CONCLUSION | | | |
| REFE | CRENCES | 45 | |
| APPENDICES | | 51 | |
| PLAT | 'ES | 55 | |

LIST OF TABLES



LIST OF PLATES

| ige |
|-----|
| 5 |
| 9 |
| 5 |
| 5 |
| 6 |
| 6 |
| 7 |
| 8 |
| 9 |
| 0 |
| |

Ċ

LIST OF APPENDICES

| Appendix | Title | Page |
|----------|--|------|
| 1 | Analysis of variance for leaf fresh weight | 49 |
| 2 | Analysis of variance for stem fresh weight | 49 |
| 3 | Analysis of variance for root fresh weight | 49 |
| 4 | Analysis of variance for leaf dry weight | 50 |
| 5 | Analysis of variance for stem dry weight | 50 |
| 6 | Analysis of variance for root dry weight | 50 |
| 7 | Analysis of variance for chlorophyll content | |
| | • Week 2 | 51 |
| | • Week 4 | |
| 8 | Analysis of variance for plant height | 52 |
| | • Week 2 | |
| | • Week 4 | |
| | | |
| | | |
| | | |

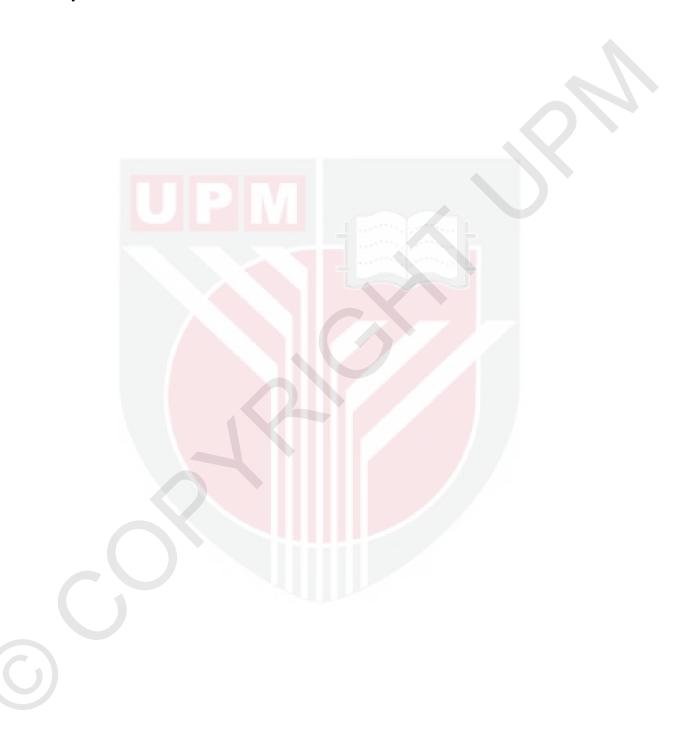
LIST OF FIGURES

| F | ligure | Title | Page |
|---|--------|---|------|
| | 1 | The effect of different type of bacterial inoculum and | |
| | | rates of Urea fertilizer on fresh weight of leaves. | 21 |
| | 2 | The effect of different type of bacterial inoculum and | |
| | | rates of Urea fertilizer on fresh weight of stem. | 22 |
| | 3 | The effect of different type of bacterial inoculum and | |
| | | rates of Urea fertilizer on fresh weight of roots. | 23 |
| | 4 | The effect of different type of bacterial inoculum and | |
| | | rates of Urea fertilizer on dry weight of leaves. | 25 |
| | 5 | The effect of different type of bacterial inoculum and | |
| | | rates of Urea fertilizer on dry weight of stem. | 26 |
| | 6 | The effect of different type of bacterial inoculum and | |
| | | rates of Urea fertilizer on dry weight of roots. | 27 |
| | 7 | The effect of different type of bacterial inoculum and | |
| | | rates of Urea fertilizer on chlorophyll content (Week 2). | 30 |
| | 8 | The effect of different type of bacterial inoculum and | |
| | | rates of Urea fertilizer on chlorophyll content (Week 4). | 30 |
| | 9 | The effect of different type of bacterial inoculum and | |
| | | rates of Urea fertilizer on plant height (Week 2). | 32 |

| 10 | The effect of different type of bacterial inoculum and | |
|----|--|----|
| | rates of Urea fertilizer on plant height (Week 4). | 32 |
| 11 | The effect of different type of bacterial inoculum and | |
| | rates of Urea fertilizer on the Nitrogen (N) content in | |
| | top part of the plant. | 33 |
| 12 | The effect of different type of bacterial inoculum and | |
| 13 | rates of Urea fertilizer on the Nitrogen content in the roots. The effect of different type of bacterial inoculum and | 34 |
| | rates of Urea fertilizer on the Phosphorus (P) content in | |
| | top part of the plant. | 36 |
| 14 | The effect of different type of bacterial inoculum and | |
| | rates of Urea fertilizer on the Phosphorus (P) content in | |
| | the roots. | 37 |
| 15 | The effect of different type of bacterial inoculum and | |
| | rates of Urea fertilizer on the Potassium (K) content in | |
| | top part of the plant. | 39 |
| 16 | The effect of different type of bacterial inoculum and | |
| | rates of Urea fertilizer on the Potassium (K) content | |
| | in the roots. | 40 |
| 17 | The effect of different type of bacterial inoculum and | |
| | rates of Urea fertilizer on the Protein content, % | |
| | in the plants. | 43 |

ABSTRACT

Green mustard (Brassica sp.) is a type of plant in the mustard category of the family Cruciferae. This plant is one out of five types of broad leaf vegetables commonly grown in Malaysia every year. Nitrogen is one the most yield limiting nutrients for most crop production in the world except for legumes, which have the ability to fix their own N. A study on the effect of *Pseudomonas spp.* and UPMB10 and different rate Urea fertilizer on protein content and growth of green mustard was conducted. The main objective is to study if the rate of nitrogen fertilizer and used of bacterial inoculum can increase protein content and growth of the plant. There were 10 treatments including two control treatments with four replications arranged in a Completely Randomized Design (CRD). The treatments were; T1 (Not inoculated + 0 g Urea), T2 (Not inoculated + 0 g Urea), T3 [Inoculated with UPMB-10 (Bacillus sphaericus) + 0 g Urea], T4 [Inoculated with UPMB-10 (*Bacillus sphaericus*)+0.49 g Urea], T5 [Inoculated with UPMB-10 (Bacillus sphaericus) + 0.98 g Urea], T6 [Inoculated with UPMB-10 (Bacillus sphaericus) + 2.94 g Urea], T7 (Inoculated with Pseudomonas spp. + 0 g Urea), T8 (Inoculated with *Pseudomonas spp.* + 0.49 g Urea), T9 (Inoculated with *Pseudomonas* spp. + 0.98 g Urea), T10 (Inoculated with Pseudomonas spp. + 2.94 g Urea). The parameters involved in this project were the plant fresh and dry weight, chlorophyll content, plant height, nutrient composition and protein content. The results indicate that used of bacteria utilization by injection inoculation method significantly improved the growth of the plant. The fresh weight of the plant increase by 20%, dry weight increase by 14%, plant height increase by 22% and chlorophyll content of the plant increase by 9% as compared to the control treatment. While rate of Urea (N) fertilizer improved the nutrient composition (N- 19%, P- 3%, K- 15%) and protein content of the plant increase by 30%.



ABSTRAK

Sawi hijau (Brassica sp.) adalah sejenis tumbuhan dalam kategori mustard daripada keluarga Cruciferae. Tumbuhan ini adalah salah satu daripada lima jenis sayursayuran berdaun luas yang selalu ditanam di Malaysia. Nitrogen adalah satu nutrien yang paling mengekang hasil pengeluaran tanaman di dunia, kecuali kekacang yang mempunyai keupayaan untuk mengikat sendiri N. Satu kajian mengenai kesan Pseudomonas spp. dan UPMB10 dan kadar baja urea yang berbeza pada kandungan protein dan pertumbuhan sawi hijau telah dilaksanakan. Objektif utama adalah untuk mengkaji jika baja urea dan inokulum bakteria yang digunakan boleh meningkatkan kandungan protein dan pertumbuhan tumbuhan. Terdapat 10 rawatan termasuk dua rawatan kawalan dengan empat replikasi disusun dalam rekabentuk rawak lengkap (CRD). Rawatan ialah; T1 (Tidak disuntik + 0 g urea), T2 (Tidak disuntik + 0.98 urea), T3 [disuntik dengan UPMB-10 (Bacillus sphaericus) 0 g urea], T4 [disuntik dengan UPMB-10 (Bacillus sphaericus) + 0.49 g urea], T5 [disuntik dengan UPMB-10 (Bacillus sphaericus) + 0.98 g urea], T6 [disuntik dengan UPMB-10 (Bacillus sphaericus) + 2.94 g urea], T7 (disuntik dengan Pseudomonas spp. + 0 g urea), T8 (disuntik dengan *Pseudomonas spp.* + 0.49 g urea), T9 (disuntik dengan *Pseudomonas* spp. + 0.98 g urea), T10 (disuntik dengan Pseudomonas spp. + 2.94 g urea). Parameter yang terlibat dalam projek ini adalah berat segar dan berat kering, kandungan klorofil, ketinggian tumbuhan, komposisi khasiat dan kandungan protein. Keputusan menunjukkan bahawa penggunaan bakteria kaedah suntikan inokulasi dengan ketara meningkatkan pertumbuhan tumbuhan. Berat segar dengan kenaikan 20%, peningkatan berat kering sebanyak 14%, peningkatan ketinggian sebanyak 22% dan kandungan

CHAPTER 1

INTRODUCTION

Agriculture sector remain as one of the important sector contributing to Malaysia's economy. It provides an employment to 16 percent of the population and contributes about 12 percent to the national GDP. In spite of the policy emphasizing on developing vegetable production to meet the domestic requirements, Malaysia remain as a net importer of vegetables. Since Peninsular Malaysia seldom experience droughts and hurricanes, its tropical climate is very favorable and suitable for the production of various fruits and vegetables.

Vegetables are grown for their leaves, roots, flower or fruits but leafy vegetables are mainly grown for their leaves although other parts of the plants are also edible. It is harvested as a whole with the shoots or stems still attached. These plants are harvested before flowering takes place that is at the vegetative stage because they are grown mainly for leaves. If the leafy vegetables reached flowering stage, it is too fibrous and old for consumption. For example, *Brassica chinensis* locally known as leaf mustard are never allowed to reached the flowering stage before harvesting. It is usually harvested with their shoots and have a higher market price.

Department of Agriculture Malaysia (2012) stated that during the year of 2011, the planted area for *Brassicas* are about 9171.58 hectares, harvested area about 8672.42 hectares and production of 128647.14 metric tonnes. To achieve high level of production, many methods have been used including the use of chemical fertilizer and compost. In additon to these method, the use of beneficial microorganisms can also be done. These microorganisms such as *Pseudomonas, Bacillus, Azotobacter,* and *Rhizobium,* can be classified as nitrogen fixing microorganisms which is bacteria and includes cyanobacteria or blue green algae. With the aid of an enzyme complex-nitrogenase, both of these types of bacteria can reduce dinitrogen.

Nutritionally, leafy vegetables are a great source of fibre and vitamins and provide good roughage for our digestive system but mostly they are low in calories and protein content. It is well known that proteins are of prime importance to health, but they are deficient in diets of most people in the developing countries. Protein is an important component in our diet and lots of people obtain protein by consuming meat, fish, or egg but not vegetables. The proteins of vegetables are built from amino acids but other related simple nitrogenous compound also occurs. To biosynthesize basic building blocks of plants such as amino acids for protein, DNA and RNA, nitrogen (N) is required. Bacteria such as Rhizobium, are able to perform biological nitrogen fixation in which atmospheric nitrogen (N_2) is converted into ammonia (NH_3) and plants are able to use to synthesize protein.

Therefore, this project is conducted to achieve the following objectives :

- i. To study if the rate of nitrogen fertilizer in the form of Urea influence the total protein content in green mustard
- ii. To determine the effect of nitrogen fixing organism on total protein content of green mustard
- iii. To observe the effect of different bacteria used and rate of nitrogen fertilizer on growth performance of green mustard

REFERENCES

Ahemad M., Kibret M., 2014. Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. *Journal of King Saud University – Science* (2014) 26, 1–20.

Aletor O., A. A. Oshodi, K. Ipinmoroti., 2001. Chemical composition of common leafy vegetables and functional properties of their leaf protein concentrates. *Food Chemistry* 78:63–68.

Aletor V. A. and O. A. Adeogum., 1994. Nuttrient and anti nutrient component of some tropical leafy vegetables. *Food Chemistry* 53:175-179.

Anem M., March 20, 2011. Sawi Hijau. Retrieved from http://animhosnan.blogspot.com/2011/03/sawi-hijau.html

Chen Bao-Ming, Zhao-Hui Wang, Sheng-Xiu Li, Gen-Xuan Wang, Hai-Xing Song, Xi-Na Wang, 2004. Effects of nitrate supply on plant growth, nitrate accumulation, metabolic nitrate concentration and nitrate reductase activity in three leafy vegetables. *Plant Science* 167:635–643.

Chin H. F., 2007. Malaysian Vegetables in Colour; Leafy Vegetables 9-21.

Choudary S. and P.C. Trivedi., 2008. Biofertilizers; Boon For Agriculture., 1-7; Potential Uses Of Bacterial And Fungal Biofertilizers., 206-215.

Gamon, J. A., & Surfus, J. S., 1999. Assessing leaf pigment content with a reflectometer. *New Phytologist*, 43:105–117.

Islam M. F., M.Z. Islam, M.E. Haque1, P. Sarker and M.F. Kabir., 2010. Performance of bioinoculant, urea and cowdung on growth, nodulation and biomass yield of akashmoni (Acacia auriculiformis) in old Bramaputra floodplain soil. *Journal of Agroforestry Environment*. 3 (2): 111-116.

Jacob A. & H. V. Uexkull., 1958. Fertilizer Use Nutrition and Manuring of Tropical Crops, Manure: Inorganic Fertilizers, 25-28.

Johansson E., L. Haby, M. L. Prieto-Linde, and S.-E. Svensson., 2013. Influence of fertilizer placement on yield and protein composition in spring malting barley. *Journal of Soil Science and Plant Nutrition*. 2013, 13 (4), 895-904.

Juma, N.G. Tabatabai, M.A. Phosphatase activity in corn and soybean roots: conditions for assay and effects of metals. *Plant and Soil*, 107:39-47, 1988.

Kimura, M., & Rodriguez-Amaya, D. B., 2003. Carotenoid composition of hydroponic leafy vegetables. *Journal of Agricultural and Food Chemistry*, 51, 2603–2607.

Kloepper J.W., Lifshitz R. and Schroth M. N., 1986. Pseudomonas inoculants to benefit plant production. *Animal Plant Science*, 60-64.

Kmiecik, W., Lisiewska, Z., & Jaworska, G., 2001. Effect of storage conditions on the technological value of dill (Anethum graveolens L.). *Folia Horticulturea*, 13: 33–43.

Lim A. H. and P. Vimala., 2012. Growth and yield responses of four leafy vegetables to organic fertilizer. *Journal of Tropical Agriculure and Food Science*. 40(1)(2012): 1–11.

Maryam Boroujerdnia, Naser Alemzadeh Ansari., 2007. Effect of Different Levels of Nitrogen Fertilizer and Cultivars on Growth, Yield and Yield Components of Romaine Lettuce (Lactuca sativa L.) *Middle Eastern and Russian Journal of Plant Science and Biotechnology* 1(2), 47-53.

Mc lachlan, K.D. Acid phosphatase activity of intact roots and phosphorus nutrition in plants: I. Assay conditions and phosphatase activity. *Australian Journal of Agricultural Research*, 31:429-440, 1980a.

Mc lachlan, K.D. Acid phosphatase activity of intact roots and phosphorus nutrition in plants: II. Variations among wheat roots. *Australian Journal of Agricultural Research*, 31:441-448, 1980b. Mia, Shamsuddin, Z.H., Amir, H.G., M.A.B., Halimi, M.S., Zakaria, W. and Marziah., 1999. Symbiotic and associative N2 fixation with vegetable soybean, oil palm and bananas. In *Biotechnology for sustainable utilization of biological resources in the tropics*, 14, eds.

Muraleedharan H., S. Seshadri, and K. Perumal, 2010. Biofertilizer (Phosphobacteria), 4-7.

Okon Y., Labandera-Gonzales C.A., 1994. Agronomic applications of Azospirillum: an evaluation of 20 years worldwide field inoculation, *Soil Biology and Biochemistry* 26:1591–1601.

Parani K. and B.K. Saha, 2012. Prospects of Using Phosphate Solubilizing
Pseudomonas as Bio Fertilizer. *European Journal of Biological Sciences* 4 (2): 4044.

Schachtman D. P., Robert J. Reid, and S.M. Ayling., 1998. Phosphorus Uptake by Plants: From Soil to Cell. *Plant Physiology* 116: 447–453.

Shereen, A., Shaheen and Aly A. A., 2011. Response of Rooted Olive Cuttings to Mineral Fertilization and Foliar sprays with Urea and Gibberlline. *Nature and Science* 9(9):76-86. Siri Panduan Kualiti Sawi Hijau, September 2008. Retrieve from

http://www.fama.gov.my/documents/10157/dd865ec1-4b5b-4ab8-9e28c835bf045faa

Su, Q., Rowley, K. G., Itsiopoulos, C., & O'Dea, K., 2002. Identification and quantitation of major carotenoids in select components of the Mediterranean diet: Green leafy vegetables, figs and olive oil. *European Journal of Clinical Nutrition*, 56:1149–1154.

Takebe M., Ishihara T., Matsuna K., Fojimoto J., Yoneyama T., 1995.Effect of nitrogen application on the content sugars, ascorbic acid, nitrate and oxalic acid in spinach (Spinacia oleracea L.) and kamatsuna (Nrasica compestris L.). *Japanese Journal of Soil Science and Plant Nutrition* 66:238-246.

Tehrani M., Malakouti M. J., 1997. Recommendation of nitrogen fertilizer according to soil nitrate. First of national congregation decreasing poison consumption and best utilization of chemical fertilizers, *Ministry of agricultural, Karaj, Iran*, 182.

Tei F, Benincasa P, Guiducci M., 2000. Effect of nitrogen availability on growth and nitrogen uptake in lettuce. *Acta Horticulturae* 533:385-392.

University of Minesota Extension, 2014. Potassium For Crop Production. Retrieved from http://www.extension.umn.edu/agriculture/nutrient-

management/potassium/potassium-for-crop-production/

Vessey J. K., 2003. Plant growth promoting rhizobia as biofertilizer. *Plant and Soil* 255: 571–574.

Zakry, F. A. A., A. R. Anuar, Z. Z. Zin, Z. H. Shamsuddin, and A. R. Khairuddin., 2005. Use of 15N isotope dilution technique in field evaluation of N_2 fixation in young oil palm inoculated with Bacillus sphaericus (UPMB-10), 3-5.

