UNIVERSITI PUTRA MALAYSIA

EFFECTS OF LEAF SURFACE CHARACTERISTICS AND SPRAY DROPLETS ON EFFECTIVENESS OF SELECTED GLYPHOSATE FORMULATIONS

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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Dedicated to:
My belated mother
(Sulong binti Mohd Noor)
For her true love, support and inspiration

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

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Laboratory and glasshouse studies were conducted to examine the effect of leaf surfaces and spray droplets on the effectiveness of glyphosate on some selected plants. The broad leaves plants selected were *Diodia ocimifolia, Borreria latifolia, Clidemia hirta, Cleome rutidosperma*, *Mikania micrantha* and *Asystasia gangetica*, while the narrow leaves consisted of *Eleusine indica, Imperata cylindrica*, *Cyperus kylingia*, *Axonopus compressus, Pennisetum polistachyon* and *Paspalum conjugatum*. The deposition of pesticide depends on the morphology of leaf surface, thus the leaf surface roughness and epicuticular wax were evaluated. The plants were categorized accordingly to the different type of roughness based on the estimation of three roughness parameters Ra (arithmetic average height parameter), Rq (root-mean-square roughness parameter corresponding to Ra), and Rz (average of high peaks and low valleys over the evaluation length). The leaf was examined by using scanning electron microscopy for the surface roughness while the epicuticular wax content of the leaf was extracted by using chloroform. The amount of

wax extracted from the plants varied between species. For broad leaves plant, *M. micrantha* (44.22μgcm^{-2}) contained the highest quantity of wax. *Clidemia hirta* (24.03μgcm^{-2}) and *A. gangetica* (23.03$\mu gcm^{-2)}$ were grouped in the plant with medium quantity of wax while *C. rutidosperma* (16.52μgcm^{-2}), *B. latifolia* (14.19μgcm^{-2}) and *D. ocimifolia* (10.75μgcm^{-2}) were grouped in plant with low quantity of cuticular wax weight. For narrow leaves plant, *E. indica* (44.23μgcm^{-2}) and *I. cylindrica* (49.88μgcm^{-2}) have the highest quantity of wax. *Pennisetum polystachion* (32.16μgcm^{-2}) and *C. kylingia* (22.85μgcm^{-2}) were categorized under the plant with medium quantity of wax whereas *P. conjugatum* (19.59μgcm^{-2}) and *A. compressus* (16.78μgcm^{-2}) were categorized with low quantity of wax. The wax on the abaxial and adaxial leaf surface of the broad leaves plants was found to be significantly different. In contrast, the amount of wax on the abaxial and adaxial leaf surface of the narrow leaves plants was more or less similar. For the leaf surface roughness of the broad leaves species, *B. latifolia* were categorized as the roughest followed by *C. hirta*, *D. ocimifolia*, *A. gangetica*, and *C. rutidosperma. Mikania micrantha* had the smoothest leaf surface among the broad leaves species. On the other hand, the narrow leaves of *P. polistachyon* were identified as the roughest followed by *I. cylindrica* and *P. conjugatum* while *E. indica*, *A. compressus* and *C. kylingia* were categorized in the smoothest surface.

The effect of leaf surface roughness and epicuticular wax on the spread area of spray droplets of glyphosate was studied by measuring the spread area of 1µL, 2µL and 3µL of micro-emulsion formulation (ME4), soluble solution formulation (AS2), Roundup® and water on the adaxial leaf surfaces of each of the plant species. The spread area of all formulations and volume of droplets varied between species. The spread area showed that the amount of wax on leaf surface played important role as compared to the leaf surface roughness. Nevertheless, the leaf surface roughness to some extends influence for the spread droplets. ME4 gave twice spread diameter area of the droplet than AS2 and Roundup®. The ratio of spread droplet showed the smaller the droplet volume, the better the spread was obtained. The ratio of spread area of 1μL, 2μL and 3μL droplet of all formulations was estimated at about 2, 3 and 4 respectively.

The efficacy of the formulations was then evaluated in the glasshouse by spraying the plants using 3 different nozzles to represent the small (250 microns), medium (350 microns) and coarse (450 microns) droplets. The spray deposition was recorded 1 hour after the application. The chlorophyll degradation was determined on the first, third, seventh and fourteenth day and mortality of weeds was recorded third, seventh, tenth and fourteenth day after spraying. Ten days after spraying, the base of plant was cut and the fresh weights of each species were recorded. Results of all those parameters varied among the species. In general, smaller droplet size, will give better deposition. The droplet size and the leaf surface characteristic were confirmed affecting the amount of spray deposition. The solution sprayed with smaller droplets size was more effective than the coarse droplet size in killing the plants for most of plant species. However, the coarse droplet spray was found to be better in controlling the plants with hairy surface as compared to fine droplet. The micro emulsion formulation (ME4) was found to give better efficacy in killing the plants than the water soluble formulation (AS2) and Roundup®.

Abstrak tesis ini dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

KESAN CIRI PERMUKAAN DAUN DAN TITIK SEMBURAN KE ATAS KEBERKESANAN FORMULASI GLYPHOSATE TERPILIH

Oleh

NORHAYATI NGAH

Februari 2010

Pengerusi : Dzolkhifli Omar, PhD.

Fakulti : Pertanian

Kajian makmal dan rumah kaca telah dilaksanakan bertujuan untuk mengkaji kesan permukaan daun dan titik semburan ke atas keberkesanan glifosat terhadap beberapa tumbuhan terpilih. Tumbuhan daun lebar yang dipilih adalah *Diodia ocimifolia, Borreria latifolia, Clidemia hirta, Cleome rutidosperma, Mikania micrantha* dan *Asystasia gangetica,* manakala tumbuhan daun tirus merangkumi *Eleusine indica*, *Imperata cylindrica*, *Cyperus kylingia*, *Axonopus compressus*, *Pennisetum polistachyon* dan *Paspalum conjugatum.* Lantaran mendapan racun perosak turut bergantung kepada morfologi permukaan daun, maka kekasaran permukaan daun dan lilin epicutikular juga dinilai. Tumbuhan dikategorikan berdasarkan kepada anggaran tiga parameter kekasaran iaitu Ra (parameter purata ketinggian aritmetik), Rq (punca kuasa dua nilai parameter kekasaran Ra), dan Rz (purata tinggi dan rendah puncak lembah ke atas nilai panjang). Daun diperiksa dengan menggunakan mikroskop elektron untuk menilai kekasaran permukaan sementara lilin epicuticular daun diekstrak dengan menggunakan kloroform.

Kandungan lilin yang diekstrak daripada daun tumbuhan adalah bervariasi antara spesies. Untuk tumbuhan daun lebar, *M. micrantha* (44.22 μgcm^{-2}) dikenalpasti sebagai tumbuhan yang mengandungi jumlah lilin tertinggi. *Clidemia Hirta* (24.03 μgcm^{-2}) dan *A. gangetica* (23.03 μgcm^{-2}) dikategorikan sebagai tumbuhan dengan kuantiti lilin yang sederhana, sementara *C. rutidosperma* (16.52 μgcm^{-2}), *B. latifolia* (14.19 μgcm^{-2}) dan *D. ocimifolia* (10.75 μgcm^{-2}) dikategorikan sebagai tumbuhan dengan kuantiti lilin epikuticular yang rendah. Untuk tumbuhan daun tirus, *E. indica* (44.23 μgcm^{-2}) dan *I. cylindrica* (49.88 μgcm^{-2}) diakui mempunyai jumlah lilin terbanyak. *Pennisetum polystachion* (32.16 μgcm^{-2}) dan *C. kylingia* (22.85 μgcm^{-2}) dikategorikan sebagai tumbuhan yang mempunyai jumlah lilin sederhana manakala *P. conjugatum* (19.59 μgcm^{-2}) dan *A. compressus* (16.78 μgcm^{-2}) berada dalam kategori tumbuhan yang mempunyai kuantiti lilin yang rendah. Jumlah lilin pada permukaan atas dan bawah daun bagi tumbuhan berdaun lebar mempunyai perbezaan yang nyata. Sebaliknya, jumlah lilin pada permukaan atas dan bawah bagi tumbuhan berdaun tirus adalah lebih kurang sama. Untuk nilai kekasaran permukaan daun daripada tumbuhan berdaun lebar, *B. latifolia* dikategorikan sebagai paling kasar diikuti oleh *C. hirta*, *D. ocimifolia*, *A. gangetica*, dan *C. rutidosperma*. *M. micrantha* mempunyai permukaan daun yang paling halus di antara spesies daun lebar. Bagi tumbuhan berdaun tirus *P. polistachyon* telah dikenalpasti sebagai paling kasar diikuti oleh *I. cylindrica*, dan *P. conjugatum* sementara *E. indica*, *A. compressus* dan *C. kylingia* dikategorikan sebagai mempunyai permukaan yang halus.

Kesan daripada kekasaran permukaan daun dan jumlah lilin epicuticular ke atas penyebaran titisan semburan glifosat telah dikaji dengan mengukur luas penyebaran 1µL, 2µL dan 3µL formulasi larutan mikro (ME4), formulasi solusi larut (AS2), Roundup ®

dan air pada permukaan atas daun bagi semua spesis tumbuhan. Keluasan kawasan penyebaran oleh semua jenis formulasi dan saiz titisan adalah bervariasi di antara semua spesis. Keluasan penyebaran menunjukkan bahawa jumlah lilin pada permukaan daun memainkan peranan penting berbanding dengan kekasaran permukaan daun. Walau bagaimana pun, dalam keadaan tertentu kekasaran permukaan daun boleh bertindak sebagai faktor penentu untuk penyebaran titisan. ME4 memberikan nilai dua kali ganda diameter luas titisan yang tersebar berbanding AS2 dan Roundup®. Nisbah penyebaran titisan menunjukkan bahawa semakin kecil saiz titisan, semakin baik penyebaran yang diperolehi. Nisbah luas penyebaran bagi isipadu 1μL, 2μL dan 3μL bagi semua formulasi adalah dianggarkan sekitar 2:3:4.

Keberkesanan formulasi kemudiannya dinilai dalam rumah kaca dengan menyembur tumbuhan menggunakan 3 jenis nozel yang berbeza untuk mewakili saiz titik semburan sederhana (250 mikron), kasar (350 mikron) dan sangat kasar (450 mikron). Mendapan penyemburan telah direkodkan satu jam selepas aplikasi. Kandungan klorofil daun ditentu ukur pada hari pertama, ketiga, ketujuh dan keempat belas manakala tahap kematian direkod pada hari ketiga, ketujuh, kesepuluh dan empat belas hari selepas semburan. 10 hari selepas semburan, pangkal tumbuhan dipotong dan berat basah dan berat kering tumbuhan direkod. Keputusan daripada semua parameter adalah bervariasi di antara spesies. Secara umumnya, semakin kecil isipadu titisan, semakin tinggi nilai endapan racun. Isipadu titisan dan ciri permukaan daun disahkan mempengaruhi jumlah endapan semburan. Solusi semburan yang disembur dengan saiz titisan yang lebih kecil adalah lebih berkesan berbanding saiz titisan yang besar untuk membunuh sebahagian besar rumpai. Namun, isipadu titisan yang kasar didapati adalah lebih berkesan untuk mengawal tumbuhan yang mempunyai permukaan berbulu berbanding dengan isipadu titisan halus. Formulasi emulsi mikro (ME4) didapati paling berkesan untuk mengawal tumbuhan berbanding perumusan larut (AS2) dan Roundup ®.

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I certify that an Examination Committee met on 23rd March 2010 to conduct the final examination of Norhayati Ngah on his Master of Science thesis entitled “Effects of Leaf Surface Characteristics and Spray Droplets on Effectiveness of Selected Glyphosate Formulations” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the candidate be awarded the relevant degree. Members of Examination Committee are as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the degree of Master of Science. The members of the Supervisory Committee were as follows:

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Professor and Dean
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Date: 10 Jun 2010

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 Universiti Putra Malaysia or other institutions.

NORHAYATI BINTI NGAH

Date:

TABLE OF CONTENTS

LIST OF TABLE

LIST OF ABBREVIATIONS

P. conjugatum	*Paspalum conjugatum*
P. polistachyon	*Pennisetum polistachyon*
I. cylindrica	*Imperata cylindrica*
A. compressus	*Axonopus compressus*
C. kylingia	*Cyperus kylingia*
E. indica	*Eleusine indica*
D. ocimifolia	*Diodia ocimifolia*
C. hirta	*Clidemia hirta*
A. gangetica	*Asystasia gangetica*
C. rutidosperma	*Cleome rutidosperma*
M. micrantha	*Mikania micrantha*
B. latifolia	*Borreria latifolia*
ANOVA	Analysis of variance
$CHCl_3$	Trichloromethane
CRD	Complete Randomized Design
DAT	Day after treatment
DSMA	Disodiun methanearsonate
EPTC	Ethyl di-n-prophylthiolcarbamate
HSD	Tukey’s Studentized Range
MCPA	Metaxon chloro phenyl acid
NPK	Nitrogen, Phosphorous, and Potash
RCBD	Randomized Complete Block Design

S.E	Standart Error
SAS	Statistical Analysis System
SEM	Scanning electron microscope
UPM	Universiti Putra Malaysia

CHAPTER 1

INTRODUCTION

Weeds reduce the yield of crop by suppressing plant growth through competition for light, soil minerals, moisture and water. It was also lowering the quality of the harvest through contamination with foreign matter. The damage is irreversible. In Malaysia, herbicides usage was 67.49 percent of the total pesticide used equivalent to RM 218 million in 2004 (Omar, 2008). Farmers usually use a variety of methods to control the weeds. The choice of methods is closely controlled by economics. The availability of farm labor at the critical times needed for weeding crops is often limited, and herbicide can often give more efficient weed control at comparable or even lower cost.

The efficiency of an herbicide application, while clearly depending on the chemical itself as well as the formulation is also dependent upon being delivered to the target site (Kirkwood, 1987). The application technique is the only means of improving targeting of herbicide sprays and yet current understanding is relatively crude (Matthews, 2000). Therefore, more research is needed to understand how spray and leaf structures interact to enable more efficient placing of sprays at the target sites.

The activity of foliage applied herbicide must ultimately depend on the concentration of active ingredient that reaches the sites of action together with the effect of the herbicide on the biochemical mechanisms that take place at these sites. The micro structure of leaf surfaces are among the factors that influence droplet retention. The amount of herbicide deposited on a crop or weed canopy during the spray application is influenced by plant morphological, spraying technique, herbicide formulation and environmental factors. The plant factors included the habit of growth, size, shape, and orientation of the leaves, as well as the characteristics of surface such as corrugation, roughness, and the presence of hairs or trichomes and the physiochemical properties of the epicuticular waxes.

The epicuticular waxes are considered to be the first and main barrier to the penetration of agriculturally important chemicals across the cuticle to their target site. The efficiency of cuticle retention and penetration, tissue absorption and, in the case of systemic compounds, herbicide metabolism or immobilization en route may reduce the amount reaching the active sites. The surface topography also influences the behavior of herbicide deposit on leaf surface. The surface roughness is an important factor to determine the spray retention; it is responsible for droplet bounce by presenting surface inclined from the horizontal, and governs the magnitude of contact angle hyteresis. Roughness can cause droplets to coalesce to form larger deposits.

Spray deposition is generally proportional to the rate of application with losses occurring due to runoff, volatility or spray misdirection; the height of the boom above the canopy and the location of the leaves in the canopy may also influence surface deposits. Spray volume and droplet size are also of considerable importance, presumably due to interaction involving such factors as retention, spreading, wetting and penetration to the surface of the foliage. In general, herbicidal efficiency increased with reduction in droplet size. The optimal effect of herbicide spraying may be achieved when maximum contact area is obtained by combination of small droplets and high volume. However, large droplets maybe preferable in situations in which fine hairs are present on the plant surface or where there is a need to exploit differential retention between a waxy crop and less waxy weeds or between broad leaved plants and narrow leaved plant species.

Droplet size is undoubtedly a key issue; the smallest droplets being the most prone to drift yet often being optimal for enhanced coverage, whereas larger droplets convey excessive amounts of pesticide and may rebound from leaf surfaces. This results in poor distribution, unless large volumes are applied to completely wet the surfaces which can cause wastages. Further knowledge of the surface retention of droplets of different sizes and their distribution, as well as information on the availability and persistence of deposits on surfaces should provide a better specification for the type of nozzle required, whether the spray liquid can be improved by including an appropriate adjuvant and whether any additional delivery system such as air assistance should be used.

This research was carried out to study the effect of roughness and wax on leaf surfaces and spray droplet sizes on the effectiveness of glyphosate. The study consisted of three parts. Part one was the study on the leaf wax and surface roughness. The second part was to study the effect of roughness and wax on leaf surface on spread droplet of glyphosate formulations. Lastly, the study was conducted to evaluate the effect of roughness and wax on leaf, spray droplet sizes and formulation on the effectiveness of glyphosate.