FINAL PROJECT REPORT/LAPORAN AKHIR PROJEK

FOR/UNTUK

KAJIAN GARIS DASAR* (BASELINE) *PELBAGAI SISA RACUN PEROSAK DALAM PRODUK ORGANISMA TERUBAHSUAI GENETIK

MEMORANDUM NO.: JBK/UPM/100-2/2/3

BETWEEN/ANTARA

THE GOVERNMENT OF MALAYSIA/KERAJAAN MALAYSIA

AND/DAN

UNIVERSITI PUTRA MALAYSIA

DEPARTMENT OF BIOSAFETY

MINISTRY OF NATURAL RESOURCES, ENVIRONMENT AND CLIMATE CHANGE

LEVEL 4, BLOCK F11, COMPLEX F

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Contents

Isi Kandungan

1. INTRODUCTION

1.1 Background

Herbicides is a class of pesticides which controls the growth of undesired plants that compete with the crops for the nutrients necessary for growth (Holt, 2013). The introduction of herbicides in the agricultural sector brings to the need for crops that tolerate the action of herbicides. Since the commercialization of genetically modified herbicides-tolerant (GMHT) crops in the mid-1990s, the area covered by these crops, as well as the number of nations where these crops are cultivated, has gradually increased (Kleter et al., 2011). Consequently, herbicides are often used in conventional agricultural operations, and the usage of GMHT crops is mostly depending on their respective specific herbicides application, which increases the possibility of herbicide residues ending up in animal feed (Muola et al., 2021). Unfortunately, herbicide-resistant weeds have emerged as a result of the repeated application of glyphosate-tolerant crops and glyphosate-only sprays in the same fields without enough variation and chemical diversity. Among the GMHT crops, soybean, cotton, maize, and canola are the most major, whereas alfalfa, papaya, squash, sugar beet, sweet pepper, and tomato are produced to a lesser level at the moment (Kleter et al., 2011). The herbicides to which GMHT crops have been approved for food consumption are divided into four categories: acetolactate synthase (ALS) inhibitors, bromoxynil, glufosinate, and glyphosate (Kleter et al., 2011). As the global demand for GM crops keep increasing, consumers will probably distrust the claim of GM foods labeled with pesticide-free labels. Also, there is an underlying risk consuming high dose of these herbicides' residues in GMHT crops. For instance, the human health risks associated with glyphosate-based herbicide formulations are metabolic alterations, DNA damage, kidney damage, reproduction toxicity, mental conditions like attention deficit hyperactivity disorder (ADHD), Alzheimer's, Parkinson's, celiac disease, autism, effect on erythrocytes, leaky gut syndrome, cancers, etc 1(Fluegge & Fluegge, 2016; Fortes et al., 2016; Mesnage et al., 2015; Mink et al., 2011; Myers et al., 2016; Swanson et al., 2014). Hence, arose the need of conducting pesticide residues analysis independently not only to comply with the claim but also to identify the maximum residue limit (MRL). Thus, we analysed 49 crop samples consisting of soybeans, wheat, rice, and corn received from Jabatan Biokeselamatan.

1. PENGENALAN

1.1 Latar belakang

Racun rumpai ialah sejenis racun perosak yang mengawal pertumbuhan rumpai yang tidak diingini yang bersaing dengan tanaman untuk mendapatkan nutrien yang diperlukan untuk pertumbuhan (Holt, 2013). Pengenalan racun rumpai dalam sektor pertanian membawa kepada keperluan untuk tanaman yang dapat menangani kesan racun rumpai. Sejak daripada pengkomersilan tanaman tahan racun rumpai yang diubah suai secara genetik (GMHT) pada pertengahan tahun 1990-an, kawasan yang dilitupi dengan tanaman ini, serta bilangan negara di mana tanaman ini ditanam, telah meningkat secara beransur-ansur (Kleter et al., 2011). Secara tidak langsung, racun rumpai sering digunakan dalam operasi pertanian konvensional, dan peggunaan tanaman GMHT sebahagian besarnya bergantung kepada penggunaan racun rumpai yang lebih khusus dan spesifik, yang meningkatkan kebarangkalian sisa-sisa racun rumpai berakhir dalam makanan haiwan ternakan (Muola et al., 2021). Pada peringkat awal penerimaan tanaman GMHT, tanaman ini pada umumnya telah menunjukkan pengurangan penggunaan racun rumpai. Namun demikian, mucul rumpai yang dapat mengatasi kesan racun rumpai (herbicide-resistant weeds), hasil daripada penggunaan aplikasi tanaman tahan glifosat (glyphosate-tolerant crops) dan hanya semburan glifosat (glyphosate-only sprays) di dalam ladang yang sama tanpa variasi dan kepelbagaian kimia yang mencukupi. Penggunaan racun rumpai telah meningkat disebabkan rumpai yang dapat mengatasi kesan racun rumpai ini. Di antara tanaman GMHT, kacang soya, kapas, jagung dan kanola adalah yang utama, manakala alfafa, betik, labu, gula bit, lada manis dan tomato dihasilkan dalam jumlah yang lebih rendah pada ketika ini (Kleter et al., 2011). Racun rumpai yang telah diluluskan unuk pemakanan dalam tanaman GMHT telah dibahagikan kepada empat kategori; perencat acetolactate synthase (ALS) (ALS inhibitors), bromoksinil, glufosinat, dan glifosat (Kleter et al., 2011). Seiring dengan permintaan global terhadap tanaman terubahsuai genetik (GM) yang terus meningkat, pengguna mungkin akan meragui dakwaan makanan yang berlabel GM bebas racun perosak (pesticide-free). Selain itu, terdapat risiko pengambilan dos yang tinggi dalam sisa-sisa racun rumpai ini dalam tanaman GMHT. Sebagai contoh, risiko terhadap kesihatan manusia yang berkaitan dengan formulasi racun rumpai berasaskan glifosat, seperti perubahan metabolik, kerosakan DNA, kerosakan buah pinggang, toksisiti terhadap sistem pembiakan, gangguan mental seperti gangguan hiperaktif deficit perhatian (ADHD), Alzheimer, Parkinson, penyakit seliak (celiac), autism, kesan terhadap eritrosit, sindrom ketirisan usus (leaky gut syndrome), kanser dan lain-lain (Fluegge & Fluegge, 2016; Fortes et al., 2016; Mesnage et al., 2015; Mink et al., 2011; Myers et al., 2016; Swanson et al., 2014). Justeru, timbul keperluan untuk menjalankan analisis terhadap sisa racun perosak secara tersendiri, yang bukan sahaja untuk memenuhi dakwaan, tetapi juga untuk mengenalpasti had residu maksimum (MRL). Oleh itu, kami telah menganalisis 49 sampel tanaman yang terdiri daripada kacang soya, gandum, beras, dan jagung yang diperolehi daripada Jabatan Biokeselamatan.

1.2 Problem to address

As the global demand for GM crops keep increasing, consumers will probably distrust the claim of GM foods labeled with pesticide-free labels. Also, there is an underlying risk consuming high dose of these herbicides' residues in GMHT crops. For instance, the human health risks associated with paraquat-based herbicide formulations are mental conditions like attention deficit hyperactivity disorder (ADHD), Alzheimer's, Parkinson's, celiac disease, autism, effect on erythrocytes, leaky gut syndrome, cancers, etc. (Fluegge & Fluegge, 2016; Fortes et al., 2016; Mesnage et al., 2015; Mink et al., 2011; Myers et al., 2016; Swanson et al., 2014). Hence, arose the need of conducting pesticide residues analysis independently not only to comply with the claim but also to identify the maximum residue limit (MRL).

1.2 Penyataan masalah

Seiring dengan permintaan global terhadap tanaman GM yang terus meningkat, pengguna mungkin akan meragui dakwaan makanan yang berlabel GM bebas racun perosak (pesticide-free labels). Selain itu, terdapat risiko pengambilan dos yang tinggi dalam sisa-sisa racun rumpai ini dalam tanaman GMHT. Sebagai contoh, risiko terhadap kesihatan manusia yang berkaitan dengan formulasi racun rumpai berasaskan paraquat (paraquat-based herbicide) seperti gangguan hiperaktif deficit perhatian (ADHD), Alzheimer, Parkinson, penyakit seliak (celiac), autism, kesan terhadap eritrosit, sindrom ketirisan usus (leaky gut syndrome), kanser dan lain-lain (Fluegge & Fluegge, 2016; Fortes et al., 2016; Mesnage et al., 2015; Mink et al., 2011; Myers et al., 2016; Swanson et al., 2014). Justeru, timbul keperluan untuk menjalankan analisis terhadap sisa racun serangga secara tersendiri, yang bukan sahaja untuk memenuhi dakwaan, tetapi juga untuk mengenal pasti had residu maksimum (MRL).

1.3 Purpose and scope of evaluation

The scope of evaluation and the specific objective is:

• To assess the level of paraquat, glyphosate and glufosinate in sampled GM based products.

1.3 Tujuan dan skop penilaian

Skop penilaian dan objektif yang spesifik adalah:

• Menilai tahap paraquat, glifosat, dan glufosinat dalam sample produk berasaskan GM.

2. METHODOLOGY / *METODOLOGI*

Overview

A pesticide residual analysis for detection of glyphosate, glufosinate, and paraquat was conducted on 49 crop samples consisting of soybeans, corn, wheat, and rice received from Jabatan Biokeselamatan, Kementerian Sumber Asli, Alam Sekitar dan Perubahan Iklim via Department of Quarantine and Inspection Services Malaysia (MAQIS). The experiment was performed to determine the adherence of LMO products to MRL set by Pesticide Board Malaysia (PBM). Each sample was prepared in triplicates then analyzed using Ultra High-Performance Liquid Chromatography (UHPLC, Dionex) and Liquid Chromatography/ Mass Spectrometry with Triple Quadrapole (LC-QQQ-MS Agilent). The methods for the preparation of standards and sample extraction were explained below.

Gambaran keseluruhan

Analisis sisa racun perosak untuk pengesanan glifosat, glufosinat, dan paraquat telah dijalankan dalam 49 sampel tanaman yang terdiri daripada kacang soya, jagung, gandum, dan beras yang diperolehi daripada Jabatan Biokeselamatan, Kementerian Sumber Asli, Alam Sekitar dan Perubahan Iklim melalui Jabatan Perkhidmatan Kuarantin dan Pemeriksaan Malaysia (MAQIS). Eksperimen ini dijalankan untuk menentukan pematuhan produk LMO terhadap Had Residu Maksimum (MRL) yang ditetapkan oleh Lembaga Racun Makhluk Perosak Malaysia (PBM). Setiap sampel telah disediakan dalam tiga replikasi dan kemudiannya dianalisis menggunakan Kromatografi Cecair Berprestasi Ultra Tinggi (UHPLC, Dionex) dan Kromatografi Cecair/ Spektrometri Jisim dengan Triple Quadrapole (LC-QQQ-MS Agilent). Kaedah untuk penyediaan standard dan pengekstrakan sampel diterangkan di bawah.

2.1 Determination of Paraquat Residue using UHPLC/ *Penentuan Sisa Paraquat menggunakan UHPLC*

2.1.1 Chemicals

Acetonitrile and methanol (both HPLC gradient grade; ≥99.9 %) were purchased from Qrec (Malaysia). Phosphoric acid (Sigma-Alrich, 96.5 %) and hydrochloric acid fuming 37 % (for analysis) were purchased from Merck (Malaysia). The standard of paraquat dichloride was also purchased from Merck (Malaysia).

2.1.1 Bahan kimia

Acetonitrile dan metanol (keduanya gred gradien HPLC; ≥99.9%) dibeli daripada Qrec (Malaysia). Asid fosforik (Sigma-Alrich, 96.5%) dan asid hidroklorik pekat 37% (untuk analisis) dibeli daripada Merck (Malaysia). Standard paraquat diklorida juga dibeli daripada Merck (Malaysia).

2.1.2 Preparation of Standards

A stock solution of paraquat dichloride was prepared at 1 mg mL^{-1} in methanol and placed in an ultrasonic bath for 5 min to achieve complete dissolution. From the standard stock solution, working standard solutions with concentrations of 2, 4, 6, 8, and 10 µg mL^{-1} were prepared. The calibration curve was plotted according to peak area versus concentration.

2.1.2 Penyediaan Piawai

Larutan stok paraquat diklorida pada kepekatan 1 mg mL^{-1} disediakan dalam metanol dan diletakkan dalam kolam ultrasonik selama 5 minit untuk mencapai penguraian yang lengkap. Daripada larutan stok piawai, larutan kerja piawai dengan kepekatan 2, 4, 6, 8, dan 10 µg mL^{-1} disediakan. Lengkung kalibrasi diplot mengikut kawasan puncak (peak area) melawan kepekekatan (concentration).

2.1.3 Method of Sample Extraction

The samples were coded based on their types (corn or soybean) (Table 1). The samples were ground into fine powder using an industrial grinder. About 2.5 g of each sample was weighed into a 15 mL polypropylene tube. Approximately, 12.5 mL of the extraction solvent comprised of methanol and 0.5 M hydrochloric acid (60:40, volume/volume) were added and blended using a Vortex mixer. Then each sample was extracted for 15 min using a horizontal shaker at 150 rpm for 15 min. The extract was then centrifuged at 4300 rpm for 15 min. Around 1 mL of the supernatant was filtrated through a 0.45 µm nylon syringe filter and injected into the HPLC system.

2.1.3 Kaedah pengekstrakan sampel

Sampel-sampel telah diberikan kod berdasarkan jenisnya (jagung atau kacang soya) (Jadual 1). Sampel-sampel telah dikisar menjadi serbuk halus menggunakan pengisar industri. Lebih kurang 2.5 g setiap sampel telah ditimbang ke dalam 15 ml tiub polipropilena. Lebih kurang 12.5 mL pelarut pengekstrakan yang terdiri daripada metanol dan 0.5 M asid hidroklorik (60:40, isipadu/isipadu) telah ditambahkan dan dicampur menggunakan penggoncang Vortex. Kemudian, setiap sampel telah diekstrak selama 15 minit menggunakan penggoncang horizontal pada 150 rpm selama 15 minit. Ekstrak tersebut kemudian diempar pada 4300 rpm selama 15 minit. Lebih kurang 1 mL supernatan telah ditapis melalui penapis jarum nilon 0.45 µm dan dimasukkan ke dalam sistem HPLC.

2.1.4 HPLC Parameter

All samples were analyzed through an HPLC system using a Dionex Ultimate 3000 (CLMO Technology Sdn Bhd). High-performance liquid chromatography was performed using a SeQuant ZIC®-HILIC column (4.6 × 150 mm, 5 µm, 200 Å; Merck) combined with a Guard column (2.1 × 20 mm; Darmstadt, Germany). The mobile phase consisted of (A) ultra-pure water, added with 0.5% phosphoric acid, and (B) acetonitrile. The gradient profile was achieved at a flow rate of 100 µL/min and initiated with an equilibration phase of 20 % A for 7 min, which was increased to 80 % A within 4 min and held for 10 min. The column was heated constantly at 40 °C throughout the analysis.

2.1.4 Parameter HPLC

Semua sampel dianalisis melalui sistem HPLC menggunakan Dionex Ultimate 3000 (CLMO Technology Sdn Bhd). Kromatografi cecair berprestasi tinggi dijalankan menggunakan kolum SeQuant ZIC®-HILIC (4.6 × 150 mm, 5 µm, 200 Å; Merck) yang digabungkan dengan kolum Guard (Guard column) (2.1 × 20 mm; Darmstadt, Jerman). Fasa bergerak (mobile phase) terdiri daripada (A) air ultratulen (ultra-pure), ditambah dengan 0.5% asid fosforik, dan (B) acetonitril. Profil gradien dicapai pada kadar aliran 100 µL/min dan dimulakan dengan 20 % A fasa penyeimbangan (equilibration phase) selama 7 minit, yang dinaikkan menjadi 80 % A dalam masa 4 minit, dan dimalarkan selama 10 minit. Kolum dipanaskan pada suhu 40 °C sepanjang analisis.

2.2 Determination of Glyphosate and Glufosinate Residue using LC-QQQ-MS/ *Penentuan Sisa Glifosat dan Glufosinat menggunakan LC-QQQ-MS*

2.2.1 Chemicals

All chemicals and solvents used were analytical of the HPLC grade. Glyphosate and glufosinate ammonium (99% purity) were purchased from Merck (Malaysia). Formic acid (98%), methanol and dichloromethane were obtained from Merck (Malaysia). Deionized water (DI) from Milli-Q system. All solutions prepared for LC were passed through a 0.45 µm nylon filter before being used as a dilution medium.

2.2.1 Bahan kimia

Semua bahan kimia dan pelarut yang digunakan adalah daripada gred analitikal HPLC. Glifosat dan ammonium glufosinat (99% ketulenan) dibeli daripada Merck (Malaysia). Asid formik (98%), metanol, dan diklorometana diperoleh daripada Merck (Malaysia). Air suling (DI) didapati daripada sistem Milli-Q. Semua larutan yang disediakan untuk LC telah melalui penapis nilon 0.45 µm sebelum digunakan sebagai medium pencairan.

2.2.2 Preparation of Standards

A stock solution of glyphosate and glufosinate-ammonium was prepared at 1 mg mL^{-1} in deionized water and placed in an ultrasonic bath for 5 min to achieve complete dissolution. From the standard stock solution, working standard solutions with concentrations of 2, 4, 6, 8, and 10 µg mL^{-1} were prepared. The calibration curve was plotted according to peak area versus concentration.

2.2.2 Penyediaan Piawai

Larutan stok glifosat dan ammonium glufosinat disediakan pada kepekatan 1 mg mL^{-1} dalam air suling dan diletakkan dalam bak ultrasonik selama 5 minit untuk mencapai penguraian yang lengkap. Daripada larutan stok piawai, larutan kerja piawai dengan kepekatan 2, 4, 6, 8, dan 10 µg mL^{-1} disediakan. Lengkung kalibrasi diplot mengikut kawasan puncak (peak area) melawan kepekekatan (concentration).

2.2.3 Method of Sample Extraction

The extraction method was based on the QuPPe (Quick Polar Pesticides Method) methodology developed in the European Union (EU) for fruits and vegetables and used water: methanol (50:50) containing formic acid as the final extraction solvent. A 5 g of sample was weighed into a 50 mL centrifuge tube. About 10 mL of deionized water and 100 µL of an internal standard solution (20 µg/mL of each analyte in water) were added. The samples were then left to stand for 30 minutes to two hours. After that, 10 mL of methanol containing 1% v/v formic acid was added. The samples were mixed for 15 minutes on a laboratory shaker and centrifuged.

2.2.3 Kaedah pengekstrakan sampel

Kaedah pengekstrakan ini berdasarkan metodologi QuPPe (Quick Polar Pesticides Method) yang dihasilakan di Kesatuan Eropah (EU) untuk buah-buahan dan sayur-sayuran, dan menggunakan campuran air: metanol (50:50) yang mengandungi asid formik sebagai pelarut pengekstrakan akhir. Sebanyak 5 g sampel ditimbang ke dalam 50 mL tiub emparan (centrifuge tube). Lebih kurang 10 mL air suling dan 100 µL larutan piawai dalaman (20 µg/mL setiap bahan terlarut dalam air) ditambahkan. Sampel-sampel tersebut kemudiannya dibiarkan selama 30 minit sehingga dua jam. Selepas itu, 10 mL metanol yang mengandungi 1% i/i asid formik ditambahkan. Sampel-sampel dikacau selama 15 minit menggunakan penggoncang makmal dan diempar.

2.2.4 LC/MS/MS Parameter

The HPLC column used for this analysis was the polymer-based apHera™ NH_2 column (15 cm x 4.6 mm I.D., 5 µm) which provided stable and robust LC separations from pH 2 to 13. The mobile phase gradient used: (A) water; (B) 20 mM ammonium carbonate at pH 9, and (C) methanol: water (50: 50). This mobile phase ensured the proper ionization of glyphosate, which has a phosphate group in its structure, with detection under negative ESI conditions with multiple transitions. In addition, ammonium carbonate buffer is volatile and is fully compatible with LC/MS instrumentation. The gradient profile was achieved at a flow rate of 0.5 mL/min and initiated with an equilibration phase of 100 % A for 2 min, to 90% B, 19% C in 0.1 min, held until 10 min, to 100% A in 0.1 min and held for 5 min. The column was heated constantly at 35 °C throughout the analysis.

Table 1: Types of samples and their coding.

No	No Rujukan JBK	Type of sample	Coding
1	JBK/B/08/22 (104)	corn	1C
2	JBK/B/09/22 (106)	corn	2C
3	JBK/B/09/22 (115)	corn	3C
4	JBK/B/10/22 (117)	corn	4C
5	JBK/B/10/22 (118)	corn	5C
6	JBK/B/10/22 (122)	corn	6C
7	JBK/B/10/22 (127)	corn	7C

8	JBK/B/10/22 (136)	corn	8C
9	JBK/B/10/22 (137)	corn	9C
10	JBK/B/10/22 (139)	corn	10C
11	JBK/B/10/22 (140)	corn	11C
12	JBK/B/10/22 (141)	corn	12C
13	JBK/B/10/22 (142)	corn	13C
14	JBK/B/10/22 (146)	corn	15C
15	JBK/B/10/22 (147)	corn	16C
16	JBK/B/10/22 (149)	corn	17C
17	JBK/B/10/22 (144)	corn	144
18	JBK/B/10/22 (148)	corn	148
19	UN-18C	corn	18C
20	JBK/B/09/22 (107)	soybean	1S
21	JBK/B/09/22 (108)	soybean	2S
22	JBK/B/09/22 (109)	soybean	3S
23	JBK/B/09/22 (111)	soybean	4S
24	JBK/B/10/22 (123)	soybean	5S
25	JBK/B/10/22 (126)	soybean	6S
26	JBK/B/10/22 (131)	soybean	7S
27	JBK/B/10/22 (138)	soybean	8S
28	JBK/B/10/22 (151)	soybean	10S
29	YMMU1076380	soybean	OCS
30	JBK/B/12/22 (179)	soybean	1A
31	JBK/B/12/22 (180)	corn	2A
32	JBK/B/10/22 (193)	corn	3A
33	JBK/B/10/22 (191)	wheat	4A
34	JBK/B/10/22 (192)	wheat	5A
35	JBK/B/10/22 (190)	wheat	6A
36	JBK/B/12/22 (188)	corn	7A
37	JBK/B/10/22 (186)	corn	8A
38	JBK/B/10/22 (187)	rice	9A
39	JBK/B/12/22 (189)	corn	10A
40	JBK/B/12/22 (181)	corn	11A
41	JBK/B/12/22 (183)	corn	12A
42	JBK/B/10/22 (184)	corn	13A
43	JBK/B/10/22 (145)	corn	14A
44	JBK/B/10/22 (143)	soybean	15A
45	JBK/B/10/22 (164)	corn	16A
46	JBK/B/10/22 (185)	corn	17A
47	JBK/B/10/22 (166)	soybean	18A
48	JBK/B/10/22 (169)	corn	19A
49	JBK/B/10/22 (170)	soybean	20A

2.2.4 Parameter LC/MS/MS

*Kolum HPLC yang digunakan untuk analisis ini adalah kolum apHera™ NH2 berasaskan polimer (15 cm x 4.6 mm I.D., 5 µm) yang menyediakan pemisahan LC yang stabil dan kukuh dari pH 2 hingga 13. Gradien fasa bergerak (mobile phase) yang digunakan: (A) air; (B) 20 mM ammonium karbonat pada pH 9, dan (C) metanol: air (50:50). Fasa bergerak (mobile phase) ini memastikan ionisasi yang betul bagi glifosat, yang mempunyai kumpulan fosfat dalam strukturnya, dengan pengesanan di bawah keadaan ESI negatif dengan transisi berganda. Tambahan itu, penampan (buffer) ammonium karbonat mudah meruap dan mengikut ketepatan instrumen LC/MS. Profil gradien dicapai pada kadar aliran 0.5 mL/min dan dimulakan dengan fasa keseimbangan (*equilibration phase) *100 % A selama 2 minit, ke 90% B, 19% C dalam masa 0.1 minit, dikekalkan hingga 10 minit, ke 100% A dalam masa 0.1 minit dan dikekalkan selama 5 minit. Kolum dipanaskan secara seragam pada suhu 35 °C sepanjang analisis.*

Jadual 1: Jenis-jenis sampel dan kodnya

No	*No Rujukan JBK*	*Jenis sampel*	*Kod*
1	*JBK/B/08/22 (104)*	*jagung*	*1C*
2	*JBK/B/09/22 (106)*	*jagung*	*2C*
3	*JBK/B/09/22 (115)*	*jagung*	*3C*
4	*JBK/B/10/22 (117)*	*jagung*	*4C*
5	*JBK/B/10/22 (118)*	*jagung*	*5C*
6	*JBK/B/10/22 (122)*	*jagung*	*6C*
7	*JBK/B/10/22 (127)*	*jagung*	*7C*
8	*JBK/B/10/22 (136)*	*jagung*	*8C*
9	*JBK/B/10/22 (137)*	*jagung*	*9C*
10	*JBK/B/10/22 (139)*	*jagung*	*10C*
11	*JBK/B/10/22 (140)*	*jagung*	*11C*
12	*JBK/B/10/22 (141)*	*jagung*	*12C*
13	*JBK/B/10/22 (142)*	*jagung*	*13C*
14	*JBK/B/10/22 (146)*	*jagung*	*15C*
15	*JBK/B/10/22 (147)*	*jagung*	*16C*
16	*JBK/B/10/22 (149)*	*jagung*	*17C*
17	*JBK/B/10/22 (144)*	*jagung*	*144*
18	*JBK/B/10/22 (148)*	*jagung*	*148*
19	*UN-18C*	*jagung*	*18C*
20	*JBK/B/09/22 (107)*	*kacang soya*	*1S*
21	*JBK/B/09/22 (108)*	*kacang soya*	*2S*
22	*JBK/B/09/22 (109)*	*kacang soya*	*3S*
23	*JBK/B/09/22 (111)*	*kacang soya*	*4S*
24	*JBK/B/10/22 (123)*	*kacang soya*	*5S*
25	*JBK/B/10/22 (126)*	*kacang soya*	*6S*
26	*JBK/B/10/22 (131)*	*kacang soya*	*7S*
27	*JBK/B/10/22 (138)*	*kacang soya*	*8S*
28	*JBK/B/10/22 (151)*	*kacang soya*	*10S*

29	*YMMU1076380*	*kacang soya*	*OCS*
30	*JBK/B/12/22 (179)*	*kacang soya*	*1A*
31	*JBK/B/12/22 (180)*	*jagung*	*2A*
32	*JBK/B/10/22 (193)*	*jagung*	*3A*
33	*JBK/B/10/22 (191)*	*gandum*	*4A*
34	*JBK/B/10/22 (192)*	*gandum*	*5A*
35	*JBK/B/10/22 (190)*	*gandum*	*6A*
36	*JBK/B/12/22 (188)*	*jagung*	*7A*
37	*JBK/B/10/22 (186)*	*jagung*	*8A*
38	*JBK/B/10/22 (187)*	*beras*	*9A*
39	*JBK/B/12/22 (189)*	*jagung*	*10A*
40	*JBK/B/12/22 (181)*	*jagung*	*11A*
41	*JBK/B/12/22 (183)*	*jagung*	*12A*
42	*JBK/B/10/22 (184)*	*jagung*	*13A*
43	*JBK/B/10/22 (145)*	*jagung*	*14A*
44	*JBK/B/10/22 (143)*	*kacang soya*	*15A*
45	*JBK/B/10/22 (164)*	*jagung*	*16A*
46	*JBK/B/10/22 (185)*	*jagung*	*17A*
47	*JBK/B/10/22 (166)*	*kacang soya*	*18A*
48	*JBK/B/10/22 (169)*	*jagung*	*19A*
49	*JBK/B/10/22 (170)*	*kacang soya*	*20A*

3. FINDINGS / *HASIL KAJIAN*

3.1 Determination of Paraquat Residue using UHPLC

Figure 1 and Figure 2 show the chromatograms and calibration curve of paraquat standards plotted according to peak area versus concentration, respectively. The R^2 is 0.9985 which is considered as passed.

3.1 Penentuan Sisa Paraquat menggunakan UHPLC

Rajah 1 dan Rajah 2 masing-masing menunjukkan kromatogram dan lengkung kalibrasi piawai paraquat yang diplot mengikut kawasan puncak (peak area) melawan kepekatan (concentration). Nilai R^2 *adalah 0.9985 yang dianggap lulus.*

3.1.1 Chromatograms and calibration curve of paraquat standard:

3.1.1 Kromatogram dan lengkung kalibrasi piawai paraquat:

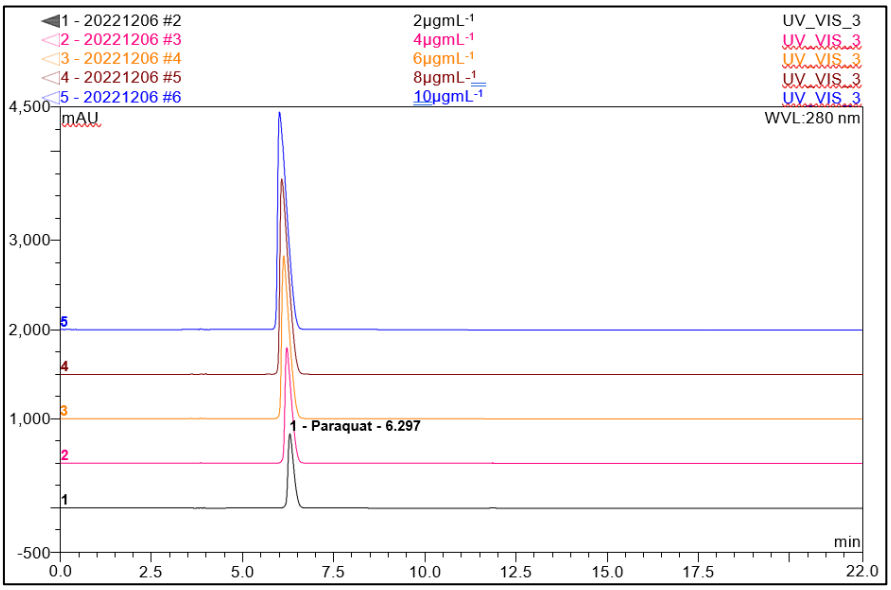

Figure 1: Chromatograms of paraquat standards at 2, 4, 6, 8, and 10 g mL^{-1}.

Rajah 1: Kromatogram piawai paraquat pada 2, 4, 6, 8, and 10 g mL^{-1}.

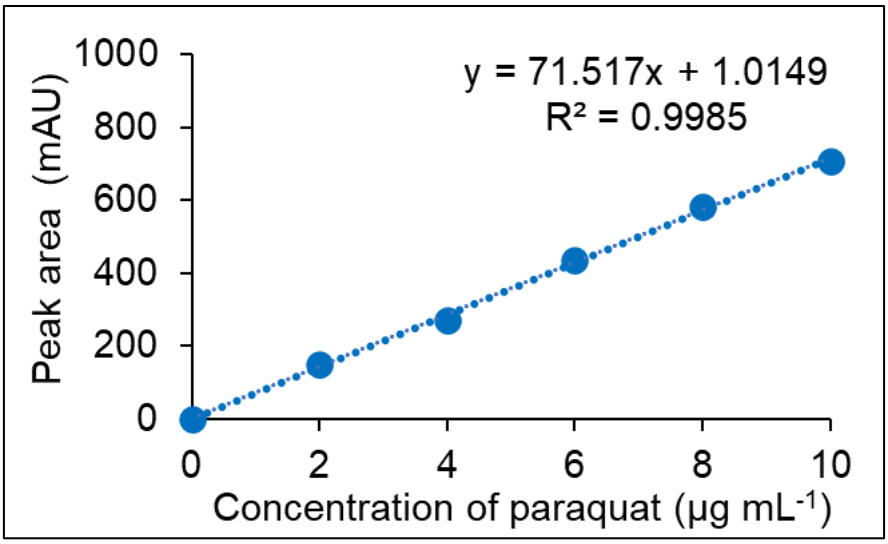

Figure 2: Calibration curve of paraquat standards at 2, 4, 6, 8, and 10 g mL^{-1}.

Rajah 2: Lengkung kalibrasi piawai paraquat pada 2, 4, 6, 8, dan 10 g mL-1.

Meanwhile, Table 2&3 show the peak area and amount of paraquat residue detected in the crop samples.

Sementara itu, Jadual 2 dan 3 menunjukkan kawasan puncak (peak area) dan jumlah sisa paraquat yang dikesan dalam sampel tanaman.

Table 2: Peak area and amount of paraquat residue in crop samples (Batch 1).
Jadual 2: Kawasan puncak dan jumlah sisa paraquat dalam sampel tanaman (Batch 1).

Sample No.	Sample Name	Ret.Time min Paraquat UV_VIS_3	Area mAU*min Paraquat UV_VIS_3	Height mAU Paraquat UV_VIS_3	Amount ppm Paraquat UV_VIS_3	Type Paraquat UV_VIS_3	Plates (EP) Paraquat UV_VIS_3
1	2 ugmL^{-1} Para	6.297	151.7288	832.13	2.1175	BMB	7736
2	4 ugmL^{-1} Para	6.213	270.5574	1298.45	3.7758	BMB	5663
3	6 ugmL^{-1} Para	6.127	436.7135	1826.93	6.0946	BMB	4114
4	8 ugmL^{-1} Para	6.077	583.8956	2190.94	8.1486	BMB	3200
5	10 ugmL^{-1} Para	6.013	708.7089	2440.96	9.8905	BMB	2598
6	1C	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
7	2C	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
8	3C	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
9	4C	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
10	5C	6.187	0.1866	0.50	0.0026	BMB	1398
11	6C	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
12	7C	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
13	8C	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
14	9C	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
15	10C	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
16	11C	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
17	12C	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
18	13C	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
19	16C	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
20	17C	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
21	18C	6.397	0.0166	0.09	0.0002	BMB	6002
22	144	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
23	148	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
24	1S	6.340	0.3577	1.12	0.0050	BMB	2163
25	2S	6.147	0.6803	1.28	0.0095	BMB	599
26	3S	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
27	4S	6.220	0.6201	1.82	0.0087	BMB	1780
28	5S	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
29	6S	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
30	7S	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
31	8S	6.187	0.3651	0.93	0.0051	BMB	1385
32	10S	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
33	OSC	6.273	36.0941	87.68	0.5037	BMB	1339
34	15C	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	Average:	**6.206**	**182.494**	**723.568**	**2.547**		**3165**
	Rel.Std.Dev:	**1.763 %**	**141.336 %**	**133.081 %**	**141.336 %**		**71.000 %**

Table 3: Peak area and amount of paraquat residue in crop samples (Batch 2).
Jadual 3: Kawasan puncak dan jumlah sisa paraquat dalam sampel tanaman (Batch 2).

Sample No.	Sample Name	Ret.Time min Paraquat UV_VIS_3	Area mAU*min Paraquat UV_VIS_3	Height mAU Paraquat UV_VIS_3	Amount ppm Paraquat UV_VIS_3	Type Paraquat UV_VIS_3	Plates (EP) Paraquat UV_VIS_3
1	2 µgmL^{-1} Para	6.297	153.7589	834.47	2.1351	BMB	7675
2	4 µgmL^{-1} Para	6.213	272.5594	1300.27	3.7848	MB	5644
3	6 µgmL^{-1} Para	6.127	438.8391	1828.54	6.0937	MB	4108
4	8 µgmL^{-1} Para	6.077	586.8874	2193.06	8.1495	MB	3192
5	10 µgmL^{-1} Para	6.013	711.7441	2443.60	9.8833	MB	2595
6	19A	6.780	0.0003	0.01	0.0000	Ru	n.a.
7	3A	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
8	5A	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
9	20A	5.820	0.4852	2.52	0.0067	BMB	5644
10	18A	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
11	2A	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
12	1A	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
13	17A	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
14	4A	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
15	10A	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
16	11A	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
17	9A	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
18	8A	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
19	7A	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
20	16A	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
21	13A	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
22	15A	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
23	12A	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
24	6A	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
25	14A	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	Average:	**6.190**	**309.182**	**1228.926**	**4.293**		**4810**
	Rel.Std.Dev:	**4.869 %**	**90.751 %**	**80.961 %**	**90.751 %**		**39.026 %**

3.1.2 Discussions

High-Performance liquid chromatography with a UV-Vis detector was used for the detection and quantification of paraquat residue in each crop sample. The SeQuant ZIC®-HILIC column was equilibrated with acetonitrile and water containing 0.5% of phosphoric acid as mobile phases. From the standard chromatogram, paraquat was detected at 280nm with regression correlation (r^2) of 0.998. Paraquat peaks that emerged at 6.3 ± 0.2 min were collected. Table 2&3 showed the amount of paraquat residues in the various samples of corn, soybean, wheat and rice from different countries. From the results, the 2 corn samples namely, 5C (JBK/B/10/22 (118) and 18C (UN-18C) showed the presence of paraquat residue of 0.0026 and 0.0002 µg ml^{-1}, respectively (Table 2-Batch 1). While 5 soybean samples, namely 1S (JBK/B/09/22 (107), 2S (JBK/B/09/22 (108), 4S (JBK/B/09/22 (111), 8S (JBK/B/10/22 (138), and OSC (YMMU1076380) showed the presence of paraquat residues of 0.0050, 0.0095, 0.0087, 0.0051, and 0.5037 µg ml^{-1}, respectively (Table 2-Batch 1). In addition, only 1 soybean sample, namely 20A (JBK/B/10/22 (170) showed the presence of paraquat residues of 0.0067 µg ml^{-1} (Table 3-Batch 2). Overall, the level of paraquat in these samples is very low compared to its ADI and MRL.

3.1.2 Perbincangan

Kromatografi cecair prestasi tinggi dengan pengesan UV-Vis digunakan untuk pengesanan dan kuantifikasi sisa paraquat dalam setiap sampel tanaman. Kolum SeQuant ZIC®-HILIC diselaraskan dengan acetonitril dan air yang mengandungi 0.5% asid fosforik sebagai fasa bergerak (mobile phase). Daripada kromatogram standard, paraquat dikesan pada 280nm dengan korelasi regresi (r^2) sebanyak 0.998. Puncak paraquat yang muncul pada masa 6.3 ± 0.2 minit telah dikumpulkan. Jadual 2 dan 3 menunjukkan jumlah sisa paraquat dalam pelbagai sampel jagung, kacang soya, gandum, dan beras dari negara-negara yang berbeza. Hasilnya, 2 sampel jagung iaitu 5C (JBK/B/10/22 (118)) dan 18C (UN-18C) menunjukkan kehadiran sisa paraquat masing-masing sebanyak 0.0026 dan 0.0002 µg ml^{-1} (Jadual 2-Batch 1). Manakala 5 sampel kacang soya iaitu 1S (JBK/B/09/22 (107)), 2S (JBK/B/09/22 (108)), 4S (JBK/B/09/22 (111)), 8S (JBK/B/10/22 (138)), dan OSC (YMMU1076380) menunjukkan kehadiran sisa paraquat masing-masing sebanyak 0.0050, 0.0095, 0.0087, 0.0051, dan 0.5037 µg ml^{-1} (Jadual 2-Batch 1). Selain itu, hanya 1 sampel kacang soya iaitu 20A (JBK/B/10/22 (170)) menunjukkan kehadiran sisa paraquat sebanyak 0.0067 µg ml^{-1} (Jadual 3-Batch 2). Secara keseluruhan, tahap paraquat dalam sampel-sampel ini sangat rendah berbanding dengan ADI dan MRLnya.

3.2 Determination of Glyphosate and Glufosinate Residue using LC-QQQ-MS
3.2 Penentuan Sisa Glifosat dan Glufosinat menggunakan LC-QQQ-MS

3.2.1 Glyphosate standard and calibration curve:
3.2.1 Piawai Glifosat dan lengkung kalibrasi:

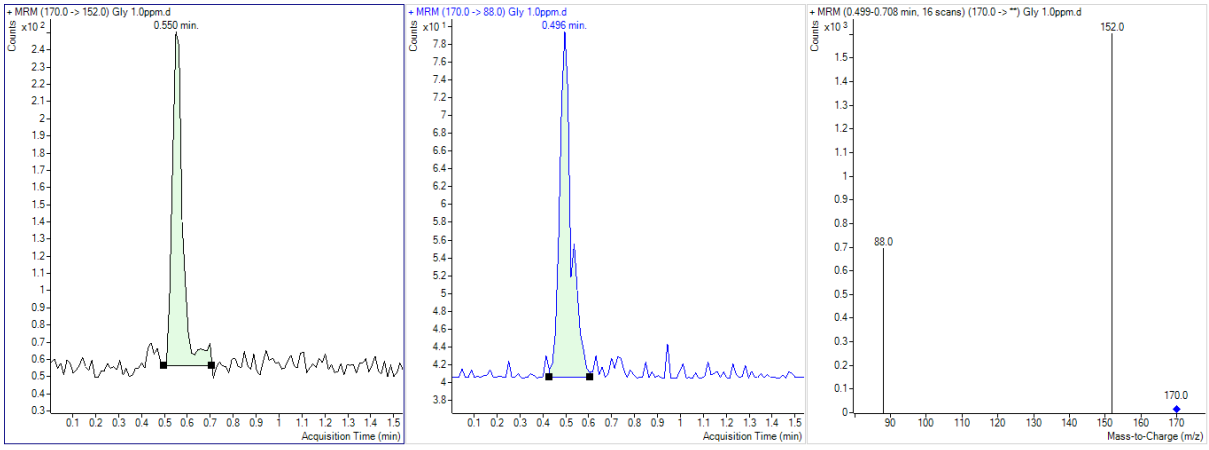

Figure 3: Chromatogram and fragmentation pattern of glyphosate standard at 1.0 µgmL^{-1}.
Rajah 3: Kromatogram dan corak penguraian piawai glifosat pada 1.0 µgmL^{-1}.

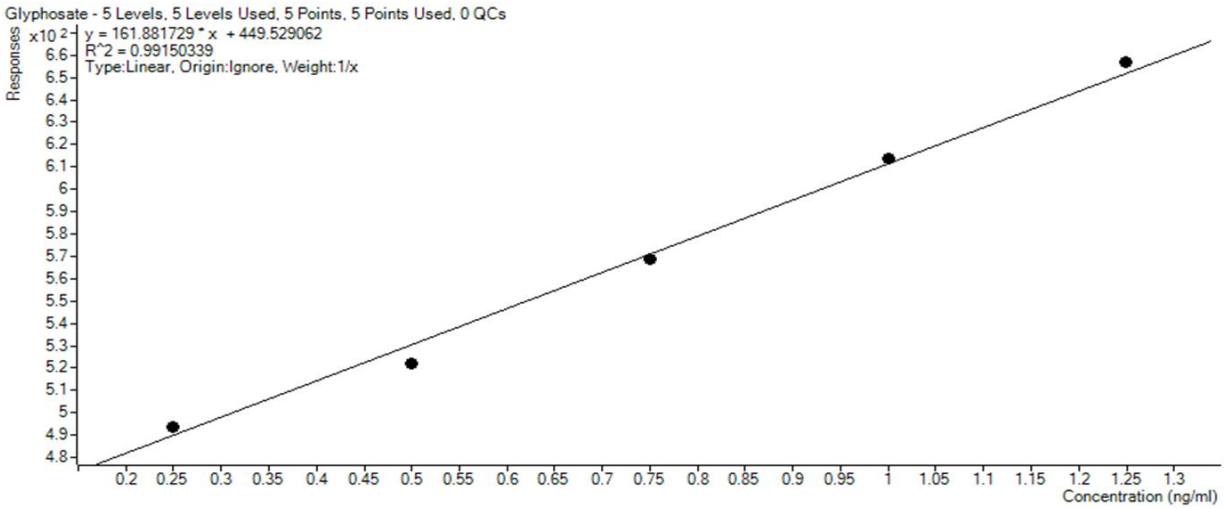

Figure 4: Calibration curve of glyphosate standards at 0.25, 0.5, 0.75, 1.0, and 1.25 µg mL^{-1}.
Rajah 4: Lengkung kalibrasi piawai glifosat pada 0.25, 0.5, 0.75, 1.0, dan 1.25 µgmL^{-1}.

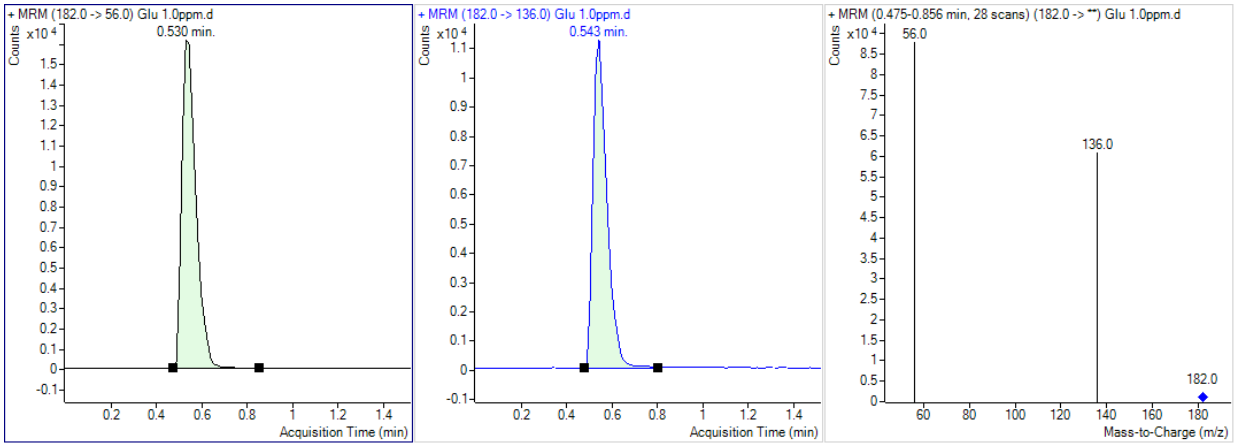

Figure 5: Chromatogram and fragmentation pattern of glufosinate standard at 1.0 µgmL^{-1}.
Rajah 5: Kromatogram dan corak penguraian piawai glufosinat pada 1.0 µgmL^{-1}.

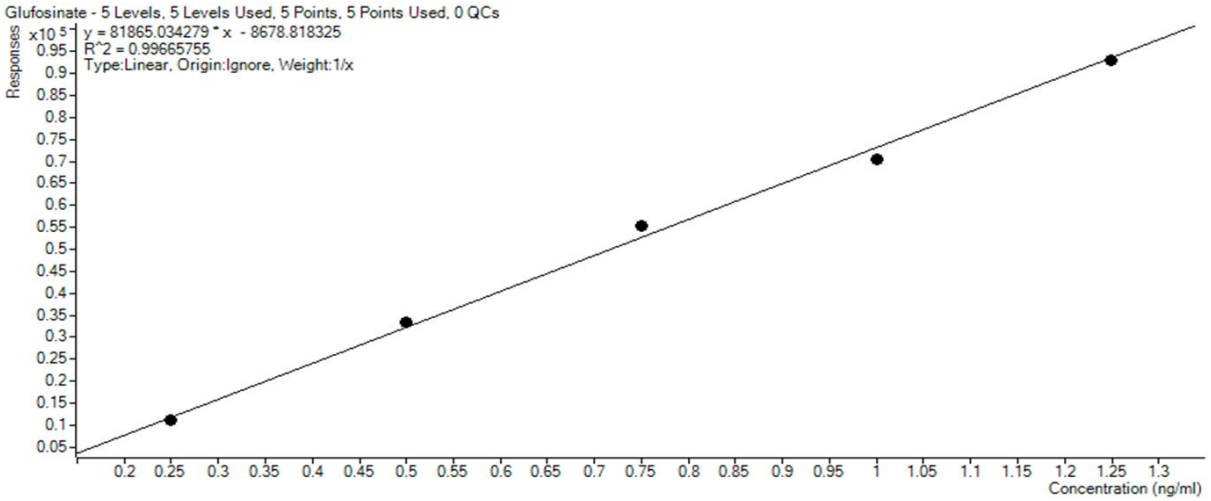

Figure 6: Calibration curve of glufosinate standards at 0.25, 0.5, 0.75, 1.0, and 1.25 µg mL^{-1}.
Rajah 6: Lengkung kalibrasi piawai glufosinat pada 0.25, 0.5, 0.75, 1.0, dan 1.25 µg mL^{-1}.

Table 4: Amount of glyphosate and glufosinate residue in crop samples.
Jadual 4: Jumlah sisa glifosat dan glufosinat dalam sampel tanaman.

No	Sample code / *Kod sampel*	**Pesticides residue/ *Sisa racun perosak* (mg/kg)** *Test method: In-house QuEChERS GCMSMS* Glyphosate, Glufosinate
1	1C	N.D.
2	2C	N.D.
3	3C	N.D.
4	20A	N.D.
5	11A	N.D.
6	18A	N.D.
7	4C	N.D.
8	9A	N.D.
9	17A	N.D.
10	1A	N.D.
11	5C	N.D.
12	8A	N.D.
13	6C	N.D.
14	3A	N.D.
15	4A	N.D.
16	7C	N.D.
17	15A	N.D.
18	8C	N.D.
19	9C	N.D.
20	13A	N.D.
21	10C	N.D.
22	11C	N.D.
23	12C	N.D.
24	2A	N.D.
25	13C	N.D.
26	OCS	N.D.
27	6A	N.D.
28	16A	N.D.
29	7A	N.D.
30	15C	N.D.
31	19A	N.D.
32	5A	N.D.
33	16C	N.D.
34	17C	N.D.
35	144	N.D.
36	148	N.D.
37	18C	N.D.
38	10A	N.D.
39	12A	N.D.
40	1S	N.D.
41	2S	N.D.
42	3S	N.D.
43	4S	N.D.
44	14A	N.D.
45	5S	N.D.
46	6S	N.D.
47	7S	N.D.
48	8S	N.D.
49	10S	N.D.

3.2.2 Discussions

Sharp chromatogram standards of glyphosate and glufosinate were monitored in Figures 3 and 5. Meanwhile, Figures 4 and 6 displayed regression relation (r^2) values of 0.992 and 0.997 for glyphosate and glufosinate, respectively; which is considered as passed. Glyphosate peak emerged at 0.55 min with fragmentation at 88, 152, and 170 m/z while glufosinate peak emerged at 0.53 min with fragmentation at 56, 136, and 182 m/z. With reference to Table 4, it can be concluded that there was an absence of glyphosate and glufosinate in all samples.

3.2.2 Perbincangan

Kromatogram tepat piawai glifosat dan glufosinat dapat diperhatikan dalam Rajah 3 dan 5. Sementara itu, Rajah 4 dan 6 menunjukkan nilai hubungan regresi (r^2) masing-masing sebanyak 0.992 dan 0.997 untuk glifosat dan glufosinat; yang dianggap lulus. Puncak glifosat muncul pada 0.55 minit dengan penguraian pada 88, 152, dan 170 m/z manakala puncak glufosinat muncul pada 0.53 minit dengan penguraian pada 56, 136, dan 182 m/z. Merujuk kepada Jadual 4, boleh disimpulkan bahawa tiada kehadiran glifosat dan glufosinat dalam semua sampel.

4. Overall remarks/*Catatan keseluruhan*

All samples sent by the Department of Biosafety, Ministry of Natural Resources, Environment and Climate Change to UPM have pesticide residues (paraquat, glyphosate and glufosinate) that are lower than the maximum residue limit (MRL) specified by Codex. The sample number is recommended to be increased in the future so that it can represent the actual amount of GM crop commodities available worldwide so that the maximum residue limit of pesticides in the commodity can be set accurately.

Kesemua sampel yang telah dihantar oleh Jabatan Biokeselamatan, Kementerian Sumber Asli, Alam Sekitar dan Perubahan Iklim ke UPM mempunyai residu racun perosak (paraquat, glifosat dan glufosinat) yang lebih rendah dari had residu maksimum (MRL) yang ditentukan oleh Codex. Jumlah sampel disarankan supaya ditambah pada masa akan datang supaya ia dapat mewakilit jumlah sebenar komoditi tanaman GM yang terdapat di seluruh dunia supaya had residu maksimum racun perosak dalam komoditi tersebut dapat ditentukan dengan lebih tepat.

5. References/ *Rujukan*

1. Heydebreck, F. Monitoring of Paraquat in Soya Products Intended for Animal Feed. *International Journal of Food Contamination*. 2021; 8(4).
2. Shimelis O. I. LC/MS/MS Method for Determination of Glyphosate, AMPA, and Glufosinate in Cereals. *Reporter US Volume 34.4.*

6. PROJECT TEAM/ *KUMPULAN PROJEK*

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