INVESTIGATION OF IMIDAZOLINONE HERBICIDE TOWARDS WEEDY RICE (*Oryza sativa* L.) AND EFFECTS OF ITS RESIDUAL CONCENTRATION IN SOIL

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FACULTY OF SCIENCE
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KUALA LUMPUR

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THESIS SUBMITTED IN FULFILMENTOF THE REQUIREMENTSFOR THE DEGREE OF DOCTOR OF PHILOSOPHY

INSTITUTE OF BIOLOGICAL SCIENCES
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ABSTRACT

INVESTIGATION OF IMIDAZOLINONE HERBICIDE TOWARDS WEEDY RICE (*Oryza sativa* L.) AND EFFECTS OF ITS RESIDUAL CONCENTRATION IN SOIL

Clearfield® Production System (CPS) for rice was introduced in Malaysia in 2010 as the current best solution to combat weedy rice (*Oryza sativa*) especially in direct-seeding system using imidazolinone-based herbicide (commercial name: OnDuty®). Although CPS has widely implemented in many rice fields in Malaysia, some of the major concerns of CPS are the probability of weedy rice to become resistant to OnDuty® and herbicide residual effects to rice agro-ecosystems and other environments. Therefore, this study was aimed to (i) evaluate the resistant status of selected weedy rice biotypes to imidazolinone herbicide; (ii) determine association between weedy rice morphological characteristics with imidazolinone resistant; (iii) develop an HPLC method to extract imidazolinone herbicide from soil; and (iv) assess imidazolinone herbicide residues in CPS rice agro-ecosystem. Fresh weedy rice seeds from 17 biotypes were collected from IADA Barat Laut Selangor rice granaries. The resistant status of these biotypes with two control cultivars (i.e. MR220 [imidazolinone susceptable] and MR220-CL [imidazolinone tolerant]) were assessed by (i) seed bioassay and (ii) plant growth response methods with different concentrations of imidazolinone herbicide. Resistant levels based on injuries and major phenotypic traits of weedy rice were recorded. Germination test on seeds showed that all weedy rice biotypes have more than 60% germination rate on all herbicide concentrations. However, the viability of the un-germinated seedlings was relatively low indicating that this herbicide is effective as a pre-germination herbicide. Only 24% of weedy rice biotypes showed susceptibility to one-dose OnDuty® application while the rest displayed various resistant levels. The number of resistant weedy rice reduced to only

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six biotypes after double-dose application with more than 80% survival rate. This indicates that certain biotypes of weedy rice in Malaysia with wide phenotypic variation has developed resistant to imidazolinone herbicide in CPS rice fields. Samples were also taken from three Clearfield® rice fields as IMI- herbicides which have been used for six years at three different locations in Sawah Sempadan,Tanjung Karang, Malaysia. High performance liquid chromatography (HPLC) with UV detection and solid-phase extraction (SPE) cartridges, with two mobile phases was used to evaluate soil samples collected from CPS rice fields to assess imidazolinone herbicide residues (imazapic and imazapyr) in the soil. Results revealed minor herbicide residues in the CPS soil except areas near the edge of the fields where values recorded were above the residual threshold for imidazolinone. The average percentage recovery for imazapyr and imazapic varied from 76-107% and 71-77%, with 0.1-5 µg/mL fortification level, respectively. In the extracted soil sample residues of imazapic and imazapyr were found to fall within 0.04-0.5µg/mL for imazapic and from 0.03-1.9 µg/mL for imazapyr, respectively. This study showed rapid evolutionary of weedy rice to develop resistant or adapt with environmental changes in rice agro-ecosystems. Various possibilities of weedy rice 'escape' from CPS were discussed including weedy rice genotypic/phenotypic variation, and farmer's attitude towards CPS. Despite low imidazolinone residues recorded in the CPS fields, the environmental impact of this system cannot be neglected, and further monitoring need to be done.

Key words: Weedy rice, imidazolinone, herbicide resistant, and herbicide residues.

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ABSTRAK

PENYELIDIKAN HERBISID IMIDAZOLINONE TERHADAP PADI ANGIN (*Oryza sativa L.*) DAN KESAN KEPEKATAN SISA HERBISID DALAM TANAH

Sistem Pengeluaran Clearfield® (CPS) bagi padi telah diperkenalkan di Malaysia pada tahun 2010 sebagai penyelesaian terbaik buat masa ini untuk memerangi padi angin (*Oryza sativa*), terutama dalam sistem tanaman secara tabur terus menggunakan herbisid berasaskan imidazolinon. Walaupun CPS telah dilaksanakan secara meluas di kebanyakan sawah padi di Malaysia, beberapa kebimbangan utama sistem ini adalah kebarangkalian padi angin menjadi rintang terhadap kesan OnDuty® dan kesan sisa herbisid kepada ekosistem pertanian padi dan persekitaran lain. Oleh itu, kajian ini bertujuan untuk (i) menilai status kerintangan biotip padi angin yang dipilih untuk racun rumpai imidazolinone (OnDuty®); (ii) menentukan perkaitan antara ciri-ciri morfologi padi angin dengan kerintangan terhadap imidazolinon; (iii) membangunkan kaedah HPLC untuk mengekstrak sisa racun rumpai imidazolinone dari tanah; dan (iv) menilai sisa racun imidazolinone dalam eksosistem pertanian menggunakan CPS. Benih padi segar dari 17 biotip padi angin dikumpulkan dari IADA Barat Laut Selangor. Status kerintangan biotip ini dengan dua kultivar kawalan (iaitu MR220 [rentan imidazolinone] dan MR220-CL [rintang imidazolinone]) dinilai dari segi (i) bioasai benih dan (ii) tindak balas pertumbuhan dengan kepekatan racun rumpai imidazolinon yang berbeza. Tahap kerintangan adalah berdasarkan kecederaan dan sifat fenotip utama padi yang direkodkan. Ujian percambahan pada biji benih menunjukkan bahawa semua biotip padi angin mempunyai kadar percambahan lebih dari 60% pada semua kepekatan bancuhan racun rumpai yang digunakan. Walau bagaimanapun, kelangsungan hidup anak benih yang tidak bercambah agak rendah menunjukkan racun rumpai ini berkesan sebagai racun pra-percambahan. Hanya 24% daripada biotip padi angin menunjukkan kerentanan untuk satu-dos penggunaan racun OnDuty® manakala yang selebihnya menunjukkan pelbagai tahap daya rintang. Bilangan padi angin rintang adalah dikurangkan kepada hanya enam biotip selepas penggandaan dos diberikan dimana kadar kelangsungan hidup adalah melebihi 80%. Ini menunjukkan bahawa beberapa jenis biotip padi angin di Malaysia dengan variasi fenotip yang luas telah menjadi rintang terhadap racun imidazolinone di sawah CPS. Sampel juga diambil dari tiga sawah berbeza yang telah mengamalkan kaedah Clearfield® selama enam tahun di tiga lokasi berbeza di Sawah Sempadan, Tanjung Karang, Malaysia. Kromatografi cecair prestasi tinggi (HPLC) dengan pengesanan UV dan prosedur pengekstrakan fasa pepejal (SPE) bersama dua fasa mobil telah digunakan untuk menilai sampel tanah yang diambil sawah CPS bagi menentukan sisa herbisid imidazolinone (imazapic dan imazapyr) di dalam tanah. Keputusan mengesahkan terdapat sedikit sisa herbisid di tanah CPS kecuali di kawasan berhampiran pinggir bendang di mana nilai sisa racun yang direkodkan adalah melebihi paras ambang sisa imidazolinon yang selamat. Pemulihan peratusan purata untuk imazapyr dan imazapic berbeza-beza dari 76-107% dan 71-77%, dengan tahap kestabilan 0.1-5 µg / mL. Dalam sisa sampel tanah yang diekstrak daripada imazapic dan imazapyr didapati sisa racun berada dalam lingkungan 0.04-0.5 µg / mL untuk imazapic dan dari 0.03-1.9 µg / mL untuk imazapyr masing-masing. Kajian ini menunjukkan evolusi pesat padi angin untuk menjadi rintang atau menyesuaikan diri dengan perubahan persekitaran dalam ekosistem pertanian padi. Pelbagai kemungkinan bagi padi angin untuk terselamat dari sistem CPS telah dibincangkan termasuk variasi genotip / fenotip padi angin, dan sikap petani terhadap sistem CPS. Walaupun sisa imidazolinone yang rendah direkodkan dalam sawah CPS, impak alam sekitar sistem ini tidak boleh diabaikan, dan pemantauan lanjut perlu dilakukan.

Kata kunci: Padi angin, Imidazolinone, kerintangan herbisid, sisa herbisid.

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LIST OF SYMBOLS AND ABBREVIATIONS

ALS	:	Acetoacetate synthase
HPLC	:	High Performance Liquid Chromatography
IMI	:	Imidazolinone
MARDI	:	Malaysian Agricultural Research and Development Institute
CL1	:	MR 220 CL1
CL2	:	MR 220 CL 2
RSD	:	Relative Standard Deviation
RPLC	:	Reversed – phase liquid chromatography
S/N	:	Signal-to-Noise Ratio
WR	:	Weedy Rice

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CHAPTER 1: INTRODUCTION

1.1 Overview of Weedy Rice Infestation in the World

Weedy rice (*Oryza sativa*) is one of the most conspecific and serious weeds in rice cultivation. Generally, weedy rice is any spontaneously and strongly shattering rice that takes place where rice cultivars is grown (Xia et al., 2011). It is considered a dangerous problem in rice growing fields globally including Australia, Italy, South-east Asia, South, North America, and southern Europe (Kaloumenos et al., 2013; Mortimer, 2000; Sudianto et al., 2016). Recently, weedy rice infestations can lead to extreme yield losses as a result of competition to cultivated rice which can be from 5% to 100% reduced yield (Shivrain et al., 2009). Weedy rice can be highly competitive against cultivated rice and can cause severe yield losses (Chauhan, 2013a; Delouche & Labrada, 2007). A study in Thailand, found that grain yield decreases linearly for every percent of the weedy rice infestation (Maneechote et al., 2005). The duration of interference with cultivated rice, and weedy rice density also affect weedy rice competitiveness (Kwon et al., 1991). Rice yield can plummet to 50% in a rice field with 24 weedy rice plants per m^2 during the first 40 days and this can increase up to 75% in the case of season-long competition (McDonald, 1999). A significant effect also observed in the greenhouse experiment when the competition had duration longer than 70 days (Estorninos et al., 2005). Noldin (2000) estimated that only two red rice seeds per kg planted in a rice field free of red rice could produce 100kg red rice per ha within three seasons. The increase of weedy rice population to 40-50% could cause more than 50% of rice yield loss (Maneechote et al., 2005). Research reported that one weedy rice plant per m^2 can lead to yield losses of about 100 kg to 755kg /ha^{-1} in some types of rice varieties (Ottis et al., 2005). Weedy rice played as a strong competitor among rice species (Baki & Mispan, 2010; Sudianto et al., 2016). In addition, these competitions lead to reduce selling price in the market (Andres et al., 2014). Weedy rice was first observed in the US since mid-1800, 1960 in China and early 1990 in many other countries (Table 1.1). It is present almost in any fields where rice crop is cultivated, and is independent of the region (Terano et al., 2016).

To make it worse, weedy rice belongs the same species of cultivated rice (Goulart et al., 2012; Shafiee et al., 2013). At the same time, the high diversity of weedy rice morphologically and genetically are contributing to invasive weedy rice infestation and negative consequences in fields (Mispan & Baki, 2008). An example, in India, has about six agro-climatic zones where diverse rice seeds are cultivated. Therefore, weedy rice plants from these areas in most cases are different in their morphological traits (Rathore et al., 2016).

Table 1.1: Weedy rice reported in rice production countries.

Country	**Year**	**References**
The United States	1846	(Olsen et al., 2007)
Malaysia	1988	(Watanabe et al., 1996)
Sir Lanka	1990	(Somaratne et al., 2014)
Philippine	1990	(Chauhan & Johnson, 2010)
Vietnam	1994	(Chauhan & Johnson, 2010)
European countries	1970	(Tarditi &Verseci, 1993)
China	1960	(Gressel & Valverde, 2009)
Italy	1990	(Fogliatto et al., 2012)

Source: adapted from (Azman, 2017)

1.2 Overview of Weedy Rice Infestation in Malaysia

The weedy rice was firstly reported in Muda rice granaries in early 1988 (Sudianto et al., 2016; Wahab & Suhaimi, 1991), but it has become the more noxious problem in the rice fields by 2000s (Azmi et al., 2007). The infestation of weedy rice in Malaysia

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become significant mainly after the cultivation shift from traditional transplanting to direct-seeding rice and the cultivation of semi-dwarf rice (Baki & Shakirin, 2010; Sudianto et al., 2016; Mispan et al., 2019). The emergence and fast spread of weedy rice were also resulted from poor land preparation which increased the survival fate of weedy rice (Figure 1.1) in the seedbank (Azmi & Karim, 2008; Chauhan, 2013a; Mispan et al., 2019).

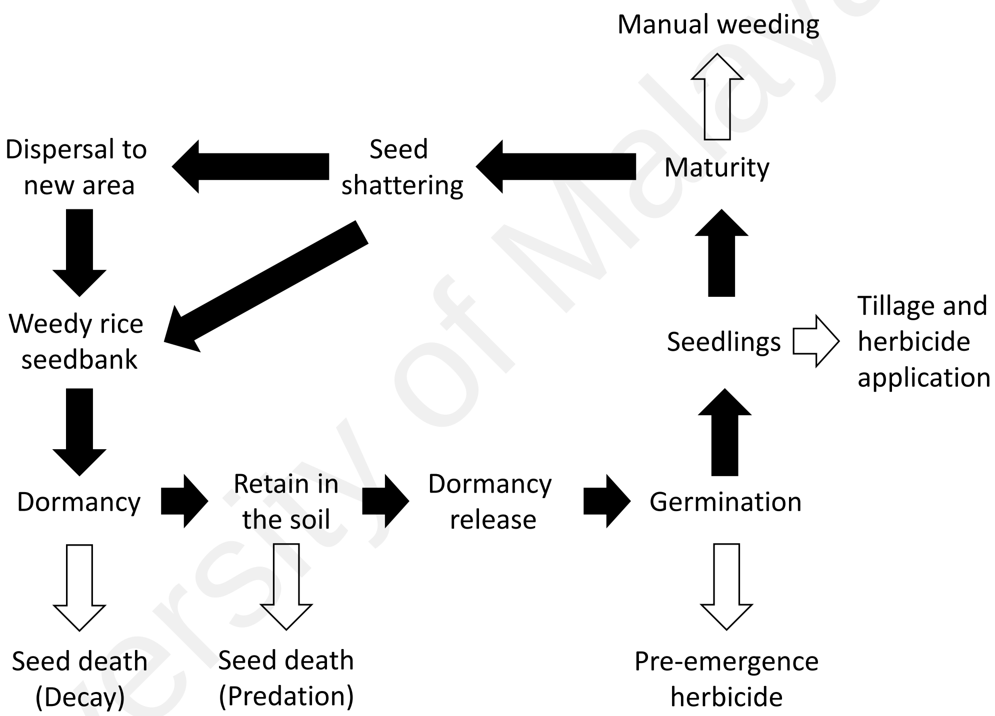

Figure 1.1: Weedy rice fate in the rice field. The weed survival cycle of weedy rice to escape for successive seasons is presented by black arrows. Weedy rice can be "withdrawn" from the field by natural and human activities as indicated with white arrows.

The weedy rice infestation in the country was fast spread to many rice granaries in Malaysia since the first observance including MADA, Kedah; Kerian-Sg. Manik, Perak; Ketara, Terengganu; and Seberang Perak, Perak (Baki et al., 2000). Later, weedy rice was recorded throughout Peninsular Malaysia in a majority of rice field regions (Azmi et al., 2005) with wide phenotypic variations (Azmi & Baki, 2003). In Tanjung Karang, Selangor and Besut, Terengganu, half of their granaries were attacked with weedy rice in the early 2000 (Azmi et al., 2000). While in Muda, it has been reported that in 2004, weedy rice was prevalent in all areas with the majority registered infestation level ranging from 0 to10%, 11 to 20% and more than 20%. (Azmi et al., 2005a). It was estimated that infestation of 5% of weedy rice in Malaysian rice granaries led to a reduction of yield production of 64,880 tons of rice (Baki, 2004).

The weedy rice infestation was not significant in 1995 but the infestation was skyrocketed in 1996 with more than 19,900 ha of rice farms were infested in Peninsular Malaysia. In 2001, it was reported that weedy rice was present in 82% of Muda farm blocks but reduced in 2002 with only 59% of the farm blocks having at least a 10% infestation rate. The infestation tremendously increased in 2005 where 91% of the farm blocks were infested with 88% of the farms having at least 10% infestation (Baki et al., 2000; Baki, 2006). Most weedy rice accessions in Malaysia have a variance to mean ratio and Lloyd's patchiness index less than 1, giving indication that the weedy rice has restricted distribution while some others showed a uniform distribution throughout the rice granaries in Peninsular Malaysia (Baki et al., 2000; Baki & Mispan, 2010).

1.3 Clearfield® Production System (CPS) of Rice in Malaysia

In the early 90's to middle 2000's, most farmers practiced hand-weeding in their direct-seeded farm by rouging the weedy rice (Azmi & Karim, 2008). Since weedy rice was generally taller in stature than cultivated rice, farmers can easily identify them and slashing the weedy rice panicles before harvest (Baki & Mispan, 2010). Selective weeding was effective in controlling weedy rice infestation during this time period especially in the Sekinchan area (Abdul Hamed, 1994). However, this method was found useless especially in a wide area (Azmi & Karim, 2008), leading in damage of

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cultivated rice during implementation. High labor costing also caused many farmers to abandon their rice fields (Mispan, 2015). To make it worse, the recent report on the emergence of new biotypes of weedy rice (NBWRs) which morphologically mimics commercial cultivated varieties (*i.e.* MR220 and MR219) especially in height, making weedy rice almost unrecognizable (Mispan & Baki, 2008) and caused hand-weeding impossible. Although the NBWRs infestation is still in the early stages, their distribution pattern was found to be like the previously weedy rice emergence in Malaysia (Baki & Mispan, 2010).

Herbicide-tolerant rice cultivar has been proposed to Malaysian farmers to be the current best solution to combat weedy rice especially in direct-seeding system (Azmi et al., 2012). Clearfield® Rice Production System (CPS) was introduced in Malaysia in 2010. An imidazolinone tolerant variety (IMI-TR) rice was developed by crossing United States IMI-TR Line No. 1770 with local cultivar, MR220, using conventional breeding technique (Azmi et al., 2012). Introduction of CPS as a pilot study in Seberang Perak rice granaries has become popular with other rice growing states (*i.e.* Selangor and Kedah) because of the success of this system to control many grasses weed species including weedy rice while boosting rice production (Sudianto et al., 2013). This system used Imidazolinone (IMI) herbicides (OnDuty®) which is a selective herbicide that inhibits the ALS enzyme and the branched chain of three amino acids: isoleucine, leucine, and valine. It stops protein synthesis, and eventually destroy any susceptible weeds including weedy rice (Sudianto et al., 2013).

1.4 Problem Statement

Rice industry in Malaysia faces serious challenges in managing weedy rice (*Oryza sativa* L.) since it was first observed in 1988. Unfortunately, there is no simple control

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method for weedy rice. Recommended practices in Malaysia adopted various integrated weedy rice management strategies mainly on land preparation and pre-harvest period controls. Multiple tillage, chemical applications via pre-emergence and pre-sowing herbicide, and manual weeding are the usual weedy rice control practices by a majority of Malaysian farmers. The conspecific nature of weedy rice with cultivated rice increased the difficulty to control the weed with several weedy rice biotypes have already mimic the local varieties. Introduction of Clearfield® Rice Production System (CPS) in 2010 has shifted the current weedy rice management strategies to an herbicide-tolerant crop approach.

However, up to date, the imidazolinone resistant status of weedy rice in Malaysia is limited despite various reports of the occurrence of IMI resistant weedy rice in other CPS implementing countries such as the United States (Burgos et al. 2008) and Italy (Scrabel et al., 2012; Rosas et al., 2014). Malaysia with tropic condition has high risks of gene flow and evolution of resistant weedy rice populations because of multiple cropping rice in a year and freezing temperature, which would reduce the density of volunteer rice, do not occur (Shivrain et al., 2008; Burgos et al., 2014).

The constant implementation of imidazolinone herbicide in the CPS fields in Malaysia since 2010 might leave a certain level of impact to the environment. However, there is still no solid reports on the status of weedy rice imidazolinone (IMI) herbicide residue level in CPS fields and the impact of herbicide residues to rice agro-ecosystem although CPS has already implemented in Malaysia for more than five years. Personal communications with local farmers in IADA Barat Laut Selangor rice granary indicates that the residue from CPS farms has already caused damages to their side farming (e.g.

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corn, tapioca, and banana), poultry and livestock. These stated challenges on CPS application need to be addressed and taken seriously by all related parties in Malaysia.

This study is aimed to address these problems related to the impact and effect of the implementation of Clearfield® Rice Production System (CPS) in Malaysia especially on the ecological and environmental perspectives.

1.5 Objectives of the Study

Investigation of the current status of the resistant level of weedy rice towards IMI herbicide in CPS fields in Malaysia and understanding the effects of its residues in rice field soils will provide an alternative insight towards better weedy rice management in the future. Therefore, this study was devoted:

i. To determine the status of weedy rice herbicide resistant to Imidazolinone (OnDuty®) herbicide in IADA Barat Laut Selangor rice granary.
ii. To evaluate the association of weedy rice phenotypic variations with the resistance to IMI- herbicide.
iii. To develop HPLC method for the extraction and clean-up of IMI herbicide (imazapyr and imazapic) from Clearfield® rice fields soil.
iv. To evaluate IMI herbicide residues in Clearfield® rice fields soil.

1.6 Research Flow Chart

To achieve the mentioned objectives, we trying to draw flow chart of the procedures and the steps as shown in Figure 1.2 is carried out.

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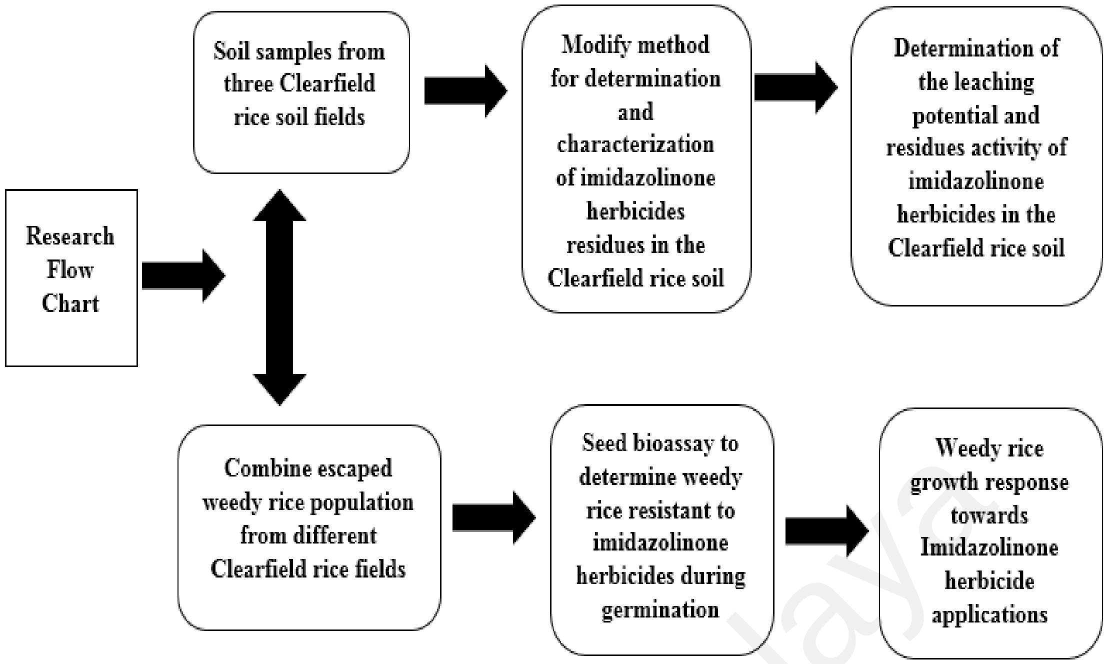

Figure 1.2: Flow chart of research methodology to study the resistant status of weedy rice and its impact to rice agro-ecosystem.

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CHAPTER 2: LITERATURE REVIEW

2.1 Rice Industry in the World

Rice (*Oryza sativa* L.), is considered one of the most crucial food crops, it gives about one-fifth of the calories consumed by people worldwide (Vaughan et al., 2003). It is the most crucial and largely grown in tropical and subtropical regions of the world. It is considered as the major source of income for more than 100 million households in Asia and Africa (Juraimi et al., 2013). It is regarded as a staple food for more than 50% the world's population (Rajamoorthy & Munusamy, 2015). It is cultivated in 154million ha globally, with annual yields of approximately 420 million/tones (m/t) with average productivity of 4 t/ha^{-1} (Parameswari & Srinivas, 2017). Asia countries are predicted to increase rice demand by more 30% in the year 2020 (Olofsdotter et al., 2000). However, there is a big variation and distribution in rice globally production leading some countries to consider the main producer for this valuable crop as shown in Table 2.1. Because of the significant of rice grains for supplying essential food, there is a high demand for higher grain yield per hectare. Approximately 90% of the world rice cultivated in Asia especially by six countries as China, India, includes about 80% of the total world production. China country started to shift up their production from hectare in the year 1970s, when started trade of hybrid rice cultivars (Bond & Walker, 2011). It has the largest producing area of rice followed by India, Indonesia, Bangladesh, Vietnam, and other countries.

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Table 2.1: World Rice production in 2016/2017.

Country	Rice production in metric/ton
China	144,850,000
India	106,500,000
Indonesia	36,600,000
Bangladesh	34,515,000
Vietnam	27,800,000
Thailand	18,600,000
Burma	12,500,000
Philippines	11,500,000
Brazil	7,820,000
Japan	7,790,000
United State	7,117,000
Pakistan	6,640,000
Cambodia	4,700,000
Egypt	4,554,000
Korea, south	4,200,000
Nepal	3,100,000

Source: (Agriculture, 2017).

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Rice production globally was about 472.40 million metric tonnes (mmt), and in the year 2016/2017 reach about 480.02 (mmt) (Chauhan, 2012; Rao et al., 2007). It is considered the main food intake for millions of people in the World and Asia specifics, such as China and Bangladesh. About 150 million hectares annually harvested which contributed to approximately 530 million tons of rice at an average yield of 3.5 t/ha^{-1}. This percentage can provide about 20% of the world's food demand and necessary calorie supply (Pacanoski & Glatkova, 2009). Rice has substituted the major total calories in the Asian countries as China about 30%, India 30%, Indonesia 30%, Bangladesh 50%, Vietnam 70% Philippines 60%, Malaysia 30% and South Korea 50% (Timmer, 2010). Studies showed that the total percentage of the cultivated rice area estimated at about more than 154 million ha, which about estimated 88% globally (Rao et al., 2007).

Rice is regarded as the main meal of many Malaysians (Siwar et al., 2014). A study showed that the annual need for rice per person is ~82.3 kg (Hakim et al., 2014; Yusoff & Panchakaran, 2015). Other studies revealed that Malaysians consume ~2.5 plates of rice-day (Rajamoorthy & Munusamy, 2015). Since a long time, despite continuous improvement of Malaysia government plan to shift up the self- sufficient in rice production, for example, from the year 2010, the percent of self-production fluctuate from 70 to 80% (Siwar et al., 2014). The rice production has increased with minimal percent, but not achieves the demand percent. Therefore, the government is put heavy effort to increase rice production yield with diverse means. Recently, many studies revealed that Malaysia will increase the demand for rice from the main producing countries because many suggestions to decline supply in the future. Therefore, the government should encourage the local rice cultivation, import new cultivar, and shift down it is dependency on imported paddy by decreasing export (Rajamoorthy & Munusamy, 2015).

2.2 Weeds in Rice Agro-Ecosystem in Malaysia

The popular weed flora in rice production includes sedges, grasses and broad leaf species. It differs based on the climatic circumstances and season conditions. Grassy weeds leading to yield losses as example are *Echinochloa colona, Eclipta prostrata, Echinochloa crus-galli*, *Leptochloa chinensis* Nees, *Oryza sativa* L. f. spontanea Roshev and *Ischaemum rugosum* (Raj & Syriac, 2017). Also, diverse invasive weed species in Malaysian fields were spread and cause a lot of yield losses. Weeds in rice ecosystem is considered a notorious weeds and dynamic in nature (Buhler, 2002). They were difficult to control especially in the early stage between crop rice and reveals a big variability of morphological traits because of the diversity of weeds, which exceeds more than 70 types (Song et al., 2014). Among these weeds, weedy rice is the most serious weed in Malaysian rice production landscape.

2.2.1 The Origin of Weedy Rice

One of the important weeds in the rice agro-ecosystem is weedy rice. The weedy rice origin is not completely understood, and the origin is still under several investigations. Weedy rice from different regions may have distinct evolutionary origins (Grimm et al., 2013). Weedy rice population includes a big group of wild rice types belonging to multiple species. The wild species *Oryza barthii* and *O. longistaminata* or weedy ecotypes from cultivated *O. glaberrima* are among the worst weeds in West Africa whereas *O. granulata*, *O. officinalis*, *O. rufipogon* and *O. nivara* are weedy or wild species in South-East Asian countries (Olofsdotter et al., 2000).

In most cases crossbreeding between cultivated and wild species facilitates weed evolution and development through time. The researchers have a debate about the origin of weedy rice. There are many scenarios of the origin, therefore, different hypotheses have been proposed to explain its origin including hybridization phenomenon between the types of cultivars, hybridization between cultivars with wild rice and through natural selection between weedy rice, or weedy rice appear from escaped domesticated rice seeds (Kane & Baack, 2007; Rao et al., 2007).

Since weedy rice was believed as a natural hybrid between crop rice and wild rice, it could be potential to utilize as a connection cycle to transmit alleles from gene pool site to rice plants (Perera et al., 2012). Recent SSR data have showed genetic contributions

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from wild rice populations (*O. rufipogon*) to weedy rice backgrounds in Malaysia and Thailand (Pusadee et al., 2013; Song et al., 2014). Also, in Thailand, the origin of weedy rice has been shown to originate from different hybridization between popular wild rice and cultivated rice (Wongtamee et al., 2017). In Vietnam, about 14% of the farmers and researchers in agriculture field show that most origins of weedy rice in the fields come from contamination rice seeds before the planting (Delouche & Labrada, 2007). A lot of studies on morphological and genetical characteristics proved that the origin of weedy rice varies according to the areas and sites. For example, using a special marker, it was revealed that the weedy rice of US is related to the aus and indica varieties, which both connected to Asia types (Gealy et al., 2009; Reagon et al., 2010). On the other hand, China weedy rice was related to distinct japonica cultivated rice (Cao et al., 2006).

2.2.2 Weedy Rice Distribution in Malaysia Granaries

In general, the introduction of direct seeding method and the usage of mechanical technology in modern rice cultivation in Malaysia has led to the high spread of weedy rice across rice fields in the country (Sudianto et al., 2016). Different phenotypic properties of weedy rice were present in many areas in Malaysia, such as the Kerian Sungai Manik, Kemubu, Muda, and Tanjung Karang rice granaries (Baki & Mispan, 2010), which lead to increase the infestation process and distribution. The percentage of weedy rice in the Muda region was 82% in 2001, which drops to 59% in 2002. Another study in 2005 reported that the weedy rice makes up 88% of farm blocks (Baki, 2006). From the first detected weedy rice in 1987 in Tanjung Karang rice fields, it began spreading to other areas, such as Muda granary, which was first observed in 1990, Besut area in 1995, and Seberang Perak in 2001 (Baki, 2006). Weedy rice can spread via multiple ways including contaminated machinery and animals with weedy rice seeds

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(Baki, 2004; Baki, 2006; Sadohara et al., 2000). Infestation increased due to neglected agriculture practices, which allows the seeds to move, especially in direct seeding system. The shattered weedy rice seeds could find its way into rice fields and exacerbate the infestation problem to new areas (Azmi & Karim, 2008; Chauhan et al., 2010; Chauhan, 2012; Delouche & Labrada, 2007) as presented in Figure 2.1.

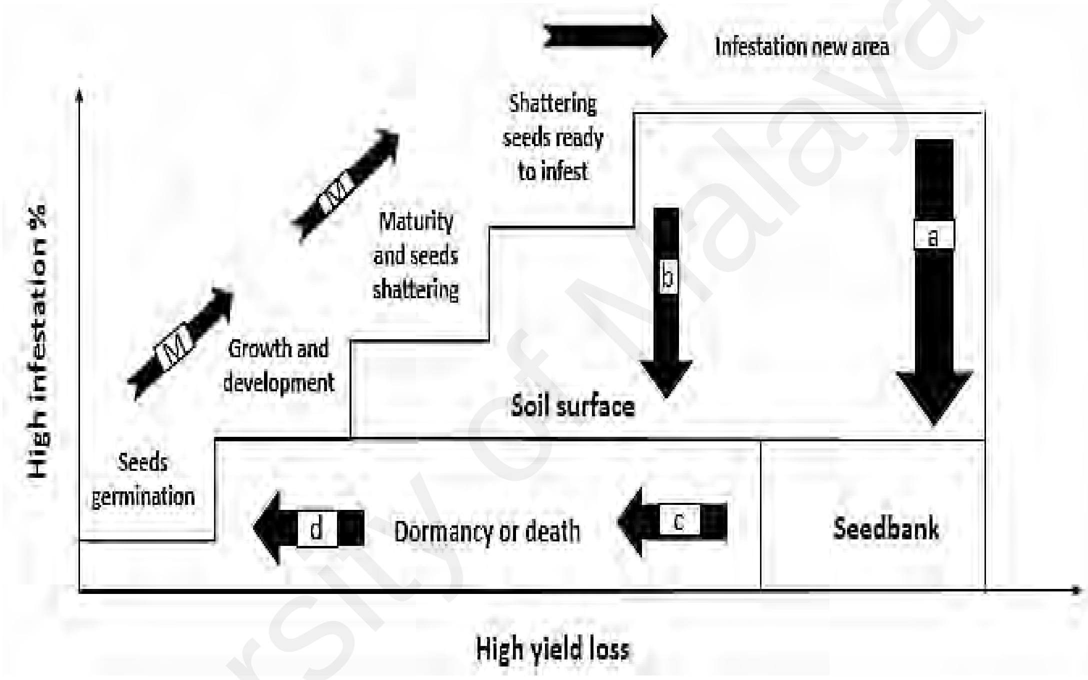

Figure 2.1: Fate of weedy rice and movement of weedy rice seeds in the seedbank. a) Seeds enter the seedbank after shattering due to deep tillage; b)Seeds remain on soil surface; c)Seeds may be go death or remain dormant; d)Seeds break dormancy and germinate again; and m)Seeds repeat the cycle continuously.

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2.2.3 Impact of Weedy Rice on Rice Production in Malaysia

Weedy rice has a direct effect on farmers income by reducing yield, interferes with rice cultivation, and negatively impact market value for the cultivated rice (Arrieta et al., 2005). The loss of yield is different from country to country and sometimes in the neighbouring fields. In general, in Asia, rice yield losses due to weedy rice infestation were recorded to be from 16 to 75% (Azmi et al., 2005). These yield losses could be reached from 5-100%, depending on the severity of the weeds, competition duration in the fields, types, the cultivar of rice, mitigation strategies, types of the herbicides used, and types of seeds (Gunawardana, 2008; Kharkwal & Shu, 2009; Shivrain et al., 2009). For example in the USA, the studies revealed that one weedy rice per m^2 is responsible for the loss between 100 to 755 kg/ha^{-1} (Ottis et al., 2003; Sudianto et al., 2013). Other study in the Caribbean revealed yield loss reach to 100% in some cultivation area (Mortimer et al., 2000; Rao et al., 2007). The percentage of weeds loses may reach to 50-60% in puddled transplanted rice and from 70-80% in direct-seeded rice (Dass et al., 2016).

The loss in the yield, different from country to country, for example, South Korea reach to 5-10% (Chen et al., 2004); while Vietnam reported an average loss about 17% (Mai et al., 2000). The elimination and mitigation process to finish the weedy rice is so complicated, due to the morphological and physiological similarity with the rice crop (Gealy et al., 2003). Therefore, weedy rice emerges along with rice crop plants lead to an adverse effect on crop yields. The multiple biotypes of weedy rice have affected more than fifty countries of Asia, Africa and Latin America (Chauhan, 2013). It is actually a global threat, leading in an annual cultivation loss of 9-10% globally (Hakim et al., 2013). In the granary Seberang Perak, the weedy rice caused huge reduction in the rice yield which estimated about RM 70000 ($17500) (Azmi et al., 2000). The yield of cultivation rice fields can be plummeted prominently, because of the seed shattering characteristic where the seeds cannot be collected (Chi et al., 2002).

Reduced yields were caused by different factors from country to country, and even area to area, as shown in Table 2.4. Also, the loss in the yield because of weedy rice in Malaysia reach 30-50% from the total rice yield (Watanabe et al., 2000). Azmi et al., (2005) revealed that yield decrease of about 1000 kg ha^{-1} was recorded in Malaysia at a

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space of approximately 35 weedy rice panicles per m^2. Researchers reported that one weedy rice plant in 1/m^2 can cause yield losses of 100 kg – 755kg /ha^{-1} in some types of rice cultivars. Also, percentage decreases in yield rely on the infestation quantity of weedy rice in rice crops. For example, when the percentage of weedy rice is (15-20) panicle/m^2, the yield loss may up to 50%-60%, (21-30) panicle/m^2 reaches 70%-80%, and the loss in yield reach 100% if it reaches (>31) panicle /m^2 (Area, 2010). Second, the size of seeds bank (Bhullar & Chauhan, 2015; Burgos et al., 2014) in the cultivation land would also increase/decrease the size. Third, traditional farmers tend to store some seeds for cultivation in the next season (Azmi & Karim, 2008; Sadohara et al., 2000). The usage of contaminated tools (Baki & Mispan, 2010; Delouche & Labrada, 2007) between farms is another factor, alongside the use of multiple types of planting. A study was done in Vietnam, showed the number of seeds, that including barnyard grass/kg of rice seeds is reached to 47 times than the recorded level (Mai et al., 1998). In the same vein, Chin (2001) revealed, the number of contaminated weedy rice seeds reached about 314 seeds/kg rice seeds. The rest farmers lean to buy certified seeds, but those farmers percentages are very low. Moreover, a common practice is machinery rentals or loans among the farmers (Arrieta et al., 2005), which also play an essential role in weedy rice proliferation (Yu et al., 2005). Therefore, the weedy rice density in the rice seeds cultivars play an important role in the percentage of the yield loss in that season.

A recent study revealed that, the yield loss of the cultivated rice because of weedy rice is comparatively higher even at weedy rice density at 1weedy rice plants/pot lead to yield loss about 43% (Karunarathna, 2017). Chauhan, (2010) shows that about half of the yield shift down at 24 weedy rice plants/m^2 competed with the rice plants during the first 40 days after emergence. In the same vein, Avila et al., (2005) showed that, the 1 red rice plant/m^2 contributed a rice yield loss of 16 kg ha.

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2.2.4 Characteristics of Weedy Rice and its Consequences on Cultivated Rice

To gain effective weedy rice control, it's essential to know the basic information about the main basic characteristics of weedy rice population in the fields. Because to tackle the infested weedy rice, should know more about its development, factors contributed, a better environment and basic traits. (Delouche & Labrada, 2007). Weedy rice population differ from site to site according to the types, numbers, characteristics, and ability to infest the new area. Therefore, to achieve the best control, it's crucial to understand well the weedy rice population properties. From the main important characteristics is the genetic variety of weedy rice (Pyšek & Prach, 2003). It is difficult to distinguish between weedy rice and commercial crop especially in the first stage of growth (Abraham & Jose, 2015). However, after the growth process and formation of tillering trait, the comparison or distinguish clear someway. The weedy rice includes plants have red or white pericarp seeds (Chauhan, 2013; Olofsdotter et al., 2000). There are different traits and characteristics distinguish weedy rice and make it more aggressiveness and competitive which lead to more rice yield losses (Ferrero & Vidotto, 1998). It has six important morphological traits as (pericarp colour, panicle shattering, hull colour grain shape class, awn distribution, and grain length class). These traits play a crucial role in manual weeding from the farmers and could have a high role in the shift down in infestation process (Sudianto et al., 2016).

The plant height is one of the major characters that gives weedy rice competitive advantage over commercial rice. The taller weedy rice plants are able to take more light and shade their adjacent cultivated rice plants (Shivrain et al., 2010). Because of this shading is high the rate of fungi and insect infestation, so, this infestation is hard to control because of taller weedy rice. In addition, the short weedy rice plants which germinate later have its effects on competition process but less than tall weedy rice

(Bayer, 1991). In the same vein, one of these traits are tillering capacity, some types have fewer tillering, but the majority have high tillering, so it bears more panicles, rapid vegetative growth, taller than rice cultivar, its leaves take a long time to drop, has high ability in fertilizer absorption, high shattering seeds and variable seeds dormancy as per in Figure 2.2 (Van et al., 2007).

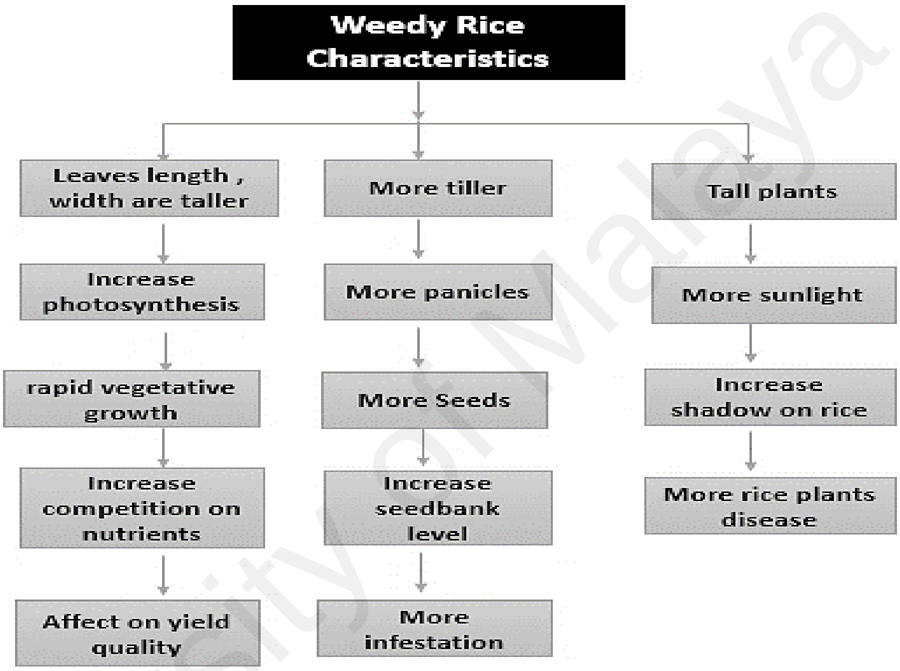

Figure 2.2: Weedy rice traits and its consequences that effect on rice cultivation crop.

In addition, from the clear traits for weedy rice are shattering which spread all the seeds within the fields. Dormancy for seeds in the soils that may be reached to three years, some die and the others complete the cycle by re-germination again (Suh et al., 2012). Seed shattering is considering one of the main characteristics and the major harm of weedy rice. Weedy rice seeds can proliferate for far distances and motivate their persistence in the seed bank before the farmer gets a chance to remove the seeds.

The time for shattering for weedy rice differs from area to area. The recent studies revealed that the seeds shattering in weedy rice started about 30 days directly after the flowing process, about 65% of the total grains (Ferrero & Vidotto, 1998). Shattered weedy rice seeds in the field soils are affected with diverse factors, as predators like birds and ants, not appropriate climate circumstances, which affect germination process and stay in dormancy for long time (Vidotto et al., 2001). The re-development of weedy rice seeds in the seedbank is dependent by the water quantity, depth of seeds and the structure of the soil (Ferrero & Finassi, 1995).

2.3 Herbicides Resistance in Malaysia

Herbicide resistant weeds in Malaysia started since the findings of glyphosate resistant goosegrass (*Eleusine indica*) in oil palm plantations (Baki, 2006). However, no reports to show the resistance of weedy rice in rice agro-ecosystem due to no specific herbicides were introduced for weedy rice until Clearfield® Production System. Weedy rice, with AA genome characterizes with close sexual reproduction and high percent in pollen with high percent fertility in the first hybrids (Naredo et al., 1998). Therefore, the percentage of gene flow between crop to crop and between crop and other ancestors are sometimes high. Table 2.2 shows the different percentage of gene flow of rice in many countries. The movement of pollens and distance played an important role in fertilization and hybridization. In addition, wind speed, their direction in pollens movement, temperature, humidity and the quantities of pollens that being produced an effect on the fertilization process. For example, indica species rice produces more pollens than japonica rice (Jia, 2002).

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2.4 Herbicides Resistant Rice

There are no clear genetic limit between the cultivated and weedy rice (Londo & Schaal, 2007) because the flowering time between the two species occur almost at the same time. Therefore, it is easily the hybridization occurs with each other. The heavy usage of traditional herbicides as glyphosate for controlling and elimination of weedy rice lead to what called glyphosate-resistance weed (Norsworthy et al., 2013) as per in Table 2.3. Introducing of the genetically modified crops has evolved great environmental risk concern all over the World (Snow et al., 2003).

Table 2.2: Gene flow occurrence between Clearfield® rice and weedy rice in commercial fields [a].

Locations	Varieties	Released	Resistant reported	Average gene flow
Louisiana, US.	CL121, CL141, CL161	2001/2002	2002	0.17%
Arkansas, US.	CL161	2002	2003	0.11%-0.76%
Brazil	IRGA 422 CL	2003	2004/2005	0.065%
Colombia	CF205	2003	2006	<1%
Costa Rica	CFX-18/CL161	2004	2007	Not available
Italy	Libero	2006	2010	Not available

[a]Adapted from (Sudianto *et al.,* 2013)

Since the drop of weedy rice dissemination and failure of the traditional herbicides i.e. butachlor/propanol which has been ineffective in decreasing the weedy rice infestation, the development of herbicide-resistant rice is very crucial events. In an experiment on a rice field showed the maximum distance about 3 meters (Olofsdotter et al., 2000). However, the isolation distance about 10 meters is safe and could stop transferring the pollens from cultivation rice and weedy rice (Khush, 1993). But, the ability of rice pollens to cross high distance about 31 m have been reported (Muker & Sharma, 1991).

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Therefore, as distance increase the probability of gene flow and introgression shift up between the cultivated rice and weedy rice. Also, a study has been documented the distances up to 43.2 meters (Song et al., 2003). The distance between weedy rice and cultivated rice is an important factor to determine the percentage of gene flow. Although the percentage is very low, which about 0.003%-0.008% but these percentages are important where rice cultivation is planted in large size areas (Shivrain et al., 2007).

Table 2.3: Examples of weed species resistance to herbicides in Malaysia.

Species	Botanical family	Herbicides	References
Fimbristylis miliacea	Cyperaceae	2,4-D	(Watanabe et al., 1996)
L. dubia var. *major*	Scrophulariaceae	Sulfonylureas	(Itoh et al., 1992)
Sagittaria guyanensis	Alismataceae	Sulfonylureas	(Itoh & Wang, 1997)
Sphenoclea zeylandica	Sphenocleaceae	2,4-D	(Itoh et al., 1992)

2.5 Weedy Rice Management Practices in Malaysia and Its Challenges

There is no simple method to control weedy rice (Delouche & Labrada, 2007). The close morphological resemblance between weedy rice and the cultivated rice has "vetoed" the application of herbicides that are able to selectively control other rice weeds. This makes weedy rice hard to control and manage chemically. An integrated approach involving the combination of cultural, physical, and chemical interventions is expected to be effective in managing the weedy rice problem in a sustainably manner (Baki & Mispan, 2010). In general, the weedy rice management strategies in Asia incorporates preventive measures, land preparation, rice establishment methods, seeding rate, weed-competitive cultivars, water management, herbicide application and crop rotation (Chauhan, 2013b).

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The adoption of direct seeding in rice cultivation was a spark of weedy rice propagation in most rice fields and farms in Malaysia (Azmi et al., 2000; Baki & Mispan, 2010). The integrated weedy rice management practice was immediately adopted, involving direct and indirect control measures as a suitable future tool (Azmi et al., 2000). The integrated approach (Table 2.4) includes shallow plowing for the land, rice plants straw burning, , pre-emergence proper herbicide application, diverse tillage, sowing of certified pre-germinated seeds, flooding, ditches/levees control, cutting panicle rouging and weed-free crop (Azmi et al., 2000; Azmi & Karim, 2008).

Current weedy rice controls in Malaysia are concentrating on: 1) managing seedbank by rice straw burning, 2) early weedy rice elimination by proper land management, and 3) manual weeding. The challenge is to add more controls to fill the gaps which can become the potential for weedy rice escape to the rice agro-ecosystem (Figure 2.3). Weedy rice cross contamination a mong the farm needs to be reduced by imposing intensive regulatory measures to the rice growers in Malaysia. The movement of machineries (*e.g.* plowers, harvesters, *etc.*) from one field to other fields need to be limited or thoroughly cleaned from any weed seeds. This can reduce the spread of weedy rice seeds especially when the harvester moves from highly infested field. Using certified seeds by farmers needs to be strictly regulated considering the major entry point of weedy rice is by contaminated seeds. Re-use of seeds from previous season and sharing seeds need to be prohibited. The government or any authorities need to find a way to impose of using only certified seeds by competent bodies to reduce the possibility of seed contamination.

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Table 2.4: Management practices recommended for weedy rice control [a].

Time	Activity	Remark
After Harvest		
1-3 d after harvest	Cut stubble	The straw and stubble should remove by service cutter.
3-7 d after harvest	Straw burning	To kill weedy rice seed and to induce new development from the seedbank.
Pre-planting		
33 d before sowing	First herbicide application	Glyphosate; Glufosinate
30 d before sowing	Dry rototilling/shallow tillage (1st tillage)	Shallow up to 7.5cm. Eradication of perennial weeds and to induce weedy rice seeds development.
15 d before sowing	Wet rototilling (2nd tillage)	To encourage weedy rice emergence.
10 d before sowing	Second herbicide application	Glyphosate; Glufosinate; Pretilachlor
2 d before sowing	Wet rototilling and land levelling	Extracted emerged weedy rice. Water level at 3cm for land levelling.
Sowing day		
0 d	Pre-germinated rice seed broadcasting	Sowing immediately after land levelling.
0-3 d after sowing	Pre-emergence herbicide application	Pretilachlor; Benthiocarb/propanil; Pretilachlor/Propanil.
7-14 d after sowing	Flooding	-
>20 d after sowing	Weedy rice monitoring and manual weeding	Cutting off panicles of weedy rice to reduce future seedbank.
Harvest		
110-120 d after sowing	Harvesting	Harvester should be cleaned when it is leaved highly infested fields to prevent the propagation of weedy rice seed.

[a] Adapted and modified from (Azmi & Muhammad, 2003; Azmi & Karim, 2008)

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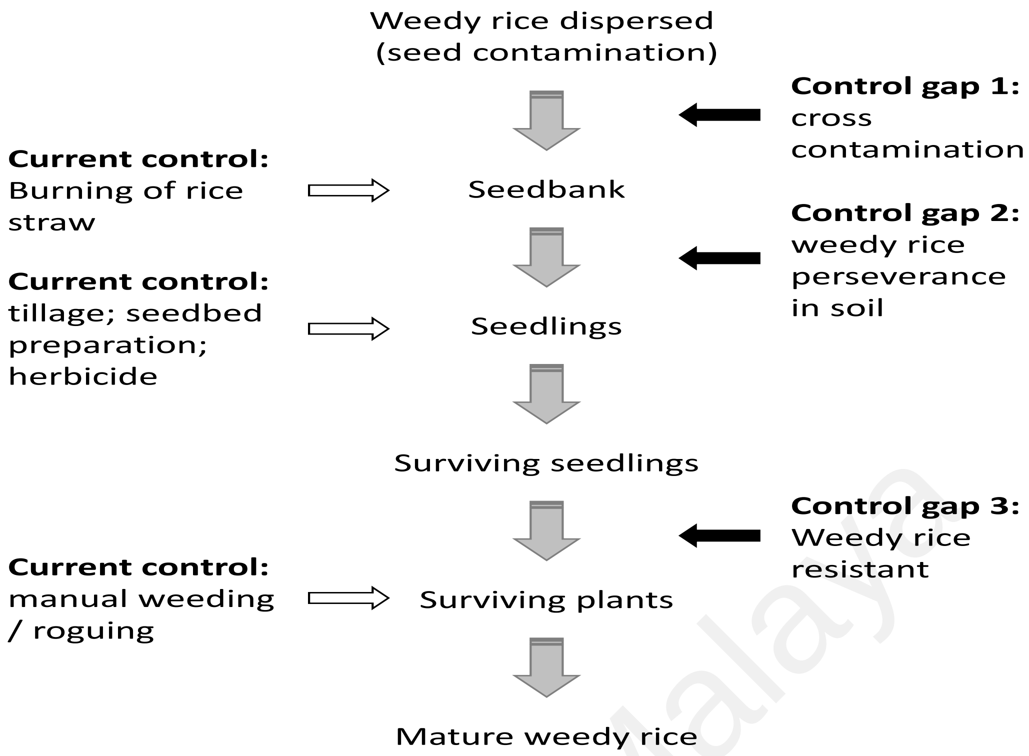

Figure 2.3: Current controls (white arrow) and potential control gaps (black arrows) for weedy rice management based on the weedy rice life cycle model from (Pandey et al., 2000).

Maintaining viability over longer period of time in the seedbank might provide several adaptive advantages for weedy rice to survive from heat and high humidity and escape seed deterioration especially in tropical areas (McDonald, 1999; Roberts, 1961). The persistence of weedy rice to deterioration of aging seed in nature especially by strong dormancy is a common trait (Noldin et al., 2006). Despite being exposed to relatively high temperature and moisture that would usually enhance seed germination and deterioration, the weedy rice problem is still severe (Baek & Chung, 2012) especially in the tropics. Physiological mechanism of seed deterioration in rice has been well studied but the knowledge of the inheritance and genetic determinants of seed longevity mechanism in weedy rice are still lacking (Miura et al., 2002; Sasaki et al., 2005), or not as advanced as studies on seed dormancy (Gu et al., 2003; Gu, Kianian, & Foley, 2005).

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Closing the gap of weedy rice resistant is crucial, and a challenge that need to be addressed. Understanding weedy rice adaptation to escape various management practices especially to chemical control is important and need a special focus (Mispan, 2015; Sudianto et al., 2013). The current status of weedy rice resistant especially to CPS need to be clarified and mitigation strategies need to be immediately set up to reduce greater damage due to the resistance (Hamdani, 2015; Jaafar et al., 2014).

2.6 Clearfield® Rice Production System (CPS)

Introducing of IMI-herbicides has provided an efficient tool to selectively control WR in the rice fields (Scarabel et al., 2012). IMI-herbicides are a class of herbicides used for protecting of a wide variety of agricultural crops, but they can harm other types of crops. Members of the IMI-herbicide family have similar structural properties entered round the IMI-ring and an attached aromatic system bearing a carboxylic acid moiety. They belong to group 2 herbicides, which are relatively new broad spectrum herbicides, and can be used to control weeds and grasses in a variety of agricultural areas (Krynitsky et al., 1999; León et al., 2018). IMI-herbicides were developed in the 1970s and were field-tested, mostly in the USA by the American Cyanamid Company. They were also tested in South America and Japan.

The IMI family includes a group of herbicides comprising of imazapyr, imazapic, imazethapyr, imazamethabenz, imazamox, and imazaquin (Grey et al., 2012). They work as selective herbicides that inhibit the acetolactate synthase (ALS) gene, also known as acetohydroxyacid synthase, or (AHAS) and branched chains of three amino acids: isoleucine, leucine, and valine (Shivrain et al., 2009). Also, IMI-herbicides are used as non-selective herbicides in non-crop areas, or in forestry and plantation crops, such as oil palm and rubber (Ramezani et al., 2009). They were absorbed via weeds'

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organs and then diffused by the phloem and xylem organs of weeds, moving to the meristematic tissue. IMI herbicides block the biochemical pathway of the substrate to the catalytic site which is essential in the branched-chain amino acid synthesis process. Moreover, IMI stops protein and nucleic acid (DNA) synthesis, thereby slowing down the plant's cell division rate and impeding the transport of important materials to growth points. Eventually, it causes a decrease in regular plant growth and kills any susceptible plants (weeds), including WR (Croughan, 2003; Sudianto et al., 2013). However, WR plants may exhibit a variety of responses according to the doses used and the time of application.

IMI class herbicides are characterized by their chemical effects at minimum concentrations, their significant influence on weed control, generally low mammalian toxicity, and their increased persistence in soil and water (Alister & Kogan, 2005). In view of this, IMI-herbicides were selected to control a broad spectrum of weed species in Malaysia (Sondhia et al., 2015). This includes the IMI-herbicide family including imazapyr, which is a generic name for [2-(4-isopropyl-4-methyl-5-oxo-2-imidazoline-2-yl) nicotinic acid], with the trade names *Arsenal* and *Chopper* (Helling & Doherty, 1995), and imazapic, the generic name of [2-(4,5dihydro-4-methy-4-(1-methylethyl)-5-oxo-1Himidazol-2-yl)5-methyl-3-pyridinecarboxylic acid], with the trade names *Cadre* and *Plateau* (Azmi et al., 2012). These herbicides are regarded as the main groups of the IMI herbicide family because of their low application rates, decreased environmental hazards, high soil persistence, and selectivity for a wide range of crops such as rice and wheat (Marcia, 2014). Also, IMI-tolerant rice allows the use of imazethapyr (5-ethyl-2-(4-isopropyl-4-methyl-5-oxo-4,5-dihydro-1H-imidazol-2-yl) nicotinic acid) with the trade label Newpath® in fields in the USA and Brazil for the control of WR and other severe weeds (Fish et al., 2015).

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In the 1990s, global evolution and the presentation of non-transgenic resistant herbicide-tolerant rice such as IMI-resistant rice (Clearfield® rice) (Meins et al., 2003) was discovered due to the heavy infestation of WR in paddy fields, which accelerated yield losses. Clearfield® rice varieties were first introduced by Louisiana State University in the USA in 2002 by introducing Clearfield® rice 121 and Clearfield® 141 (Sudianto et al., 2013). Likewise, Clearfield® rice cultivars, developed by mutation breeding without the addition of any foreign gene, were commercialized in 2002 (Croughan et al., 1996). The main reason for developing Clearfield® rice was to control weedy *Oryza* species. Diverse IMI herbicide-resistant rice cultivars containing the mutations Ala122Thr, Gly654Glu, and Ser653Asn in the ALS gene have recently been commercialized (Roso et al., 2010).

The adoption of Clearfield® rice is increasing annually. For example, in 2004, ~19% of long grain rice acres of the total cultivation land in Arkansas, 27% in Louisiana, 15% in Texas, 13% in Missouri, and 23% in Mississippi were cultivated using Clearfield® rice (Shivrain et al., 2006). This method was also adopted by other countries, such as Malaysia, due to the need to control the heavy infestation of WR. Azmi et al., (2008) reported that the CPS technology was launched on the 8th July in 2010 at the Malaysian Agricultural Research and Development Institute (MARDI), whereby the Malaysian government released two corps cultivars: MR220CL1 and MR220CL2. Both varieties were obtained from crosses between CL1770 from Louisiana State University (LSU) and a Malaysian local rice variety MR220 (Azmi et al., 2008). Sudianto et al., (2013) revealed using DNA analysis techniques that the genetic similarity between the two cultivars (MR 220CL1 and MR 220CL2) used in Malaysia reached 98.5%. The CPS technique package was used to overcome the problems caused by WR (Bakar et al., 2016). This is composed of three major components: Clearfield® rice, OnDuty™ (imidazolinone) herbicide, and stewardship guide (Azmi et al., 2012).

The global status of the CPS system has been used in specific countries which used IMI-herbicides and different types of Clearfield® cultivars, based on specific conditions; for example, the CPS system supported by the Malaysian government was commercialized using imazapic and imazapyr herbicides, and MR220-CL1/MR220-CL2 cultivars. Because of the lack of crop rotation and the practice of a monoculture system (Azmi et al., 2012), Italy officially marketed the herbicide imazamox and the cultivar CL161 in 2006, which was cultivated on about 52,000 ha from an area of about 235,000 ha (equivalent to more than one-fifth of the total rice area) (Scarabel et al., 2012). The USA used cultivars CL152 and CL162, along with imazethapyr herbicide, which was developed for use in USA paddy fields and other crops due to its efficacy against WR (red rice) (Solomon et al., 2012). In addition, this technology has been used in approximately one million ha in the USA and Brazil (Gealy et al., 2003).

The introduction of Clearfield® rice provided the selective control of WR in fields, which, together with integrated management practices, increased the rice yield in Brazil by approximately 2500 kg/ha, which was an increase of 50% (Merotto et al., 2006). Clearfield® rice technology is also used in Arkansas, USA, to mitigate significant red rice infestation (Burgos et al., 2008). Cassol et al. (2015) reported that Clearfield® technology was used in Rio Grande do Sul in 2012, resulting in more than 50% of rice acreage being planted. Also, in Brazil, this IMI herbicide resistant-cultivar was used on an area equivalent to approximately 1.1 million ha due its effectiveness in controlling the population of red rice (Singh et al., 2017), a known plant pest in many countries, which causes increased yield loss and decreased crop quality (Dauer et al., 2017). The

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CPS system is also used in paddy fields in Colombia, Brazil, Nicaragua, and Costa Rica. For example, about 22% of the rice cultivated area in Costa Rica was planted using Clearfield® rice, while types CL121, CL141 and CL161 were applied in Louisiana (USA) (Sudianto et al., 2013).

Therefore, there are four main reasons for developing Clearfield® rice and IMI herbicides: 1) to eliminate WR plants in paddy fields; 2) to increase the yield of rice cultivation systems via optimum WR controls; 3) to reduce the amount of land required to satisfy the global rice demands; and 4) to decrease the usage of fossil fuel in agricultural production (Mannion, 1995). Clearly, using herbicide-resistant rice varieties has been proven to be one of the most effective methods by which to eliminate WR from fields (Song et al., 2017). The CPS system provides an efficient tool to selectively control WR in the post-emergence stage (Novakova, 1994). Before Clearfield® rice was developed, there were no marketed herbicides that would selectively control this weed without injuring the rice crop.

CPS technology is more readily attainable than transgenic herbicide-tolerant crops. For instance, there was a quick expansion of CPS to develop IMI-tolerant diverse crops. It was also noted that farmers have been using CPS systems since 2001. However, glyphosate-tolerant rice and wheat have still not been commercialized, even though they have also been developed (Gealy et al., 2003). Azmi et al. (2012) reported a wide scale evaluation of the CPS system for cultivation areas (47.62 ha) in the off-season year 2010 in Malaysia and it was found that yield production increased from 4.93 ton ha^{-1} to 5.69 ton ha^{-1}, where the returns ranging from 5 to 8 times more, translating to a difference of USD1000 to USD1600.

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The introduction of Clearfield® rice in 2002 in the US made the selective control of red rice possible via the use of IMI herbicides. Reports showed that imazethapyr herbicide could reduce the population of WR (red rice) plants in US fields by more than 90% (Singh, et al., 2017). Also, Pellerin et al., (2003) reported that imazethapyr reduced the population of WR by 98% in US rice fields. CPS technology is used in Arkansas to eliminate red rice. It was reported that the level of control reached 90% when CPS was used (Burgos et al., 2008). The effectiveness of IMI herbicides in eliminating weeds was studied in the USA, by a study reporting that imazapic is effective in eliminating certain weeds, such as peanut (*Arachis hypogaea* L.) (Ducar et al., 2004). Also, a study of imazapic showed that it is capable of eliminating many types of weeds, such as jointed goat grass and downy brome. Rainbolt et al. (2004) reported that the herbicide imazamox can eliminate the presence of red rice by up to 99%. In the USA, where the efficacy of the CPS system on diverse types of weeds that infest crops has been proven; for example, many weeds that infest rice, including barnyard grass (*Echinochloa crus-galli)*, and severe types of weeds in wheat, such as cheat (*Bromus secalinus* L.) can be controlled. Beside to the aforementioned weeds, the list also involves Italian ryegrass (*Lolium multiflorum* Lam), shatter cane (*Sorghum bicolor),* and Johnson grass (*Sorghum alepense)* ((Tan et al., 2005).

IMI herbicides are prevalent in CPS technology on their own, as imazethapyr in Arkansas (USA) and imazamox in Italy, or as mixtures, for example, imazapyr and imazapic were used in Clearfield® rice cultivars in Malaysia. Furthermore, imazapyr and imazapic were used on IMI-resistant wheat in Australia (Tan et al., 2005). The synergistic nature of the IMI herbicide family (Table 2.5) was developed via various experiments to increase the efficiency of IMI herbicides in controlling WR and reducing the risk of injuries (Blouin et al., 2010).

Table 2.5: Synergism method efficiency by IMI herbicides application effect on the targeted weeds.

Imidazolinone effect	Types of application and modification	Targeted weeds	References
[a]High control	Imazethapyr + propanil + pendimethalin Imazethapyr + nicosulfuron /imazaquin + imazapyr	Barnyard grass Barnyard grass	(Kumar et al., 2008) (Klingman et al; Masson & Webster, 2001)
	Imazapic + imazapyr Imazapic + atrazine	Red rice; weeds in barley and ryegrass; smooth brome; Kentucky bluegrass Texas atrazine	(Webster et al., 1999); (Alister & Kogan, 2005). (Bahm, Barnes, & Jensen, 2011) (Ducar et al., 2004)
[b]Medium control	Imazethapyr + quinclorac Imazethapyr + bentazone+aciflurfen	Broad leaf, signal grass Barnyard grass	(Jason, 2011; Klingman et al., 1992; Pellerin et al., 2004; Webster, 2001)
	Imazethapyr + pendimethalin + metolachlor	Barnyard grass	(Arnold et al.,1993)
	Imazapic + clethodim	Crabgrass	(Burke et al., 2004)
[c]Minimal control	Imazethapyr + paraquat.	Bristly starbur, Prickly sida, Small flower, Nutsedge	(Richburg, Wilcut, & Vencill, 1996)
	Imazethapyr + paraquat.	Sicklepod weeds	(Wilcut et al., 1994)
	Imazethapyr + propanil	Irwin and Barneby and Flordia beggar weed	(Richburg et al., 1995).
	Propanil +Imazethapyr Propanil+Imazethapyr +Molinate Imazethapyr +Halosulfuron, Imazethapyr+Carfentrazone.	Indian jointvetch *Sesbaniaexaltata* and *Aeschynomena indica*	(Masson & Webster, 2001) (Wei et al., 2001)

a) High control: IMI has high efficiency control in this mixing procedure;
b) Medium control: IMI has medium efficiency control in this mixing procedure;
c) Minimal control: IMI has low efficiency control in this mixing procedure.

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2.6.1 Environmental Concerns of CPS

CPS technology could be harmful to humans, domestic animals, and other crops if not used properly, as per the recommendations of the manufacturer (Ibrahim et al., 2017). There are several environmental concerns regarding the misuse of this technology.

2.6.1.1 Weedy Rice Resistant Potential towards Imidazolinone Herbicide

The development of new herbicide-resistant weeds and mitigating gene flow from crops to weeds are important; however, the introduction of a new herbicide-resistant crop to the field must be comprehensively studied. Genetically, WR plants are closely related to commercial rice and are very similar to paddy plants especially on physiological and morphological similarities (Baki & Mispan, 2010). The repeated use of the same herbicide and modes of action in the same field, especially in mono-cropping systems, could impose what is known as selection pressure on WR. This could occur due to the possibility of gene flow from Clearfield® rice to WR. Subsequently, this newly formed WR gains the IMI-herbicide-tolerance characteristics from the cultivated rice.

In addition, a spontaneous mutation could also occur because of the significant usage of IMI-herbicides (Sudianto et al., 2013) as tabulated in Figure 2.4. The gene flow from cultivated rice crops is fast becoming a major problem. Escaped WR plants could be exchanged and hybridized with the alleles of cultivated rice during the flowering process (Dauer et al., 2017). This forms herbicide resistant WR, which increases the cost of rice production and decreased cultivation field and yield. Herbicide-resistant weeds are not something new and have been around for quite some time. Previously, when traditional herbicides were resorted to, the usage of glyphosate leads to the glyphosate-resistance of weed (Norsworthy et al., 2013) Highly resistant weeds could cause high yield losses and create a complicated weed dynamic in the fields.

The use of new CPS technology creates a strong bias towards the introgression of resistance alleles of one plant population into the gene pool of another (Li et al., 2017; Singh et al., 2017). The most significant effect of IMI herbicides is prompting

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modifications to certain crop structures and developments. Notably, these modifications are not immediate, and could have carryover effects, which are usually overlooked by farmers (Qi et al., 2017). WR resistant mechanism could be induced by increasing the selection of previously existing alleles in specific genes, known as spontaneous mutation, and the complicated outcrossing of WR plants with Clearfield® rice (Busconi et al., 2012). Wang et al. (2006) revealed that introgression is possible due to both WR plants and Clearfield® rice being related to diploids (2n = 24) in the 'AA' genome.

Similarly, the herbicide resistance character in Clearfield® rice is governed by a single dominant gene (Kaloumenos et al., 2013). It should also be pointed out that the result of the outcrossing gene is rather diverse, variable, and mostly less than 1%. However, within sequential generations between WR plants, these introgression alleles could lead to the increased development of a more aggressive WR population (Burgos et al., 2014). The presence of genetic variation in the WR population leads to genes moving from cultivated rice to the WR population, leading to the development of increased resistance. The interaction exchange increases as the distance decreases, and the increasing CO_2 could enhance the competition from wild WR in rice production; therefore, consumable rice production reduces, and the gene flow in the fields becomes easier (Ziska et al., 2012). The distance for fertilization and movement of pollen in the air is crucial regarding hybridization (Ziska et al., 2012). Jia (2002) showed other factors that influence fertilization, including wind speed and direction affecting pollen movement, the quantities of pollens being produced, environmental conditions such as temperature and humidity, and the rice cultivar variety. For example, *Indica* rice produces more pollen than *Japonica* rice. Muker &Sharma(1991) reported the ability of rice pollen to cross approximately 31 meters (m). However, Khush (1993) pointed out that an isolation with a suitable distance of ~10 m is safe, and could prevent the transfer

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of pollens from the cultivation rice and WR. The sexual compatibility between cultivated rice and WR plants was found to be perfect for gene ingression, (Engku et al., 2016).

Therefore, the presence of herbicide-resistant weeds in agro-ecosystems increases the interest in the environmental risk of herbicides (Table 2.6) in the future (Qi et al., 2017). In most countries, scientists and farmers are concerned about the resistance trait being passed on from Clearfield® rice to WR (Shivrain et al., 2009). Weiqiang et al., (2006) reported that 0.17% of outcrossing was detected between Clearfield® rice cultivars (cultivar121, cultivar 141, cultivar161, and cultivar 8) and WR, which was proven by phenotypic and DNA marker analyses.

However, there are many solutions to overcoming gene flow between rice and WR plants. Some of these solutions were accepted, such as the suggestion to choose special conventional rice cultivars, called cleistomgamous, due to the pollination process taking place prior to flowering, rendering the rice plants able to self-pollinate. Other mitigation techniques used genes conferring traits such as non-shattering, dwarfism, and the lack of secondary dormancy as mitigation (Gressel & Valverde, 2009).

Cassol et al.(2015) reported that more than 56% of red rice plants in Brazil were resistant to imazethapyr and imazapic. Merotto et al. (2006) reported that this resistant incidence emerged because of the IMI-herbicide resistance in WR and the escalation of production costs. Many rice growers and farmers in Brazil had to leave the business, selling off or renting their lands out, which led to the escalation of the average farm size. In these WR populations, the mechanism of resistance was modified target site with approximately 80% of these WR plants had the same mutation as the IMI-tolerant

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cultivar, which was the most widely used in the fields (Roso et al., 2010). The resistance mechanism could be due to activated metabolic reactions of mixed-function oxidases which remove toxics of this type of herbicides. For example, sulfonylurea resistance in lettuce is due to the modified site of action, acetolactate synthase (Carol et al., 1990). In certain regions, Clearfield® rice seeds were removed from the fields, which resulted in it being replaced with other cultivars such as CFX- 18/ CL 16 in Costa Rica in 2004 (Sudianto et al., 2013).

Table 2.6: Examples of weed species resistant to herbicides globally.

Species	Family	Herbicide	Country	References
Fimbristylis miliacea	Cyperaceae	2,4-D	Malaysia	(Watanabe et al., 1996)
Sagittaria montevidensis	Alismataceae	Bensulfuron	Australia	(Graham et al., 1994)
Echinochloa crus-galli	Poaceae	Propanil	USA	(Smith et al., 1992)
Monochoria korsakowii	Pontederiaceae	Sulfonylureas	Japan	(Kohara, 1996)
Limnocharis flava	Butomaceae	2,4-D	Indonesia	(Heap, 2014)
Scirpus mucronatus	Cyperaceae	Cinosulfuron	Italy	(Sattin et al., 1999)

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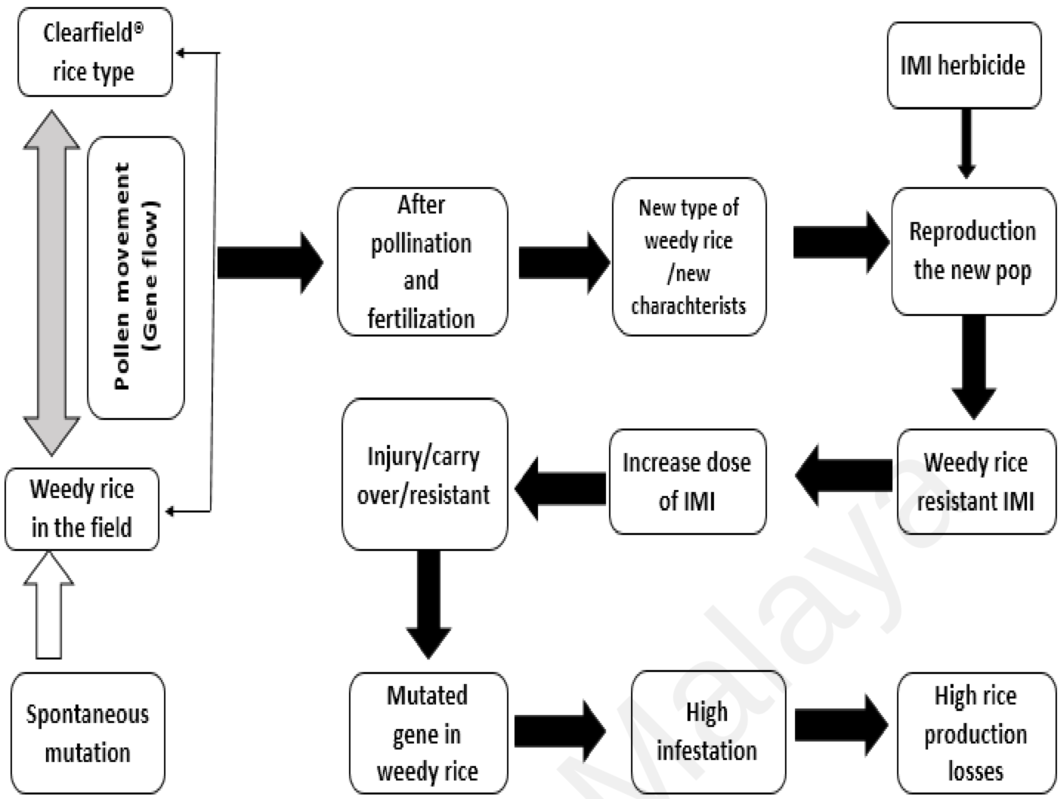

Figure 2.4: Development of IMI resistance in weedy rice population in the fields. Adapted from: (Sudianto et al.,2013). a) Grey Arrow represent gene flow due computability between rice and weedy rice; b) White Arrow represents inter-mutation within weedy rice genes; and c) Black Arrows represent weedy rice resistant pathway mechanism.

Introducing of Clearfield rice cultivars represents a promising alternative to control weedy rice plants and decrease rice crop losses in the fields. However, Clearfield rice cultivars could be a short-term solution, due to the selection of herbicide resistant WR. According to (Burgos et al., 2008), more than 55% of rice farmers showed that the beginning of development of resistant weedy rice in the field.

2.6.1.2 Imidazolinone Herbicide Residues in Soil and Water

The World Health Organization (WHO) has defined residues as "any substance or mixture of substances in food for man or animals resulting from the use of a pesticide and includes any specified derivatives, such as degradation and conversion products, metabolites, reaction products, and impurities that are considered to be of toxicological significance”. Herbicides play an important and much needed role in food production worldwide, which improves the production of high crop yields at a low cost. It is evaluated that without the use of pesticides, approximately half of the world’s agricultural production could be lost (Ramezani et al., 2009). The environmental fate of herbicides after their application is a major concern for producers responsible for maintaining good-quality water. Concern should also be given to the interaction and destination of these dangerous chemical particles, which may be adsorbed in different forms, such as minerals and organic compounds, and to their reaction with other compounds, forming complexes. Refatti et al., (2017) reported that the extensive use of herbicides close to water sources is considered a risk of contamination to the environment.

There is no doubt that the fate of herbicides in the crop fields causes pollution both on the surface and in groundwater, as it is also harmful to human health by affecting the food chain; studies revealed that less than 1% of herbicide components can reach the target in plants, and the rest of the herbicide penetrates and moves through soil pores in surface and groundwater (Gavrilescu, 2005).

The continuous use of herbicides for a long period of time in agriculture systems has generated diverse consequences to the environment. Currently, the intensive use of herbicides in crop cultivation is due to the issue of weed infestation, thus accelerating the problem of pollution. Changing the method or technique in cultivation may solve the problem but may lead to another setback. As an illustration, the formulation of a new method for rice crop planting, such as the direct seeded rice method, can save labour efforts and costs, but may lead to higher levels of herbicide applications for weed

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control management (Ismail et al., 2011). The IMI herbicides family represent a new type of herbicide that can be widely used in agriculture due to its low use rate application, reduced environmental concern herbicides, and low toxicity (Ramezani et al., 2009). However, IMI-herbicides use may limit the succession of non-tolerant crops for long residual activity in the soil and which can cause an agronomic problem and the environmental complicated problem (Ulbrich et al., 2005).

The movement and persistence of IMI-herbicides in cultivation regions is very important to modify the agronomic quality of IMI-herbicides in the future and to lessen concerns about environmental pollution. Leaching (the vertical movement of herbicide components along the soil matrix) and degradation are both key factors determining the mobility of herbicides downstream. The persistence and leaching of the IMI herbicides in the soil are due to many factors such as photo-degradation, chemical degradation, microbial activity, and the hydrolysis process (Refatti et al., 2017). The two mechanisms (microbial and chemical) are correlated with water availability and high temperature (Süzer & Büyük, 2010). The photo degradation process has been considered one of the main processes, especially in tropical and subtropical areas, due to the hot temperatures and high solar radiation all day long (Ramezani et al., 2009). The nature of the chemical compounds for IMI-herbicides and the environmental conditions play a critical role in the fate of the herbicide in the environment. As an example, the availability of both acid and basic IMI-herbicides led these types of herbicides to present in triple states: cationic, anionic, and neutral (Marcia, 2014).

Quivet et al. (2006) reported that complex interactions in the soil matrix between IMI-herbicidessuch as imazapyr and metal ions such as Na^{+}, Ca^{2+} and Cu^{2+} decreased the photolysis and degradation ofimazapyr, and therefore increased the persistence time

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in the soil. These previous states are controlled by the pH of the matrix, as soil and water, as IMI-herbicides are anionic at higher pH values. Higher pH values lead to the increased dissipation and degradation of some IMI-herbicides in the matrix, such as imazethapyr and imazaquin (Marcia, 2014). In the field, IMI-herbicide (imazapyr) half-lives were estimated to be more than 325 days for dissipation in the upper layer of the soil compared to the lower layer (Azzouzi et al., 1998). Therefore, these previous triple states allow researchers to extract IMI-herbicides from the soil and water and separate them from other interfering compounds in the matrix with high efficiency. The spread and movement of small particles of herbicides due to heavy rainfall in tropical and semitropical areas increases the chance of water pollution. The general effect of contamination with most herbicides on the aquatic eco-system has been studied and reported (Ismail et al., 2011).

Studies have revealed that the lifetime (the time needed to degrade about 50% of the substance) in the soil for IMI varies. For example, imazapic remained in the soil for 90 days (Grymes et al., 1995; Ulbrich et al., 2005), imazethapyr for about60 to 360 days, and imazapyr was about 141 days (Alister & Kogan, 2005). The persistence of imazethapyr residues in the soil remains for between four and 20 weeks in clay and sandy soil, respectively (Hollaway et al., 2006). Herbicides sometimes persist for a period of time in the soil to kill weeds in the crop. This is called the critical period of weed competition. However, it should not persist for a long time because it causes injury to crops and subsequent rotational crops (Santos et al., 2014). The depletion of IMI-herbicides in the field is quicker than any external study, because the water content (soil moisture), photo degradation, biodegradation**,** and temperature fluctuation in the field affected the draining process and the persistence of chemical substances.

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Experiments and reports on availability of IMI-herbicides in the sandy soils revealed that approximately 50% of herbicides were broken and drained in two days of extensive Ultraviolet light (UV) (Aichele & Penner, 2005). Consequently, it was observed that continuous monitoring is a suitable technique for determining residues in both water and soil as the appreciation for the main factors related to IMI-herbicides in the soil are very important to comprehend the behaviour of herbicides and ascertain the suitable dosage to avoid unwanted consequences affecting the ecosystem (Table 2.7).

Table 2.7: Different studies about Imidazolinone residues in environment.

Imidazolinone	Origin	Residues	Country	References
Imazapic and Imazethapyr	Surface water	0.007 - 0.085 mg/L	Brazil	(Silva et al., 2009)
Imazapyr	Surface water	1 µg/L	USA	(Shaifuddin et al., 2014)
Imazethapyr	Soil and grains	0.001- 0.0015 µg/g	India	(Sondhia et al., 2015)
Imazethapyr	Soybean oil	0.003 µg/mL	India	(Mastan et al., 2016)
Imazethapyr	Food	0.01 - 0.02 µg/g	Japan	(Akiyama et al., 2009)
Imidazolinone	Water and soil	0.1- 0.05 ng/g	Italy	(Laganà et al., 1998)

Table 2.8 tabulated the studies exploring the different depths IMI herbicides could leach into, where it is revealed that the translocation and leaching of IMI herbicide components between soil particles and the continuation of the IMI- herbicides at diverse depths in the soil may increase their persistence, possibly due to the lower temperature in greater depth, less solar radiation, and the decrease of the activity of microorganisms in the soil (Refatti et al., 2017). Battaglin et al. (2000) showed that approximately, 2.5% of an applied pesticide was wasted in runoff during rainfall through one to two days since the herbicide applied. Therefore, low soil pH, clay soil, highly organic matter, and

low rainfall are the climatic conditions and soil types that raise the persistence of IMI-herbicides. Extensive future research should be conducted in this domain to determine these factors and to understand the maximum depth of how IMI herbicides can penetrate and its correlated environmental risks.

Table 2.8 : Imidazolinone herbicides movement and leaching depth in various studies.

Imidazolinone	**Leaching soil depth**	**References**
Imazethapyr	Below 25 cm	(Sondhia et al., 2015)
Imazapic, Imazapyr and Imazethapyr	Up to 25 cm	(Refatti et al., 2017)
Imazapic and Imazapyr	Up to 25 cm	(Neto et al., 2017)
Imazapyr	Up to 10 cm	(Börjesson et al., 2004)
Imazethapyr	Up to 70 cm	(Sondhia, 2013)

There are several methods that were proposed to determine the extraction of IMI from the soil. To understand this, the water solubility of IMI is generally relatively high (Sondhia et al., 2015), but the pKa is relatively low. This solubility of IMI-herbicides in water helps to determine the leaching potential at different depths, where the pKa values of the IMI-herbicides are 1.3–3.9 (Martins et al., 2014). However, its persistence in the soil is linked to the pH value of the soil (Marcia, 2014). Furthermore, the presence of these herbicides in the form of ions influences the extraction approach in both soil and water (Ramezani et al., 2009). Santos et al., (2014) pointed out that IMI-herbicides (imazethapyr, imazapic, and imazapyr) are used in USA fields and when applied as post-emergence herbicide, they were reported to cause high residual activities in the soil with different effects, depending on internal characters such as pKa values and lifetime of the soil.

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IMI-herbicides present a high risk of contamination for soil and water sources because of their high solubility. Moreover, due to the special physicochemical traits of IMI-herbicides, they exhibit long residual activity (retained in the soil matrix), which is highlighted as the most important feature; also, IMI-herbicides could lead to phytotoxic damage of rotational crops in succession to rice plants in the future. Curran (2001) reported that volatilization is compatible with temperature and the presence of water in the soil, as it increases with these two factors. However, IMI-herbicides are relatively non-volatile under field conditions, which increases the adsorption and persistence for a longer time in the soil. IMI-herbicides have relative half-lives of 1–5 months. This persistence could affect the next round of crops, which could reduce their quality of production.

Due to numerous complaints about the carryover effects, the recommended usage of IMI herbicide is not more than two consecutive years, leaving the soil undisturbed for at least a year (Santos et al., 2014). It should also be pointed out that IMI-herbicides do not leach easily because their translocation and movement is influenced by diverse factors, as previously mentioned. Similarly, Neto et al. (2017) also reported the transformation and leaching of IMI-herbicides to be influenced by multiple factors, such as chemical compounds and the amount of rainfall in the area. Organic matter and pH concentration are both negatively correlated with the adsorption of IMI. Studies revealed that clay soil samples, which have high concentrations of organic matter, have a tendency to hold high moisture quantities. Therefore, the microorganisms present, such as microbial flora and fauna, flourish, becoming more active in the degradation of IMI-herbicides, especially when temperature is high, as found in tropical and sub-tropical regions (Süzer & Büyük, 2010). Sondhia (2013) reported that IMI-herbicides could leach to greater depths in tropical countries with higher rainfall.

Countries such as Norway and France are prohibiting the use of IMI-herbicides due to their high persistence in soil (Shaifuddin et al., 2014). In Sweden, it was reported that IMI-herbicides residues were found 8 years after their application, and that IMI-herbicides display high activity against diverse annual weeds when applied either pre- or post-emergence. Sweden resorted to using IMI-herbicides for the long-term removal of a wide spectrum of broad-leaved weeds along railway lines in the country, with different concentrations used because the plants demonstrate a relatively wide range of sensitivity. As the accumulation takes a long time, this allows the herbicide to leach through soil pores and subsequently transport into both of natural surface and groundwater alongside railway tracks (Börjesson et al., 2004a).

Battaglin et al. (2000) revealed that there were 16 herbicide components related to IMI-herbicides that were found in water samples collected from both surface and groundwater in USA areas. On the other hand, rice fields in Brazil are considered a focus of water pollution, with the extensive use of IMI-herbicides detected in surface waters, rivers, lakes and groundwater. Herbicides such as Clomazone, for example, are the most frequently found herbicides in rice fields in studies in Arkansas and Australia (Silva et al., 2009).

Recently, Refatti et al. (2017) reported that the IMI-herbicides used in the Clearfeld® rice system could leach up to 25 cm or more. Similarly, IMI-herbicides were found to exceed the method reporting limit (MRL) of 0.01 μg/l in 83% of total water samples in US (Battaglin et al., 2000). Also, IMI-herbicides have been found to leach into groundwater in Canada streams and the pollution of groundwater with noticeable concentrations of herbicides would be unlikely (Cessna et al., 2012). However, IMI-herbicides persist for longer in the surface soil due to the adsorption mechanism

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potentially affecting the quality and yield of the next round of crops, and negatively affecting the environment. Studies and experiments revealed that low levels of imazapic herbicide are efficient to decrease the fresh weight of rice crops, sorghum, and maize (Shaw & Wixson, 1991). Also, a study has been done on IMI-herbicides to evaluate the effect and carryover in the soil and factors causing injuries to the next planting in the fields; the results show that IMI-herbicide residues in the soil have some negative effects, because IMI can persist in the soil for a long time, reaching several months (Alister & Kogan, 2005).

IMI herbicides remain in the soil for quite some time, depending on the application rate (D'Ascenzo et al.,998). Therefore, a safe re-planting period is suggested between IMI-herbicide application and the planting of non-tolerant crops. Marchesan et al. (2010) showed that plants demonstrate a wide range of sensitivity to IMI-herbicides and plant injury was still present about 70 days after application (imazethapyr), but without a decrease in grain yield. IMI-herbicide residues in the cultivation soil have affected and damaged the following crops in Canada about one year after the application of IMI-herbicides (Sullivan, 1998). Süzer & Büyük (2010) showed that IMI-herbicide residues also affect the second rotation crop, and seed yield decreased significantly, by 35.7%. The pH-value for IMI-herbicides and the type of soil play significant roles in the carryover process for the next generation of plants.

Loux & Reese (1993) reported that the carryover of IMI-herbicides (imazaquin) in Hoytville clay soil led to corn plant injury in the second year. Yield reduction was increased as pH decreased in the soil because the persistence of imazaquin increases as soil pH decreases to 4.5. IMI-herbicide residues in the soil can damage the next rotational crop, such as sugar beet, canola, cauliflower [*Brassica oleracea* (Botrytis

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group)], broccoli [*Brassica oleracea* (Botrytis group)], lettuce (*Lactuca sativa* L.), and potato (Hollaway et al., 2006). These inequalities in size and shape of potato revealed that IMI- herbicides significantly decreased potato yields and reduced marketable yields.

Due to the small application rates, the properties of these herbicides and the presence of other interfering chemicals from soil samples render the analysis of IMI at low detection limits complex. Therefore, current methods for analyzing these herbicides in soils are regarded as slow, expensive, and complex, requiring numerous steps (Martins et al., 2014).

Most extraction methods rely on the pH concentration and the nature of the extracted chemical compounds; some of the common techniques used in this context are HPLC-UV and LC/MS/MS. Recently, the solid phase extraction (SPE) was widely used to clean up the final process; this is a very simple and inexpensive tool, but its silica compound is susceptible to acids.

The Environmental Protection Agency (EPA) of the US provides legal guidelines related IMI herbicides residues which range from 0.01 to 100µ/mL (León et al., 2018). Furthermore, although IMI-herbicides are not considered carcinogenic to humans, they can cause eye and skin irritation from misuse. According to EPA classification, imidazolinone is a “Group E” compound, with no evidence of mutagenic potential related to humans. This is based on the experiments that have been carried out on animals (American Cyanamid 2000). Nevertheless, Koutros et al. (2015) recently reported that there is an increased risk and an association of bladder cancer with the use of two IMI-herbicides (imazethapyr and imazaquin) in the field.

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CHAPTER 3: METHODOLOGY

3.1 Evaluation of Weedy Rice Resistant Status to OnDuty® Herbicide

3.1.1 Weedy Rice Collection

Seeds from a total of 17 WR (*Oryza sativa* L.) populations were hand-harvested at maturity in October 2016 (off-season) from Sawah Sempadan rice district of IADA Barat Laut Selangor (N 3°25'35.0724", E 101°10'36.1704"). This area was observed to have a serious WR infestation despite many of the farmers applied CPS system to their farm (Mazlan et al., 2016). Seeds were hand-threshed, and the seed phenotype as awn, pericarp, and hull colour was recorded and placed into an individual paper bag. Seeds were air-dried at room temperature for 3 days (d) before placed in a -4 ˚C refrigerator for further experiments (to test for resistance to OnDuty^{TM} herbicide).

3.1.2 Seed Bioassay with Different IMI-Herbicide Concentration Application

The resistant of WR seeds to IMI-herbicide was tested by standard germination method (Dilipkumar et al., 2018) using a half, single, and two-doses herbicide application. A sample of ~30 seeds from each WR population was distributed in standard petri dishes a (9-cm petri dish diameter) moistened with a Whitman no. 1 filter paper in an incubator set at 40 ˚C overnight to break the dormancy. This will eliminate the possibility of false negative germination data. Seeds on each petri dish with three replications were wetted with ~5 ml concentrated (2.2 g/L) for two doses, commercially concentrated (1.1 g/L) OnDuty^{TM} herbicide for single-dose application and half concentrated (0.56 g/L) herbicide for a half-dose application. distilled water as applied to the seeds of control treatment. Samples were placed in an incubator set at 30 ˚C and 100% relative humidity in the light. Germinated seeds were determined by the emergence of radical or coleoptiles. The germination rate and a number of the viable

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seedlings (green seedlings) were counted at 1, 3, 5, 7, 10 and 14d after imbibition as tabulated in Appendix A: section (a).

3.1.3 Effects of IMI-Herbicide Application on Plant Growth

Induced non-dormant weedy rice population was germinated in an incubator to synchronize germination. The healthy seedlings were transferred into the rice water solution (Yoshida et al., 1976) in the greenhouse for two weeks to ensure seedling growth. Seedlings were transplanted in pots, with 30 plants per pot (40cm x 40cm x 25 cm) filled with a mixture of local clay and greenhouse medium with three replications. Complete Randomized Design (CRD) was used for this experiment. The seedlings were carefully thinned to ~25 per pot during 2-leaf stage. The IMI-herbicide at 220 g/ha concentration was applied to the plants at 3-leaf stage using hand-held spray. The plant response (injury and death) to the treatment was recorded as in Table 3.1 for every three days after the herbicide application.

Table 3.1: Herbicidal injury scales and its description.

Scale	Characteristics of Plants
0	Healthy plant with no herbicide injury symptom.
1	Yellowish at some part of leaf tip
2	Small percentage of leaf tip turned yellow
3	Large percentage of leaf tip turned yellow
4	Number of green leaves is higher than the yellow.
5	The number of yellow and green leaves are almost equal
6	Number of yellow leaves is higher than the green.
7	High percent of leaves are yellow
8	Some leaves yellow to green and some still green
9	Large percent of plant leaves die but very little green
10	Complete plant death

Adapted from: (Burgos et al., 2014).

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The second IMI-herbicide spray was applied to the plants at 21 days after first application with an increase dosage to 440 g/ha and the plant response was recorded for subsequent 3 days. No application of herbicide for control treatment. All materials were grown with natural temperature, humidity, and day length in the greenhouse during the experiment. Plants were watered approximately, every two days and keep it about 1-inch depth and standard fertilizer (N15:P15: K15) was applied two times at 20 and 40 days at rate after transplanting. Morphological characteristics Table 3.2 were measured and recorded when plants were at 90 days.

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Table 3.2: Morphological traits selected to characterize weedy and cultivated rice populations.

Traits	Code	Descriptions
Germination rate	GD	Rate of germinated seeds after the protrusion of the radicle from the caryopsis
Plant height (cm)	PH	Height from the base (soil surface) to the tip of the highest leaf.
Number of tillers	NT	Number of tillers per plant counted 90 days after seedling
Seed shattering	SS	Ratio of shattered seeds weight against the total seeds weight per panicle.
Panicle length (cm)	PL	Measured from the panicle base to the tip
Seeds length (mm)	SL	Average length of 15 well-developed seeds measured at maturity stage
Flag leaves erectness	FLE	Scored as erect (1), semi-erect (2), and open panicle (3)
Flag leaf width (cm)	FL	Measured at the widest portion of the blade (cm)
Leaf length (cm)	LL	Measured from beginning of leaf to the end.
Seed width (mm)	SW	Average width of 15 well-developed seeds measured at maturity stage
Heading date	HD	Days to the first panicle emergence.
Pericarp colour	PC	The colour of the pericarp
Hull colour	HC	The colour of the seed coat
1000grain weight	GW	Weight of 1000 mature grains.
Awn length	AL	Average length of awn of 20 seeds
Panicle type	PT	Scored as erect (1), semi-erect (2), and open panicle (3)

*Source: modified from (Mispan et al., 2013)

3.2 Development of a Method to Determine IMI- Herbicide from Soil

3.2.1 Chemicals, Reagents and Apparatus

Standards of imazapyr (99.5% purity) and imazapic (99.9% purity) were purchased from Sigma-Aldrich (USA), while formic acid (85%), methanol 99.9% (HPLC grade)

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and acetonitrile 99.9% (HPLC grade), acetic acid-ACS reagent (Fisher), formic acid-98% (EM Science), Sodium phosphate (Fisher), hydrochloric acid 6N (Fisher), phosphorous acid dichloromethane (DCM) 99.9% (HPLC grade), and Rotary evaporator were purchased from Sigma Aldrich (Germany). Ultrapure water was obtained from a Milli-Q Direct UV3® system (Millipore, USA), and was further purified by passing it through a 0.2 µm Whatman filter paper. The HPLC 1100 series fitted with a UV detector was used. The HPLC column used in this work was a Zorbax RX–C18 (4.6 × 250 mm, 5 µm). Its temperature was maintained at 30 °C. Centrifuge-Dupont Sorvall Model RC-5C, centrifuge bottles with cap 45 ml polypropylene (Kontes Scientific), vortex mixer (Labmart 3000), Thermo-ultra-sonic, analytical balances (AUW-220D and UX-420H from Shimadzu, Japan), 0.22 µm nylon filters, glass vials with capacity of 2 mL (Agilent, USA), and screw-capped polypropylene tubes (45 ml, Germany), DSC-186 ml tubes 500 mg (6 cm × 3 cm) SPE cartridges (supelco), anhydrous sodium sulfate, and a vacuum pump were all used in this work as well.

3.2.2 Stock Solution and Working Standards Preparation

Standards stock solutions of the herbicides imazapyr and imazapic were individually prepared in methanol at concentrations of (100 µg/ml), respectively, from (1000 µg/ml). Different fresh diluted solutions were prepared as 0.1, 0.5, 1, 5, 10, and 20 µg/ml, and diluted in methanol. All stock and working solutions were stored at -18 ºC in the dark (Marcia, 2014). Then, each of these solutions was injected (17 µL) into the HPLC system, at 251 nm, and peak areas were recorded and plotted versus the concentration of the herbicides.

Therefore, for 100 µg/mL mean 0.1 mg/mL, we need to prepare 5 mL from the two standards Imazapic and Imazapyr = 0.5 mg/5 mL.

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$$M1 \times V1 = M2 \times V2$$

$$100\ \mu g/mL \times 5\ mL = 1000\ \mu g/mL \times V2$$

The solutions were prepared at 0.1, 0.5, 1, 5, 10, and 20 µg /ML and diluted in acetonitrile.

3.2.3 Locations of Soil Samples

Sawah Sempadan-Tanjung Karang district is located on (N 3°25’35.0724”, E 101°10’36.1704”) in Kuala Selangor, Malaysia as shown in Figure 3.1. The soil samples were collected on November 2016/2017 from these fields, because the farmers there have been using IMI herbicides since its introduction in Malaysia in 2010 (Azmi et al., 2012). This area is the most prosperous agricultural district in Malaysia, and has many hectares of paddy rice (Mazlan et al., 2016).To determine the final IMI residue, the soil samples were collected after harvest time, which was ~90 days after IMI was sprayed on Clearfield rice crop.

3.2.4 Soil Collection and Preparation

The sample preparation process which involves extracting the analyte is very important and crucial. To determine the herbicidal residues from the soil samples, the samples were taken systemically from a randomly chosen area from three Clearfield® rice fields that were exposed to the herbicides. The basic approach is to analyse the depth intervals of the soil’s samples for each field. Each sample was within 0 – 20 cm and 20 – 40 cm, about 30-m distance between each two samples was taken with the helical shape method as shown in Figure 3.2. A 20 soil samples were taken, and ~500-gram soil samples were collected using special auger for collection of the soil samples for increased control and were stored in sterile zip lock polyethylene bags and coded with special code waterproof stickers.

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Three random samples were selected (two from each field), then one sample was selected randomly for examination, while the rest were stored in a refrigerator at a suitable temperature for subsequent analyses. The samples were air-dried in the special room at 35 °C for up to 5 days, grounded with a mortar and electronic machine, sieved through stainless steel sieve (2.0 mm) and stored at 4 °C. A 100 g of homogenized soil samples was stored in polyethylene bag at a temperature of ~15 °C until it was analysed for herbicidal residues. The soil physio-chemical characteristics were analysed for three random samples, and the basic properties of these soils was analysed in the lab in university Malaya. Standard methods used for determining Physical and chemical properties. Soil texture (Sand%, Silt%, and Clay%) by soil texture triangle (appendix b), pH was measured by 2 g soils sample mixed 20 ml distilled water or 50 ml soil with 50 ml water and shake around 20 mints, then calibrate the pH meter with buffer solution, then measure the samples with pH meter. Organic matters were measured by using a demonstration/measurement method for understanding light fraction organic matter in soils (George, 2013). 100 g soil was put in 2 mm sieve and water added with slightly moving because not to break down the large organic matter. Then the mixture transferred to 250-micron sieve, then refer the mixture in to small measuring beaker and pour again in to 250 micron sieve to transferred fine filter. At the end organic matter weighed after air drying.

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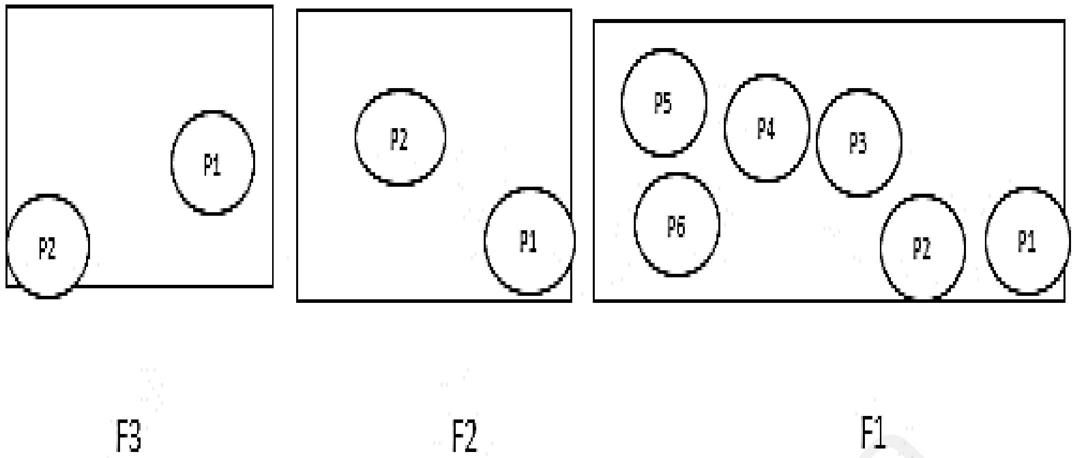

Figure 3.1: Soil sampling overview (random samples were selected with helical shape designed).

Table 3.3: Soil texture characteristics of three locations soil.

Locations	Depth (cm)	PH	Moisture %	Sand %	Silt %	Clay %	OM* %	Soil type**
Site A	0-20	6.21	38	39.0	29	30.0	2.0	Clay loam
	20-40	6.70	33					
Site B	0-20	6.81	44	24.6	35.7	39.2	1.3	Clay loam
	20-40	6.61	57					
Site C	0-20	7.10	38	25.0	35.0	38.0	1.9	Clay loam
	20-40	6.94	59					

*OM: Organic matter
**Soil type according to soil texture triangle

3.2.5 Soil Extraction Procedure for IMI- Residue Level

Analyses of the samples of soil were carried out using the modified extracted published methods proposed by (Krynitsky et al., 1999; Ramezani et al., 2009). About 5 ± 0.001 g of randomly homogenized soil sample weight with electronic scale, it provides appropriate and representative amount as some authors use (Martins et al., 2014), then the portion soil was placed in 250 ml centrifuge tube (polypropylene), 150 ml of extracted 0.5 N NaOH , then sample was kept 45 minutes in an end-over-end shaker at 30 °C to assess the homogeneity of the sample, 10 ml methanol was added to precipitate humic acids and sonicated for 10 minutes, then centrifuge the sample for 10 minutes at 7000 rpm to remove particulates.

The solution filtered and adjusted to pH=2 by 6N HCL. Clean-up is necessary to shift down the detection limits of methods and to avoid interferences from the matrix. The suspension was left to stand at room temperature for 10 minute until analysis, then transfer to 500 ml separatory funnel and extracted with 50 ml dichloromethane (DCM) for two times then combined and transferred to special flask and DCM dried by anhydrous Na_2SO_4, then filtered by activated charcoal, then the residues evaporate at 65 °C with rotary evaporator near dryness, residues was diluted with about 2 ml with Methanol:0.1% Formic acid (1:1) then DSC-18-6ml cartridge 500 mg (6 cm × 3cm) (Supelco), of adsorbing material conditioned with 3ml of each of the solvents methanol, Acetonitrile and H_2O.

After that the sample loaded through cartridge under vacuum pump, the vacuum was reduced slowly. The analytes were washed by 9 ml H_2O and 6 ml (60/40) (H_2O: acetonitrile), lastly, the vials were put in the vacuum apparatus and the cartridge eluted with 3 ml methanol/0.1 formic acid. Then the residues filtered through 0.22 µm poly tetrafluoroethylene (PTFE) membrane transferred to a 1.5 ml HPLC auto sampler vial, and storage at 4 °C until further use through HPLC device. Then filtered through 0.22 µm polytetrafluoroethylene (PTFE) membrane, transferred to a 1.5ml HPLC auto sampler vial, and storage at 4 °C until further use through HPLC device as (Figure 3.3).

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3.2.6 Accuracy, Limit of Detection (LOD), and Limit of Quantitation (LOQ)

LOD is the lowest concentration that can be detected, and it could be determined by a statistical method. This could be achieved by measuring the more dilute concentrations of analyte. These concentrations are expected to produce a response of ~3 times the background noise. LOD should be between 3 - 10. The LOQ is expected to behave similarly, but with a ratio of 10 times the background noise. Recovery studies in soils samples were conducted using the standard calibration curves equation.

These herbicides were spiked to blank soils (clean soils free from herbicides), taken from the land around University Malaya (N 3°7'8.9328" E101°39'28.494"). This soil was selected due to its similar characteristics with the tested soil samples. Acetone was added to 5 g of dried homogenized soil at different concentrations, and left to dry for 48h at room temperature to activate the introgression and equilibrium while slowly evaporating the solvent (Laganà et al., 2000; Rebelo et al., 2016), followed by extraction and analysis using HPLC-UV.

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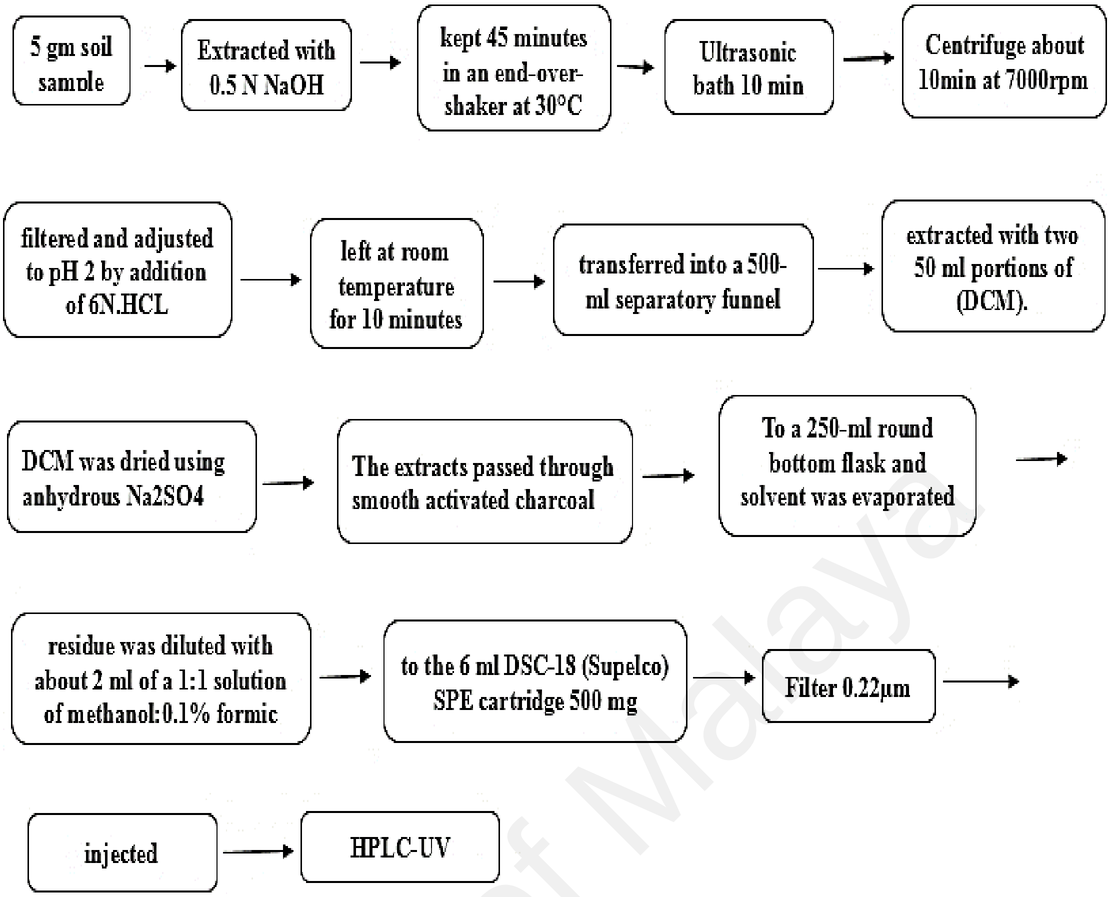

Figure 3.2: Flow chart of methodology for the extraction of IMI-herbicides from soil using Solid Phase Extraction (SPE) by DSC-18 sorbents.

3.2.7 Storage Stability

Solution stability of the test substances after preparation according to the test method should be tested related to the same method used in the lab. The stability of the standard solutions and sample extracts needs to be checked, and this is done by analysing these solutions over a period under different storage temperatures. Analyte decomposition is usually indicated by decreasing analyte peak height accompanied by the appearance of extraneous peaks. Storages stability in this study was conducted at -18 °C with standard solutions at different concentrations and soil samples spiked with 0.1 and 0.5 µg/mL imazapyr and imazapic.

3.2.8 Ruggedness of the Test

It is the effect that functioning and environmental circumstances effect on the analytical outcome. It is the degree of disparity in the final results obtained by the analysis of the same sample under a condition, such as different laboratories, different analysts, and different apparatuses. In the laboratory used for this study there is restricted chance for ruggedness testing other than changing and used multiple HPLC column brand (C_{18} and C_8) and using the same or different solvent from different sources.

3.3 Determination Residues Activity of Imidazolinone Herbicide in Clearfield Rice

The soil samples were taken on November 2016 from three Clearfield rice fields in Sawah Sempadan-Tanjung Karang district. The farmers' field was located at (N 3°25'35.0724", E 101°10'36.1704") in Kuala Selangor, Malaysia. The physicochemical characteristics of the soil were determined for the three fields, the region experiences a sub-tropical climate, with almost high daily rainfall and temperatures. IMI herbicides were used in this area for the past six years.

Field experiments were conducted at three different locations. 20 samples were collected randomly prior to harvesting the crops, which is equivalent to ~80 days. Soil samples were taken at depth of ~0-20 cm and 20-40 cm, and ~1 kg of soil was collected using an auger. The samples were directly stored in a sterile zip-lock polyethylene bag and coded using a special waterproof sticker. On the same day, the samples were placed in special room at 35 °C under the shade for up to 5 days, then, the dried samples were ground and sieved via stainless steel sieve (2 mm), and stored at 4 °C, see appendix B.

Herbicides standards were purchased from Sigma-Aldrich (USA), with purities of 95.5

and 99.9% for imazapic and imazapyr, respectively. Methanol, dichloromethane (DCM), and acetonitrile 99.9% (HPLC gradient) (Fisher), acetic acid, ACS reagent (Fisher), formic acid, 98% (EM Science), and all materials for the HPLC experiments were purchased from Sigma Aldrich (Germany). Ultrapure water obtained from a Milli-Q Direct UV3® system (Millipore, USA), was also filtered through a 0.2 µm Whatman filter paper. Other equipment includes a DuPont Sorvall Centrifuge (Model RC-5C), centrifuge bottles with cap 45ml poly-propylene (kontes Scientific), vortex mixer (Lambert 3000), and Supelco SPE cartridges.

The soil samples were analyzed using a simple modified extraction method proposed by (Krynitsky et al., 1999; Ramezani et al., 2009). In this procedure, (5 ± 0.001g) of randomly homogenized soil sample was weighed, then a portion of it was placed in a 250-ml centrifuge tube (polypropylene), followed by the addition of ~150 ml of 0.5 N NaOH. The samples were then stored, for 45 minutes in an end-over-end shaker at 30 °C to allow for equilibration. 10 ml of methanol was added to the precipitate of humic acid, followed by sonicating the samples for 10 minutes, then the samples were centrifuged for 10 minutes (at 7000 rpm) to remove particulates.

The solution was filtered and adjusted to a (pH 2.0) using 6N HCl. The suspension was left at room temperature up till analysis, where the sample solution was transferred to a 500-ml separatory funnel and extracted using 50 ml dichloromethane (DCM) twice, then mixed and transferred to the flask. DCM was dried using anhydrous Na_2SO_4, and the solution was passed through a smooth activated charcoal column. The resulting solution was transferred to a 250-ml round bottom flask and evaporated at 65 °C using a rotary evaporator at a slow flask motion to near dryness.

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The residue was diluted using ~2 ml ofmethanol:0.1% formic acid (1:1), then DSC-18-6 ml cartridge 500 mg (6 cm × 3 cm) (Supelco) of adsorbing material conditioned with 3 ml of methanol, acetonitrile, and H_2O. The sample was then loaded through the cartridge under vacuum, which was gradually reduced. The analytes were washed using 9 ml H_2O and 6 ml (60/40) (H_2O: acetonitrile). Finally, the vials were placed in a vacuum and the cartridge eluted with 3 ml methanol:0.1% formic acid solution.

The residues were then filtered through a 0.2 µm polytetrafluoroethylene (PTFE) membrane, transferred to a 1.5 ml HPLC auto sampler vial, and stored at 4 °C until the HPLC analysis. IMI standard solutions were individually prepared in acetonitrile at concentrations of (100 µg/mL), respectively, by dilution from a 1000 µg/mL stock solution. Afterward, other fresh diluted standard solutions were prepared by dilution (0.1, 0.5, 1, 5, 10, and 20 µg/mL) in acetonitrile. All stock and working solutions were stored at -18 ºC in dark conditions (Marcia, 2014).

IMI residues were analyzed using an HPLC-UV system consisting of Shimadzu high-performance liquid chromatography with LC-10AT pump and SPD-20A interfaced with LC software, and fitted with variable wavelength UV detector. The HPLC column used was C_{18} (4.6 × 250 mm, 5 µm) (USA). The gradient solvent program used mobile phase A (acetonitrile 100%) and mobile B (water, including 0.1% of acetic acid) (pH = 2.8). The initial gradient program was: 30% A (0-1 min), 30-45% (1-5 min), and 45-35% (5-13 min). A 17-µg aliquot of the samples was injected into the column.

Linearity calibration curves were constructed using different standard concentrations (0.1, 0.5, 1, 5, 10, and 20 µg/mL). The concentrations of both IMI herbicides were determined by comparing the peak area of the samples that deduced from the calibration curve. Also, the limit of detection (LOD, μg/mL) was determined as the lowest concentration that responded thrice to the baseline noise. Limit of quantification (LOQ) (μg/mL) was determined as the lowest concentration of the herbicide providing a response 10 times of the baseline. The spiked soil samples were fortified with standard solutions (0.1, 0.5, 5,10 μg/mL).

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CHAPTER 4: RESULTS

4.1 Weedy Rice Herbicide Resistant Status

4.1.1 Effects of IMI-Herbicide Application on Seeds Germination

The effect of various IMI-herbicides half, commercial, and two doses at 0.56, 1.1, and 2.2 g/L respectively, on WR seed germination and early seedling development varied greatly. 5-ml of IMI-herbicides concentrations were applied to WR seeds in petri dishes in the lab. Each treatment and control consisted of three-replications. At the same time, in control dishes, 5-ml of distilled water was substituted for the IMI- herbicide treatment. Germination counts were continued for 14 days after treatment with IMI-herbicide, the development and viability of WR seedlings was studied. WR control germination was significantly decreased by treatments with the pre-emergence IMI-herbicide.

IMI-Herbicide had a significant effect on percent germination, mean germination time and, seedlings length. The inhibition of WR seed germination and seedling growth did not completely stop as herbicide dose increased. However, with the shift up of IMI-herbicide dose, percent germination decreased obviously and then remained constant. WR seeds which treated with IMI-herbicide showed slower germination than controls in the first three days, but at 7 days' germination was almost as high at half and commercial doses as in controls. However, at about 10 days the germination was almost as the other doses or control (Figure 4.1 and Table 4.1). WR seed germination was not completely inhibited by the three doses, however, some seeds failed to break seed coats after treatment and can't proceed germination process.

In all treated populations that germinated, 88% of the total germination was completed after 3 days, 84% and 27% at half, commercial, and two doses. About 93% of the treated seeds with half- dose had germinated at 7 days, 89% for commercial dose and 72% for two doses. WR seeds of controls showed 100% germination after 7 days, similar germination value (100%) were obtained for some WR populations in half-dose as MWR_Pop07, MWR_Pop10, and MWR_Pop11.

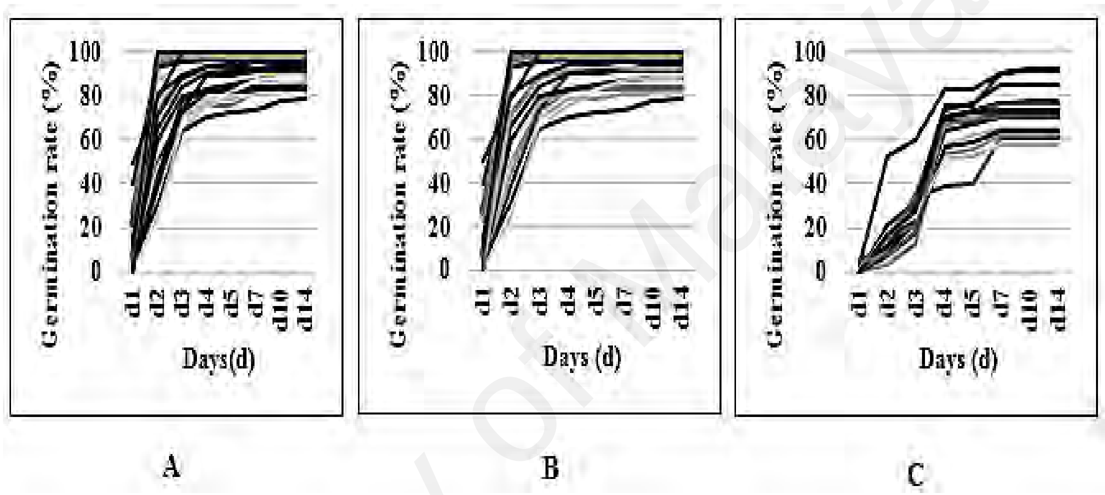

Figure 4.1: Effects of IMI-herbicide at various dosages of (A) half-dose, (B) one-dose, and (C) two-doses on the germination ratio of WR seedlings populations from Sawah Sempadan rice district of IADA Barat Laut Selangor at 14d after treatment.

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Table 4.1: Germination rate (%) of 17 weedy rice population at 14d after the application of half, one-dose, and double dose of imidazolinone herbicide.

Population	Germination rate (%)		
	Half dose	**One dose**	**Two doses**
MWR_Pop01	91	91	78
MWR_Pop02	87	82	73
MWR_Pop03	84	81	65
MWR_Pop04	99	94	70
MWR_Pop05	91	87	62
MWR_Pop06	97	96	85
MWR_Pop07	100	94.	64
MWR_Pop08	99	93	76
MWR_Pop09	99	90	72
MWR_Pop10	100	99	76
MWR_Pop11	100	95	92
MWR_Pop12	96	84	91
MWR_Pop13	85	78	61
MWR_Pop14	95	88	73
MWR_Pop15	96	88	58
MWR_Pop16	98	93	71
MWR_Pop17	97	95	70

However, in one dose 94%, 99%, and 95% and 64%, 76%, and 92% in two doses respectively. Whereas this herbicide significantly influenced seed germination, however, did not suppressed germination process. WR seeds germination was affected by herbicide rate. The average seeds germination varied from 95%, 91%, and 74% for a half, one, and two doses applications, respectively at the end of 14days (Figure 4.2). The three treatments significantly decreased with a p-value (<0.001) in germination rate when herbicide dosage increased from half to one dose, and two- doses as shown in Table 4.2 and Table 4.3.

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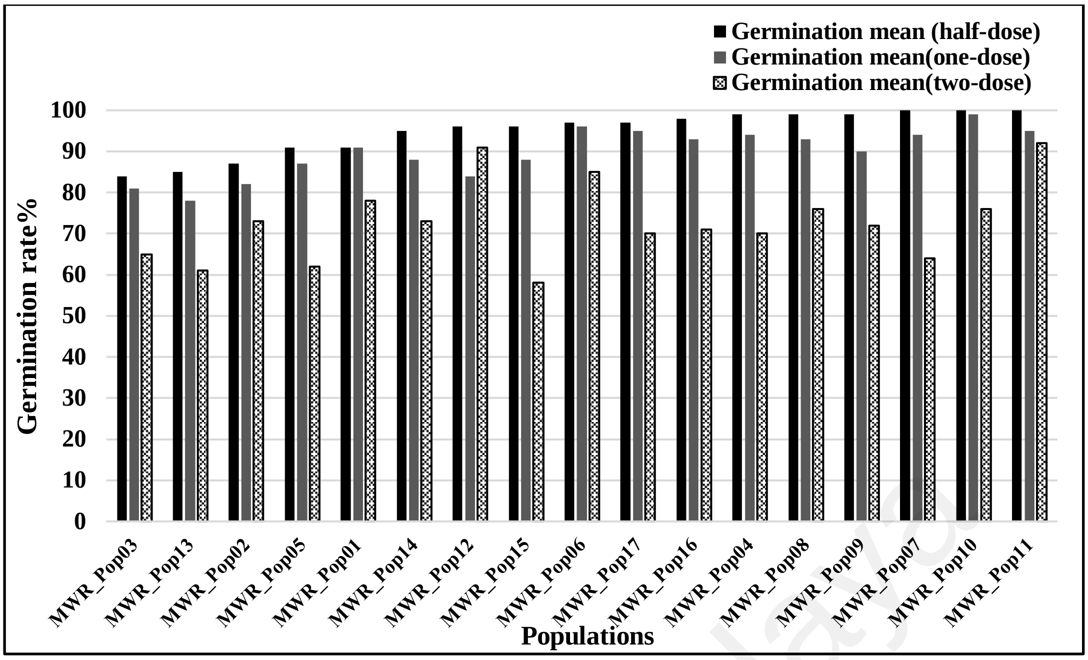

Figure 4.2: The average germination rate of 17 weedy rice population from Sawah Sempadan rice district of IADA Barat Laut Selangor for 14 days after the application of half, one-dose, and two doses of imidazolinone herbicide.

The effects of IMI-herbicides at various dosages on WR seedlings development, from the figure shows that, increasing the dosage did not stop complete seedling growth. This indicate that there was a carry-over effect of IMI- herbicides sprayed to WR plants seeds in terms of final percentage of viability. WR control efficiency was increased by decreasing the seedlings height at 14d.

Table 4.2: Summary of Anova-single factor for germination rate.

Groups	Count	Sum	Average	Variance
Germination (50% dose)	17.00	1608.57	94.62	31.27
Germination (100% dose)	17.00	1534.94	90.29	35.61
Germination (200% dose)	17.00	1240.50	72.97	96.10

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Table 4.3: Source of variation from Anova-Single factor of germination between doses.

Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit.***
Between Groups	4462.58	2.00	2231.29	41.07	<0.001	3.19
Within Groups	2607.86	48.00	54.33	-	-	-
Total	7070.44	50.00				

*SS: sum off square; *df: degree of freedom;* MS: mean square; F: F, statistics; P-value: probability (0.05); *F crit:* critical value.

The seedlings height of treated plants had average value of 2.05, 1.51, and 0.95 cm for 50%, 100%, and 200% doses, respectively. Increasing the dosage of IMI-herbicide from 0.52 g/L to 2.2 g/L appeared to have a significant influence on seedlings plant height (P<0.001). The greatest reduction in WR seedlings growth and germination occurred at IMI- herbicide rates in 2.2 g/L (440 g/ha). However, this rate exceeds those recommended (1.1 g/L) (220 g/ha) and WR control. The interactions mechanism between IMI- herbicide and growth stage (hypocotyl) showed significantly impact with p-value <0.001. In addition, the percentage of seedling green and height for the two-cultured rice (Cult_CPS_1 and Cult_MRW220) was significantlyp <0.001 decreased by increasing the dose of IMI-herbicide.

WR control efficiency was significantly decreased p < 0.05 (0.01) by treatments with the pre-emergence IMI-herbicide at 14d in the lab (Table 4.4 and Table 4.5). From the results above, the seeds germination and seedling growth were affected with increasing the IMI- herbicide dose, the percentage of germination have slowly decreased. Green seedling plants are an indicator for viable WR population, however, the viability of the seedlings for most populations was low (<10%) except MWR-pop11 (15%) for two-doses Figure 4.3, Figure 4.4, Figure 4.5, and Figure 4.6. The IMI-herbicide had a strong impact on the WR seed viability, so that about 50% of WR populations was completely

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stopped by doses higher at 2.2g/L of herbicides and the significant difference was seen among the treatments, also, see Appendix A.

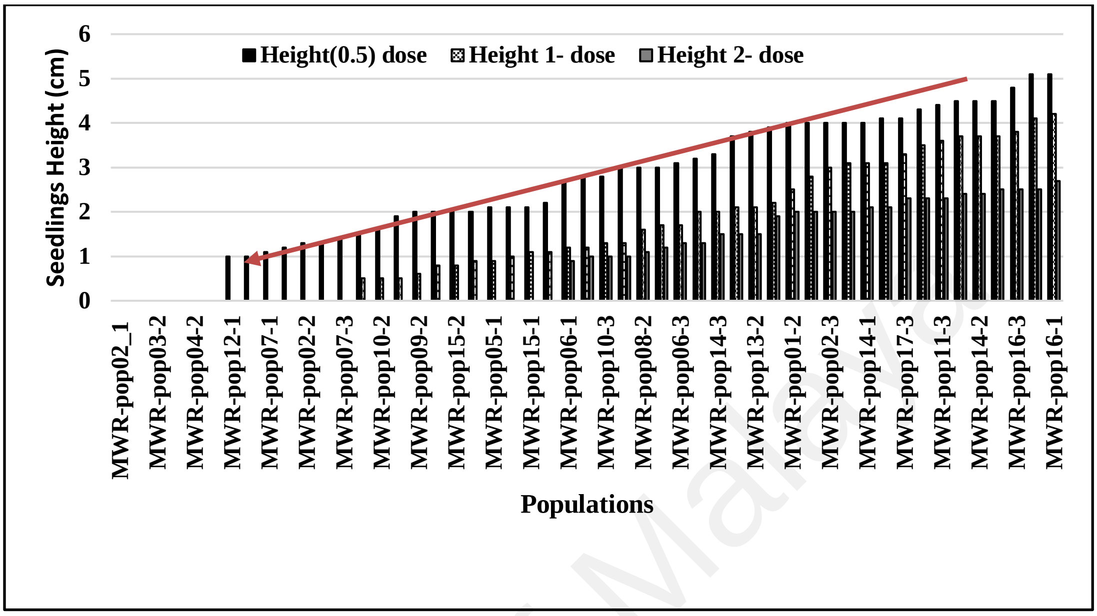

Figure 4.3: Effects of IMI-herbicides at half, one, and two doses applications on the development of WR seedlings height.

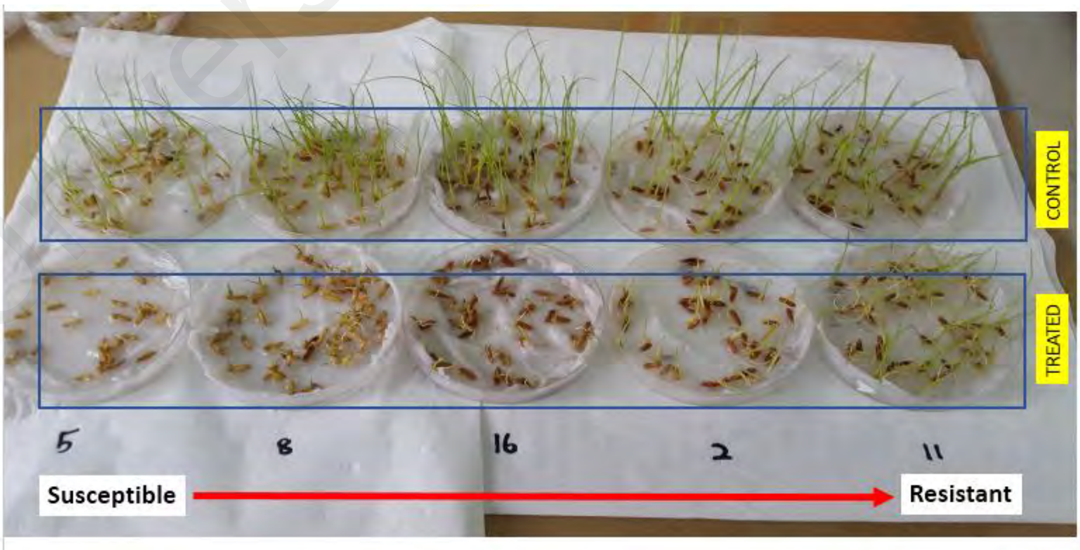

Figure 4.4: The viability of weedy rice plants to commercial (one) dose of IMI-herbicide after 14d.

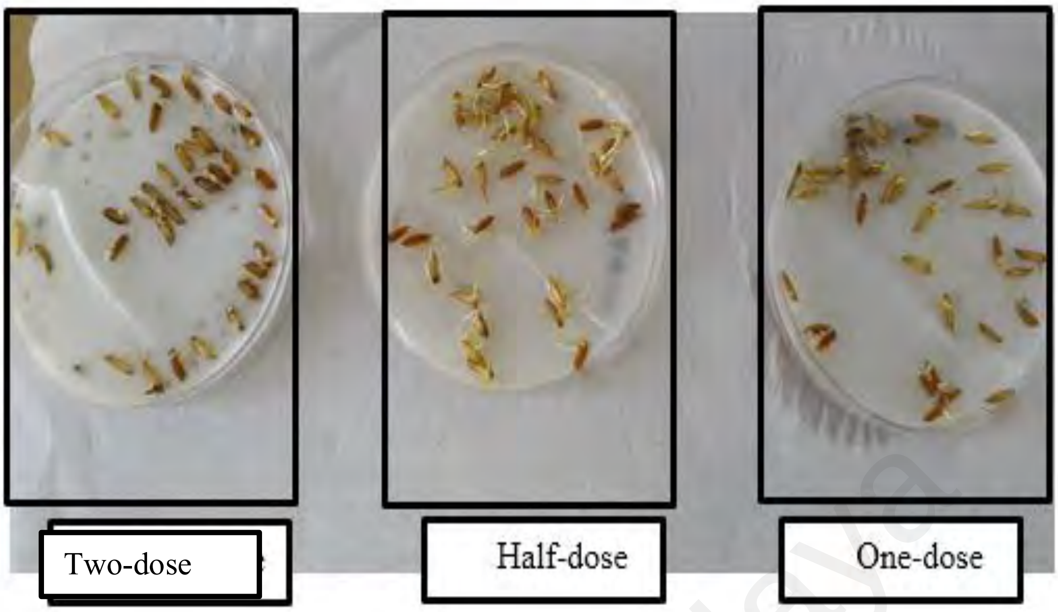

Figure 4.5: The viability of weedy rice plants to half, one, and double dose of IMI-herbicide after 14d.

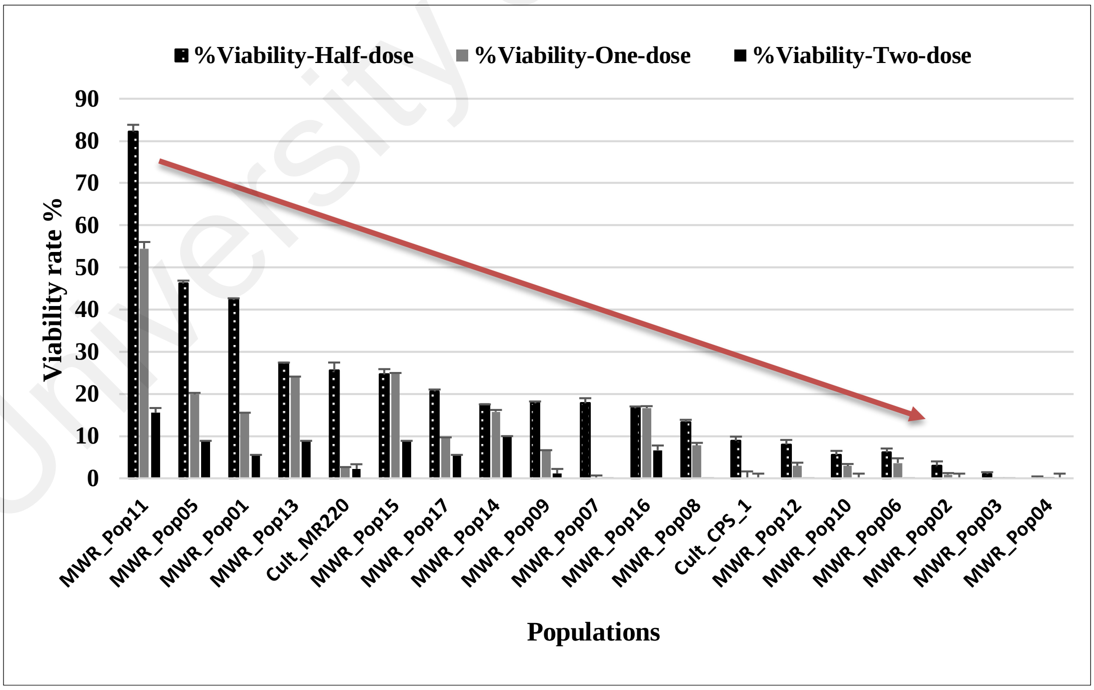

Figure 4.6: The effect of Imidazolinone herbicide on the viability of weedy rice seedlings at half, one, and two doses of IMI-herbicide after 14d.

Table 4.4: Summary of Anova-single factor for seedlings viability.

Groups	Count	Sum	Average	Variance
Viability -200%-dose	17	71.11	4.18	24.31
Viability -100%-dose	17	206.08	12.12	191.73
Viability -50%-dose	17	350.02	20.59	424.46

Table 4.5: Source of variation from Anova-Single factor of seedlings viability between doses.

Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit.***
Between Groups	2288.67	2.00	1144.34	5.36	0.01	3.19
Within Groups	10248.06	48.00	213.50			
Total	12536.73	50.00				

*SS: sum off square; *df: degree of freedom;* MS: mean square; F: F, statistics; P-value: probability (0.05); *F crit:* critical value.

4.1.2 Effects of IMI-Herbicide Application on Weedy Rice Growth

Survival of WR population varies from 8% to 100% at one-dose. Four susceptible populations (MWR-pop06, MWR-pop07, MWR-pop08, and MWR-pop11) were totally controlled by IMI-herbicide at the commercial dose (recommended field dose) respectively (Figure 4.8 and Figure 4.9). Among the suspected resistant seedlings plants, thirteen WR populations survived with one-dose, eight of them with survival percentage above the 80%.

Both treatments showed significant difference in seedling plants response for both doses (*P*= 0.002).The performance of rice cultivars revealed that Cult_CPS_1 is not affected for both doses and showed 100% survival as the opposite of Cult_MRW220 which died from the one dose of IMI herbicide.

Therefore, in the Figure 4.7 and Figure 4.8, these plants had survived at both the two doses applications of IMI-herbicide. Among the 17 collected weedy rice plants produced about 22% to 100% resistant. Two weedy rice populations were highly resistant to the two doses of IMI-herbicide with no visible injury (MWR_Pop15 and MWR_Pop16) was revealed 100% resistant. Also, 4 weedy rice populations (MWR_Pop03, MWR_Pop12,MWR_Pop13, and MWR_Pop17) was revealed (82% to 97%) resistant. Lastly, 1 weedy rice population was revealed low resistant (MWR_Pop14) about 22%. These resistant weedy rice plants also, were classified in to three categories as (high-level resistance, medium- level resistance, and low-level resistance) according to the chlorotic plants leaves percentages as shown in Table 4.6.

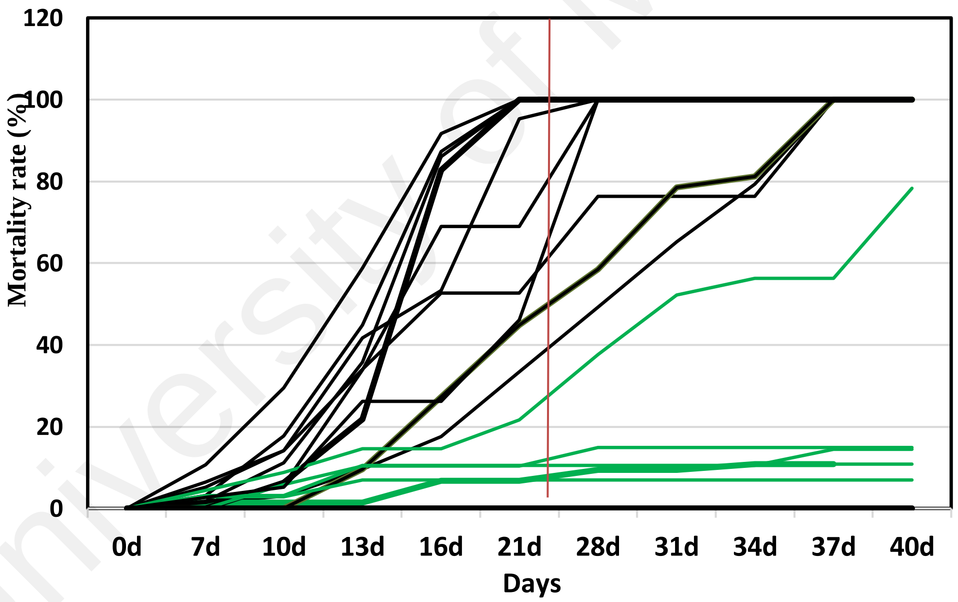

Figure 4.7: Screening of weedy rice resistant population at one dose (before 21d) and two doses (after 21d) of IMI-herbicide. Green lines indicate population with lower mortality rate for both treatments.

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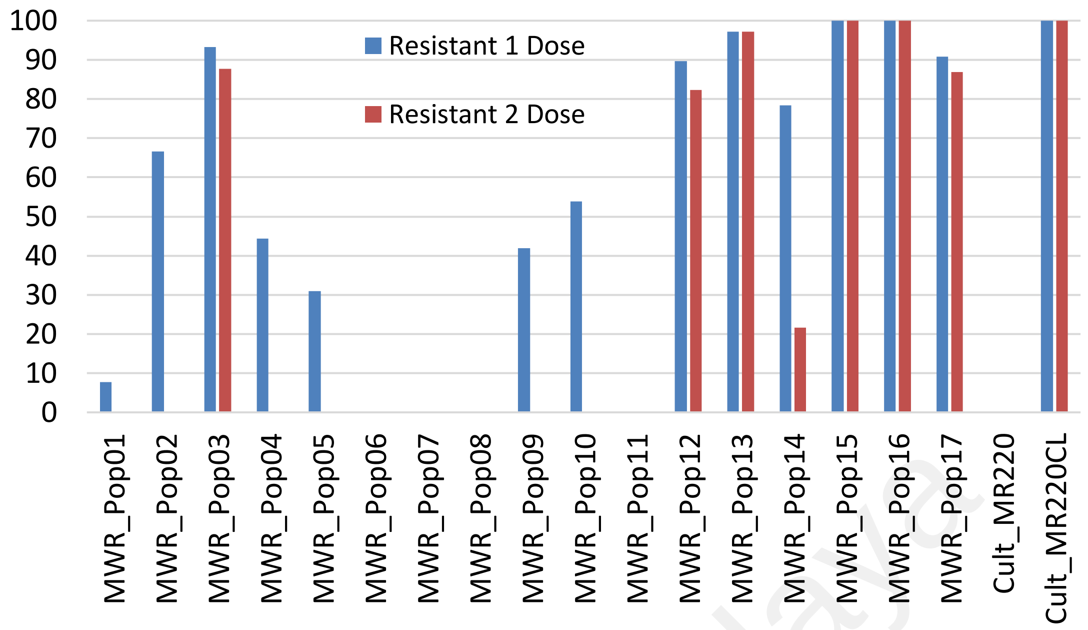

Figure 4.8: Evaluation of resistant population of weedy rice with one and two-dose of IMI-herbicide based on survival percentage.

Table 4.6: Classification of selected resistant weedy rice based on leaves chlorotic percentage after 2-dose treatment.

Weedy rice population	Chlorotic* (%)	Classification
MWR_Pop03	12	HLR
MWR_Pop12	18	HLR
MWR_Pop13	3	HLR
MWR_Pop15	0	HLR
MWR_Pop16	0	HLR
MWR_Pop17	13	HLR
MWR_Pop14	78	MLR

*HLR (high level resistance): < 15% of chlorotic plants leaves; MLR (medium level resistance): 15%-85% of chlorotic plants leaves; LLR (lowlevel resistance): > 86% of chlorotic plants leaves.

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4.1.3 Weedy Rice Phenotypic Association with IMI-herbicide Response

Regarding the plant height, all the resistant weedy rice population was significantly (*P*=0.04) taller than commercial cultivars except MWR-pop03 (Figure 4.9). Most of resistant weedy rice plants were >100 cm height except MWR-pop03 which was 97 cm. The resistant weedy rice was typically ranged from 97 cm to 151.5 cm, which was taller than the two commercial rice cultivars MR220CL and MR220 with 101 and 105cm height, respectively (Figure 4.10). This is also in agreement with a study by Burgos et al., (2014). It is exerted that 85% heading at less than 95 days, most of resistant weedy rice have panicles length (22.5-24 cm) and classified to intermediate to open panicles. Where the panicles lengths of commercial rice varieties (Cult_MRW220 and Cult_CPS_1 from 23.37 to 23.5 cm. The resistant weedy rice had leaf length range (65 to 73cm) and width range (1.2 to 1.34 cm) as tabulated in (Table 4.7). Sixteen weedy rice morphological characteristics were measured and recorded when plants were at 80-90 days. Based on morphological characterization, there are a level of variations in the weedy rice populations and trait descriptions in the variables of 17 weedy rice populations as tabulated in Table 4.8.

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Table 4.7: Summary of the average of phenotypic descriptions of 17 weedy rice populations and 2 cultivated rice varieties.

Traits*	Mean	Std Dev	Minimum	Maximum
PH	115.7	23.4	77.5	151.5
SH	57.7	26.1	3.3	93.6
PT	2.4	0.8	1.0	3.0
PL	21.6	1.9	18.5	23.9
TN	3.7	1.0	2.0	6.0
LL	61.3	9.0	36.8	73.7
LW	1.2	0.2	0.7	1.5
PC	1.4	0.5	1.0	2.0
FE	0.7	0.9	0.0	2.0
HC	1.1	0.2	1.0	2.0
HD	89.8	3.4	82.0	94.0
AN	0.4	0.5	0.0	1.0
AL	0.8	1.2	0.0	4.2
SW	16.3	1.9	13.4	19.4
LS	9.4	0.4	8.7	9.9
WS	2.4	0.0	2.4	2.5
G1D	94.9	5.4	84.1	100.0
GhD	90.8	6.0	77.9	100.0
V1D	12.5	16.1	0.0	54.4
VhD	20.2	19.7	0.0	82.2
S1D	52.4	41.2	0.0	100.0
S2D	35.6	45.9	0.0	100.0

*PH, plant height; SH, shattering; PT, type of panicles; PL, panicles length; TN, tiller; LL, leaf length; LW, leaf width; PC, pericarp colour; FE, flag leaves erect; HC, hull colour; HD, heading; AN, awn presence; AL, awn length; SW, 1000-seeds weight (gm); LS, length of the seed; WS, width of seed; G1D, Germination rate for one-dose; GhD, Germination rate for half-Dose; V1D, Viability one-dose; VhD, Viability half -dose; S1D, Survival one -dose; S2D, Survival 2-dose.

Tillering growth trait is very important agronomic stage in weedy rice plants life. We noted that all resistant weedy rice plant produced a high number of tillers, above 4 except MWR-pop14 which was 3.6 tillers. Where non-resistant weedy plants

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morphotypes produced a low number of tillers less than 4 tillers except MWR-pop11 had 6 tillers. MR220CL2 has no difference in tillering compared to resistant weedy rice.

Generally, the flag leaf for weedy rice was widely diverse between erect as MWR-pop10, intermediate as MWR-pop01, and non-erect, as MWR-pop02. Regarding to awn trait, the MWR-pop01, MWR-pop03, MWR-pop04, MWR-pop10, MWR-pop 17 have awn with diverse length between 1.44 to 4.2 cm. Also, the shattering trait is considered one the main characteristics for weedy rice, from the Table 4.8 showed that the percentage of shattering was between 35% to 93.6%. Most of the weedy rice resistant revealed high shattering except MWR-pop03 was at 38%.

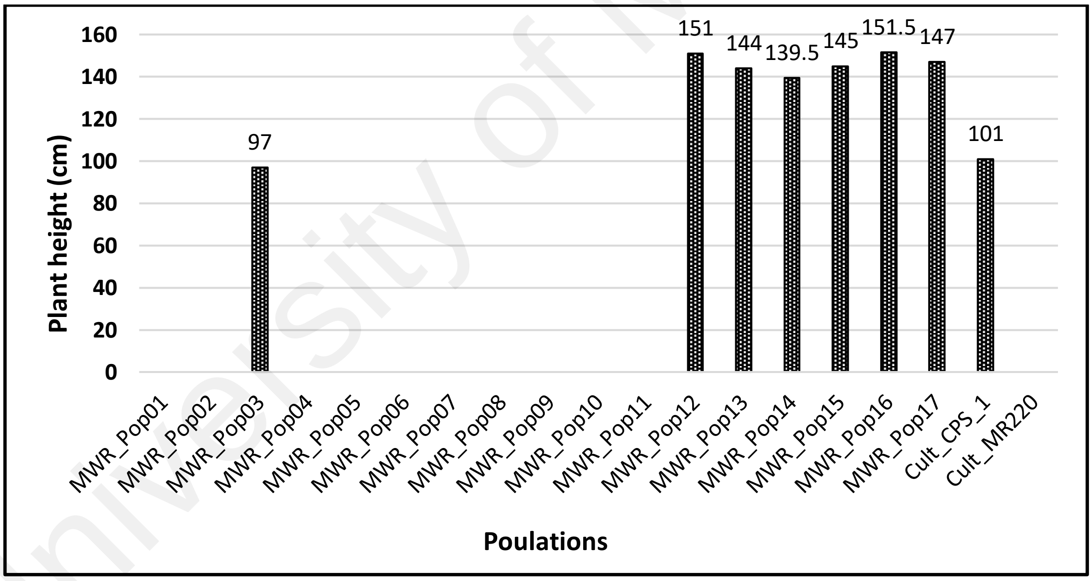

Figure 4.9: Resistant weedy rice plants height at both doses of IMI.

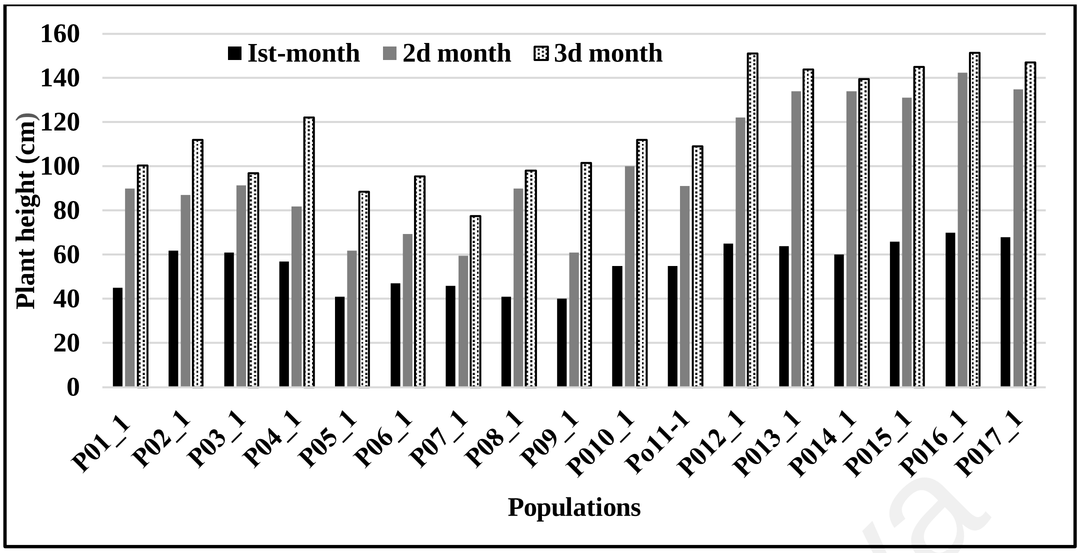

Figure 4.10: Weedy rice plants height growth at interval month.

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Table 4.8: Phenotypic descriptions of 17 weedy and 2 cultivated rice.

ID*	PH	SH	PT	PL	TN	LL	LW	PC	FE	HC	HD	AN	AL	SW	LS	WS
MWR_Pop01	100.5	64	Compact	20.75	2.66	56.11	1.46	Red	Intermediate	straw	92	Awn	1.46	14.3	9.76	2.42
MWR_Pop02	112	54	Intermediate	21.75	2	63.88	0.79	Red	Non-erect	straw	92	Awnless	None	16.1	9.5	2.42
MWR_Pop03	97	38	Open	23.25	4.5	73.66	1.22	Red	Intermediate	straw	94	Awn	2.4	17.41	9.7	2.46
MWR_Pop04	122	48	Intermediate	23	3.33	55.11	1.26	White	Non-erct	straw	93	Awn	1.44	17.4	9.36	2.4
MWR_Pop05	88.5	60	Compact	22.87	3	53.5	0.74	White	Non-erect	straw	92	Awnless	None	14.4	9.74	2.4
MWR_Pop06	95.5	35	Intermediate	22.62	3	66.22	1.02	Red	Non-erect	straw	93	Awnless	None	18.5	9.29	2.48
MWR_Pop07	77.5	67	Intermediate	23.5	2.66	53.33	0.88	Red	Intermediate	straw	93	Awnless	None	13.4	8.77	2.45
MWR_Pop08	98	35.5	Intermediate	23.25	3.66	62.77	1.08	White	Non-erect	straw	90	Awnless	None	17.54	9.35	2.45
MWR_Pop09	101.5	46.5	Compact	23	3.33	36.77	1.08	White	Non-erect	straw	90	Awnless	None	13.7	9.51	2.47
MWR_Pop10	112	64	Intermediate	20	3.33	55.5	1.5	White	Erect	straw	88	Awn	4.2	15.55	9.22	2.53
MWR_Pop11	109	64	Intermediate	23.5	6	65	1.3	Red	Intermediate	Furrowed	88	Awnless	None	14.1	9.45	2.49
MWR_Pop12	151	81.5	Intermediate	22.5	5	66.33	1.06	White	Non-erect	straw	84	Awnless	None	14.9	9.75	2.42
MWR_Pop13	144	93.6	Intermediate	23.87	4.66	68	1.34	Red	Non-erect	straw	89	Awnless	None	17.4	9.59	2.46
MWR_Pop14	139.5	75	Intermediate	22.37	3.66	66.5	1.22	Red	Non-erect	straw	88	Awnless	None	14.2	8.87	2.53
MWR_Pop15	145	91.5	Open	22.25	4.66	65.33	1.22	Red	Non-erect	straw	86	Awnless	None	18.8	8.66	2.46
MWR_Pop16	151.5	77.6	Open	23.75	4.33	71.5	1.2	Red	Non-erect	straw	87	Awnless	None	18.33	9.87	2.41
MWR_Pop17	147	91.3	Intermediate	23	4	73.55	1.3	Red	Non-erect	straw	82	Awn	3	19.41	9.37	2.4
Cult_CPS_1	101	3.3	Compact	23.5	4.33	54.55	1.28	White	Intermediate	straw	94	Awn	1	17.7	9.88	2.45
Cult_MR220	105	6	Intermediate	23.37	3.33	56.88	1.22	White	Intermediate	straw	92	Awn	0.8	15.9	9.47	2.43

*PH, plant height; SH, shattering; PT, type of panicles; PL, panicles length; TN, tiller; LL, leaf length; LW, leaf width; PC, pericarp colour, FE, flag leaves erect;HC, hull colour; HD, heading; AN, awn presence; AL, awn length; SW, 1000-seeds weight (gm); LS, length of the seed; WS, width of seed

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4.1.4 Correlations between IMI-herbicide Weedy Rice Resistant and Phenotypic Relationship

Correlation analysis among the physiological variables of weedy rice shows that the plants height was positively correlated and significant with shattering (<0.001), panicles length (0.02), and tiller (0.04), and leaf length (0.02), width of seed (0.08), flag leaf (0.019), and heading (0.0001). In the same vein, there were positive correlation between panicles length with the tiller and leaves length, (< 0.001) and (0.037). Also, the shattering trait was significant correlated with pericarp colour and heading (0.02) and (0.005) respectively. In the same time, tiller number was significant correlated with hull colour and heading with (0.012) and (0.039) respectively. Also, leaves width was correlated with awn presence and awn length with (0.008) and (0.008), respectively. Lastly, flag leaf was correlated with awn presence (Table 4.9).

However, a strong correlation between plant height and survival at 1-dose and survival at 2-dose with (0.7) and (0.64), respectively and highly significant for both doses with p-value (<0.001). In the same vein, panicles length has correlation with survival 1-dose and survival 2-dose with (0.52) and (0.72) respectively and highly significant for both doses with p-value (0.02) and (<0.001) respectively. Also, tiller number is correlated with survival 2-dose with (0.6) and p-value (0.01). In addition, leaf length is correlated with survival 2-dose with (0.5) and p-value (0.02). The pericarp colour is correlated with germination I-dose with (0.47) and p-value (0.04). Lastly, hull colour is correlated with viability 1-dose and 2-dose with (0.63) and (0.76) p-value (<0.001).

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Table 4.9: Summary of correlation coefficients (*r*) between different morphological traits of weedy rice populations.

Pearson Correlation Coefficients, N = 19, Prob > |r| under H0: Rho=0																						
Traits*	**PH**	**SH**	**PT**	**PL**	**TN**	**LL**	**LW**	**PC**	**FE**	**HC**	**HD**	**AN**	**AL**	**SW**	**LS**	**WS**	**G1D**	**GhD**	**V1D**	**VhD**	**S1D**	**S2D**
PH		0.00	0.26	0.02	0.04	0.02	0.15	0.71	0.02	0.78	<.0001	0.62	0.99	0.09	0.92	0.73	0.95	0.21	0.76	0.72	0.00	0.00
SH	0.65		0.41	0.60	0.30	0.11	0.69	0.03	0.05	0.81	0.00	0.11	0.89	1.00	0.23	0.98	0.62	0.16	0.12	0.38	0.10	0.18
PT	0.27	0.20		0.97	0.73	0.05	0.92	0.98	0.48	0.49	0.24	0.61	0.83	0.56	0.06	0.38	0.21	0.63	0.42	0.49	0.61	0.57
PL	0.54	0.13	0.01		0.00	0.04	0.06	0.78	0.38	0.32	0.06	0.79	0.91	0.35	0.25	0.74	0.77	0.25	0.61	0.41	0.02	0.00
TN	0.48	0.25	0.08	0.76		0.06	0.08	0.97	0.72	0.01	0.04	0.70	0.81	0.41	0.56	0.48	0.76	0.44	0.08	0.12	0.10	0.01
LL	0.54	0.38	0.45	0.48	0.43		0.52	0.02	0.55	0.68	0.09	0.85	0.75	0.01	0.95	0.84	0.24	0.21	0.72	0.85	0.07	0.01
LW	0.35	0.10	0.02	0.44	0.41	0.16		0.48	0.21	0.52	0.29	0.01	0.01	0.38	0.82	0.18	0.42	0.53	0.87	0.67	0.40	0.27
PC	-0.09	-0.51	0.01	-0.07	-0.01	-0.51	0.17		0.92	0.46	0.78	0.18	0.42	0.78	0.47	0.93	0.04	0.21	0.05	0.13	0.68	0.51
FE	-0.53	-0.45	-0.17	0.21	0.09	-0.15	0.30	0.02		0.16	0.06	0.03	0.26	0.19	0.62	0.57	0.83	0.33	0.83	0.22	0.18	0.65
HC	-0.07	0.06	0.17	0.24	0.56	0.10	0.16	-0.18	0.34		0.60	0.46	0.55	0.26	0.95	0.30	0.35	0.43	0.00	0.00	0.20	0.44
HD	-0.79	-0.72	-0.28	-0.44	-0.48	-0.40	-0.25	0.07	0.45	-0.13		0.41	0.68	0.46	0.52	0.96	0.28	0.94	0.34	0.52	0.08	0.08
AN	-0.12	-0.38	-0.13	0.07	-0.10	-0.05	0.59	0.32	0.50	-0.18	0.20		<.0001	0.36	0.31	0.54	0.91	0.23	0.09	0.38	0.79	0.80
AL	0.00	-0.03	0.05	-0.03	-0.06	0.08	0.58	0.20	0.27	-0.15	-0.10	0.82		.38	0.80	0.67	0.83	0.28	0.19	0.29	0.51	0.80
SW	0.40	0.00	0.14	0.23	0.20	0.57	0.22	-0.07	-0.31	-0.27	-0.18	0.22	0.21		0.82	0.31	0.82	0.85	0.36	0.10	0.05	0.01
LS	-0.02	-0.29	-0.44	0.28	0.14	0.01	0.05	0.18	0.12	0.02	0.16	0.25	0.06	0.05		0.05	0.11	0.42	0.84	0.81	0.52	0.38
WS	-0.08	-0.01	0.22	0.08	0.17	-0.05	0.32	0.02	0.14	0.25	-0.01	-0.15	0.11	-0.25	-0.46		0.44	0.78	0.89	0.89	0.80	0.49
G1D	0.02	-0.12	0.30	-0.07	0.07	-0.28	0.20	0.47	0.05	0.23	-0.26	0.03	0.05	-0.06	-0.37	0.19		<.0001	0.52	0.89	0.11	0.24
GhD	-0.30	-0.33	0.12	-0.28	-0.19	-0.30	0.16	0.30	0.24	0.19	0.02	0.29	0.26	-0.05	-0.20	0.07	0.79		0.97	0.47	0.01	0.03
V1D	0.07	0.37	-0.20	0.12	0.41	0.09	-0.04	-0.45	-0.05	0.63	-0.23	-0.40	-0.32	-0.22	0.05	0.03	-0.16	-0.01		<.0001	0.62	0.86
VhD	-0.09	0.21	-0.17	0.20	0.37	-0.05	0.10	-0.36	0.29	0.76	-0.16	-0.22	-0.25	-0.39	0.06	0.04	0.04	0.18	0.88		0.12	0.44
S1D	0.70	0.39	-0.13	0.52	0.39	0.42	0.20	-0.10	-0.32	-0.31	-0.41	0.06	0.16	0.45	0.16	-0.06	-0.38	-0.60	-0.12	-0.37		<.0001
S2D	0.64	0.32	-0.14	0.72	0.60	0.55	0.27	-0.16	-0.11	-0.19	-0.41	0.06	0.06	0.58	0.21	-0.17	-0.29	-0.49	-0.04	-0.19	0.86	

*PH, plant height; SH, shattering; PT, type of panicles; PL, panicles length; TN, tiller; LL, leaf length; LW, leaf width; PC, pericarp colour, FE, flag leaves erect; HC, hull colour; HD, heading; AN, awn presence; AL, awn length; SW, 1000-seeds weight (gm); LS, length of the seed; WS, width of seed.G1D, Germination rate for one-dose; GhD, Germination rate for half-Dose; V1D, Viability one-dose; VhD, Viability half -dose; S1D, Survival one -dose; S2D, Survival 2-dose. Listed below and above the diagonal line are *r* values and their probability (*P*) levels, respectively.

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4.2 A Simple Method to Determine and Characterize Imidazolinone Herbicide Residues from the Soil

Contamination of environmental resources by herbicides is an increasing environmental concern. Undoubtedly, soil plays a significant role in an agro-ecosystem, but information for analysis of these types of herbicide residues in the soil can be very difficult to achieve. HPLC with UV detection was chosen due to it being a fast and effective separation method. This study involves trying different columns and mobile phases for the HPLC technique. Finally, in this method a proper separation was achieved using the gradient mobile phase and C_{18} column (4.6 × 250 mm, 5 µm) was used for stationary phase separation.

Purified water was used as one of the mobile phases, due to its low cost, lack of toxicity to the environment (Laganà et al., 2000). The mobile phase acetonitrile (100%), as one of mobile phase, is the best mobile phase (Demoliner et al., 2010; Martins et al., 2014), along with purified water acidified with 10% acetic acid (pH to 2.8), due to the pH's effect on the peak shape (Singh, 2013). Therefore, acetonitrile was chosen due to it is higher solubility and higher elution strength than dichloromethane for fractionating the analytes. Acetonitrile is the best choice for the mobile phase (Singh, 2013). However, analysis was carried out using gradient solvent program using mobile phase A (acetonitrile (100%)) and mobile B (purified water acidified with 10% acetic acid (pH adjusted to 2.8). The initial gradient program was 35% A, maintained for a minute, then increased to 45% for 3 min, then decreased to 35% at 8 min. The column temperature was set to 30°C. The flow rate was 1 ml/min, injection volume was set to 17 µL, and UV detection was set to a wavelength of 251 nm.

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Simultaneously, methanol was evaporated before the sample is injected into the HPLC apparatus. Standard curve linearity and calibration was determined at six concentrations (0.1, 0.5, 1, 5, 10, and 20 µg /ml), and were prepared in the laboratory by diluting the stock solution and plotting the analytes' concentration against peak area. Each level of the concentration was analyzed repeatedly. The equation of analytical calibration was obtained by plotting the peak areas on y-axis and the concentration on the x-axis within the previous calibration levels for both Imazapic and Imazapyr. The concentration of both herbicides was calculated by comparing the peak values in the calibration, using the regression equation. The linearity of the method was determined from the correlation coefficient, as per Figure 4. 11.The equations of analytical calibration graphs, obtained by plotting peak areas against concentrations of the imazapic and imazapyr herbicides. The linear regression equations were y = 64086 x + 6626.7, with R = 0.9978 for imazapic, and y = 35078X + 3189.9, with R of 0.9998 for imazapyr respectively, showed good linearity as shown in Table 4.10.

The matrix effect has been mentioned in literature and is explained via multiple perspectives, with some reporting a shift of over 10% on the analytical results (Kemmerich et al., 2015). However, some that are less than 20% does not affect the matrix (Ferrer et al., 2011).The chemical analysis of these herbicides in soil is often problematic due to the low detection limits required and the pH adjustment during the extraction process. IMI is a weak acid as per in Table 4.11, therefore their presence in soil is influenced by pH (Schreiber et al., 2017). Soil particles were fine-grinded to increase the interaction between the solvents and soil particles, which lead to increased herbicides extraction.

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The traditional types of extractions ordinarily use the chemical compound PSA (primary secondary amine), and due to the fact that the IMI family are present in multiple forms, it acts as a weak acid/base, which allows PSA to hold over acidic herbicides (Marcia, 2014). One of the important effects occurs when the types of herbicides have pK_a values in the range 1.3-3.9 (Krieger, 2001), which includes the weak acid IMI herbicides. Based on this, the shape of the peak area during analysis was expected to be affected by the value of the pH of the mobile phase. Soil pH and the microbial activity are the main factors in the degradation process of IMI herbicides in the soil (Sondhia et al., 2015). For example, when the pH increases, the adsorption and persistence decreases.

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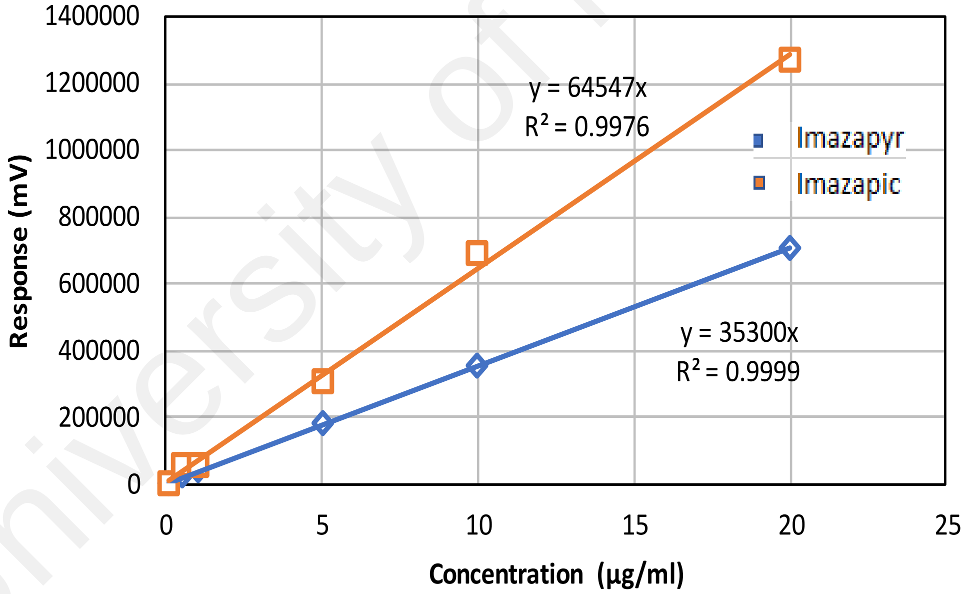

Figure 4.11: Representative calibration curve for IMI was obtained by the determination of six levels in duplicate at ranged from 0.1-20µg/mL.

Table 4.10: Peak area versus concentration (0.1 to 20 µg/mL) for imazapic and imazapyr.

Concentration (µg/mL)	[a]Area for imazapic	Std. deviation	Area for imazapyr	Std. deviation
0.1	3477	±124.02	3680	±140.00
0.5	54956	±5512.72	21381	±949.65
1	59366	±8092.74	39914	±4501.50
5	305898	±65193.93	179150	±8560.39
10	688046	±96563.22	354730	±36051.21
20	1273564	±178045.52	704156	±2816.62

* Average of three replications; "Std deviation (standard deviation).

Also, another important factor that control the residues' concentration is the depth and type of soil. IMI sorption is correlated and increased with clay content, due to increased binding of the herbicide to soil particles, where (Gianelli et al., 2014). Burnside et al., (1963) show that some herbicides can leach deep into the soil. For example, some studies revealed that the sorption of these types of IMI as imazapyr to sandy soils is very weak compared to its sorption to clay and humic soils (Lode & Meyer, 1999). The agricultural soils contain numerous impurities and old chemicals, which can persist for a long time, which would cause separation problems in the column, especially if the soil contained only very low concentrations of imazapic or imazapyr. Imazapyr and imazapic have the potential to leach into groundwater due to its persistence and mobility in soils, and very low volatility (Gianelli et al., 2014). Certified imazapic and imazapyr (USA) were used for calibration (Figure 4.12, Figure 4.13, Figure 4.14, and Figure 4.15).

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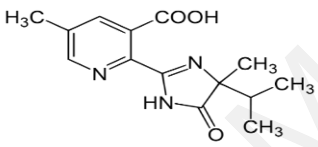
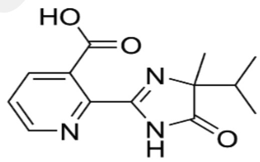
Table 4.11: The characteristics (molecular and physicochemical) of Imazapic and Imazapyr.

Name	[a] Imazapic	[a] Imazapyr
Family/chemical class	Imidazolinone	Imidazolinone
Trade name	Cadre, panoramic, plateou	Arsenal, Chopper, Habitat, Stalker
Chemical name	[2-(4,5dihydro-4-methy-4-(1-methylethyl)-5-oxo-1Himidazol-2-yl) 5-methyl-3-pyridinecarboxylic acid]	[2-(4-isopropyl- 4- methyl-5-oxo-2-imidazoline-2-yl) nicotinic acid]
Molecular weight	275.30308g/mol	261.2765g/mol
Molecular formula	$C_{14}H_{17}N_3O_3$	$C_{13}H_{15}N_3O_3$
Structural formula		
Water solubility	2200mg/L	9740 mg/L
Lifetime in soil	Around 120days	90-120 days
[b] pKa	2.1, 3.9	1.9, 3.6
[c] Goss	High potential	High potential

[a] Data quoted from (Schreiber et al., 2017; Senseman, 2007).[b] Indicates the pH value at which 50% of total molecules are associated in soil and 50% of total molecules are dissociated.[c] Method of classification of potential surface water contamination.

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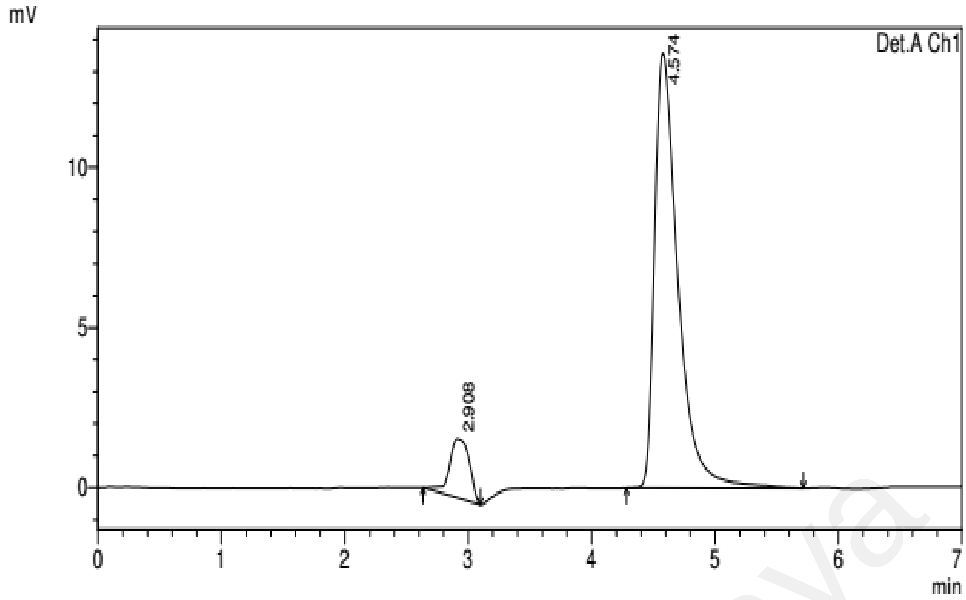

Figure 4.12: Imazapyr standard 10ppm.

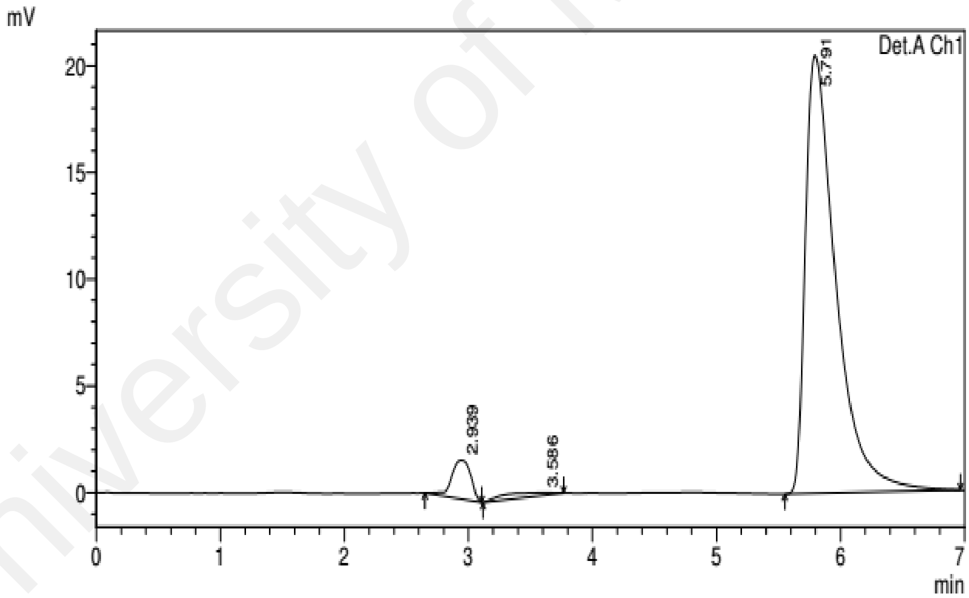

Figure 4.13: Imazapic standard 10ppm.

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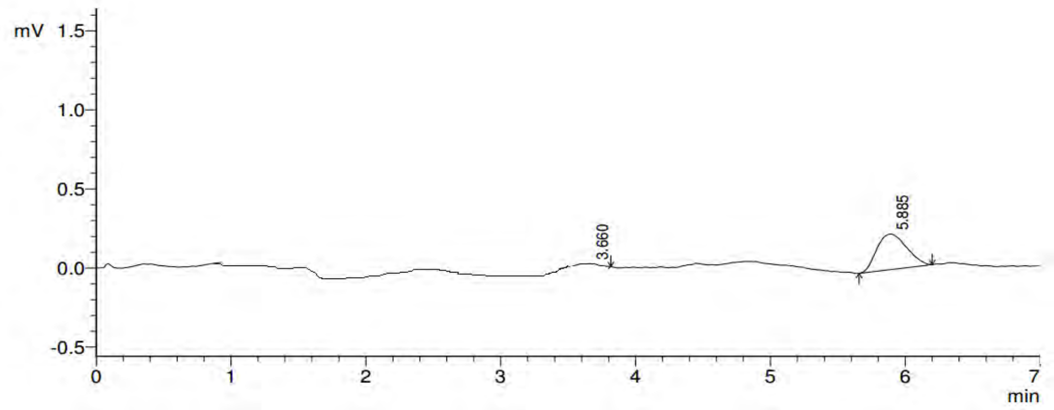

Figure 4.14: Imazapic standard 0.5 µg/ml.

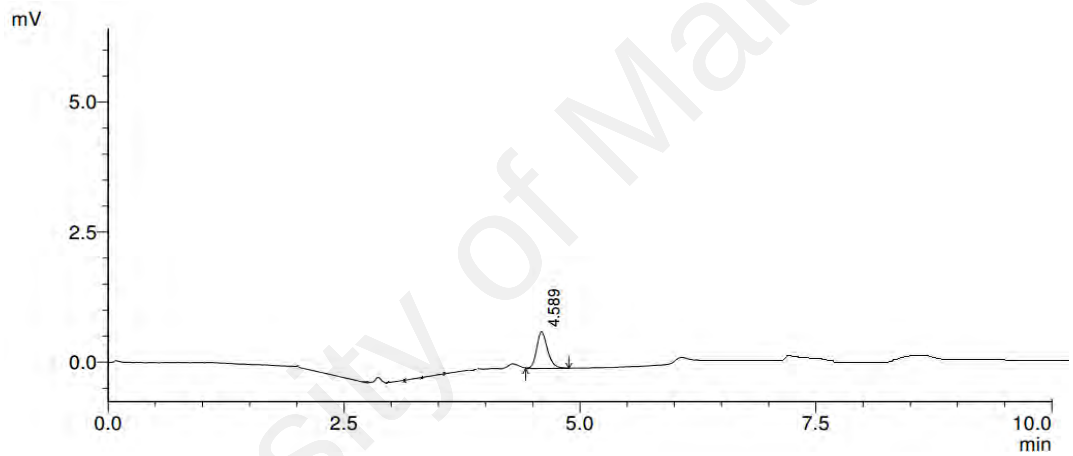

Figure 4.15: Imazapyr standard 0.1 µg/ml.

The adsorption of IMI herbicides decreases by increasing heavy rain and temperatures. The higher solubility of these types in water, high temperatures, and great rainfall in Malaysia are main factors that play important roles in the transition of residual particles of herbicides via its pores or movement to other places and shift up the degradation mechanism, as per (Fish et al., 2015; Grey et al., 2012). Malaysia has almost daily high intensity rain fall and temperatures. Studies revealed that temperatures between 35C°- 45C° and increased soil moisture enhance both the chemical and microbial degradation for herbicides (Neto et al., 2017). Different methods are applicable for extraction of IMI herbicides from soil samples, but most are not satisfactory (de Oliveira et al., 2014). Despite the fact that imazapyr and imazapic were applied in low doses, both can remain for long periods of time in the soil , which can cause agronomic and environmental problems (Kraemer et al., 2009). However, leaching is influenced by the environment, which means that when the water content decrease from the upper surface, it leads to increased pH. Also, some chemical herbicides move to the upper surface of the soil due to capillary action, which causes it to evaporate (Mangels, 1991).

4.2.1 Selectivity

Selectivity is defined as the evaluation or detection of the analyte from others analytes and different compounds that could be present at the same moment in the matrix or the sample (Ahuja, 1989). There were no matrix peaks in the chromatogram analysis that interfere with analysis of the residues as shown in Figure 4.16

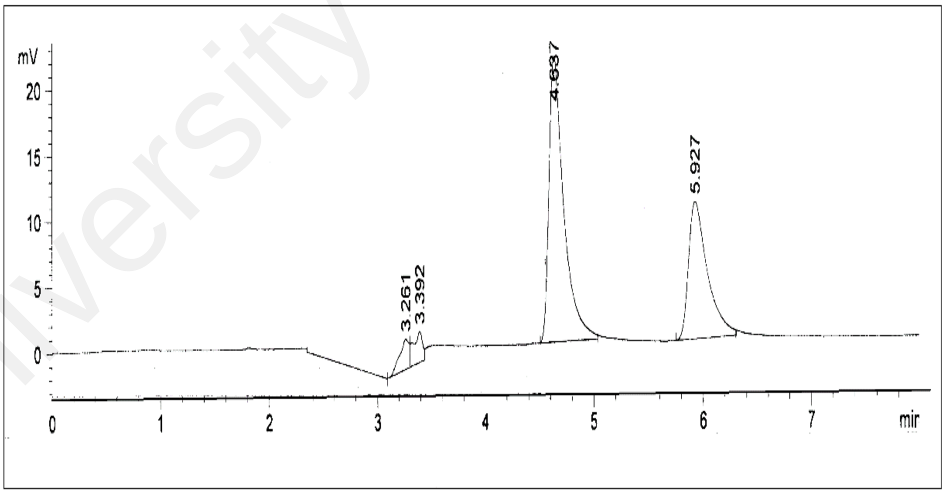

Figure 4.16: Extraction imazapic and imazapyr with good resolution.

4.2.2 Accuracy (%Recovery)-Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The achieved results revealed an excellent linearity at different concentrations of Imazapyr and Imazapic standards in the range from 0.1 to 5 μg/mL. These herbicides' concentrations are spiked to blank soils as described in the experimental section. Due to the spiking of the extracts, the final comparison between the two systems is expected to be valid. The precision and recovery for the two herbicides was calculated through the injection of freshly prepared six standards. The proportion of the area of the peak of herbicide resulting from the spiked solution to the area of the herbicide peak resulting from a standard solution prepared previously was calculated. The average percentage recoveries for Imazapyr and for Imazapic varied from 76%-107% and 71-79% with 0.1-5 µg/mL fortification level, and 0.1-10 µg/mL at fortification level, respectively, are shown in Table 4.12 and Figures 4.18 to Figure 4.23.The LOD and LOQ were found to be 1.04 and 3.09µg/ml for imazapic, and 0.17 and 0.51µg/ml for imazapyr, respectively. In the extracted soil sample, it was 0.19 µg/mL for imazapic and 0.04 µg/mL for imazapyr. This proves the slow degradation process of these residues in the soils under environmental conditions. The soil samples were taken during rice crop cultivation of about 90 days and the residues are evidently still present.

The Koc for the two herbicides were 137 and 100ml g^{-1}, respectively, which means low adsorption and high mobility, and eventually high levels of leaching. Nevertheless, both herbicide residues are still present after ~90 days, especially imazapic with 0.19 µg/ml. Simultaneously, persistence of residues in the soil does not necessarily mean that it injures sensitive crops, as persistence differs from bioavailability.

4.2.3 Repeatability and Stability

The repeatability was determined by calculating the RSD of the peak areas of the 6-duplicate injections of fortified samples which is < 15. It represents the closeness of the results from the same methods, laboratories, and tools. This is achieved via 6 concentrations, each replicated thrice to a total of eighteen times, encompassing the

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specified range of the procedure. Accuracy = mean ±SD, for imazapic 75.85 ± 3.4, and for imazapyr, it was 90.232±14.

Table 4.12: Recovery of imazapic and imazapyr from the soil. (n=3).

Fortified concentration (µg mL)	[a] Recovery (%) for imazapyr	Av. recovery for imazapyr ±SD [b]	Recovery (%) for imazapic	Av. recovery for imazapic ±SD
0.1	(96.4; 110.9; 114.0)	107.0 ± 9.4	(71.9; 70.9; 70.6)	71 ± 0.6
0.5	(72.6; 80.1; 89.5)	80.7 ± 8.4	(52.5; 82.8; 102.0)	79 ± 20.4
5	(79.2; 77.9; 73.1)	76.7 ± 3.1	(97.4; 97.4; 77.6)	90 ± 11.2

[a] The averages of three samples processed through the procedure. [b] SD: Standard deviation.

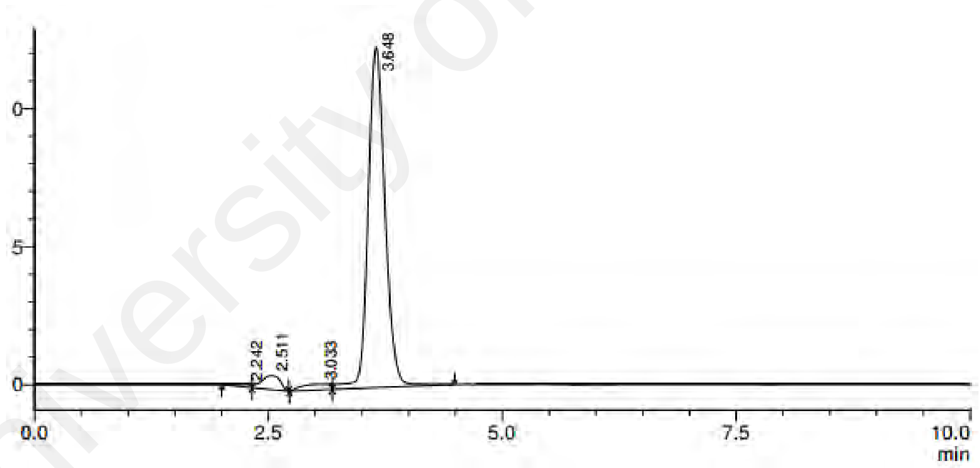

Figure 4.17: Fortification imazapic 0.1µg/mL.

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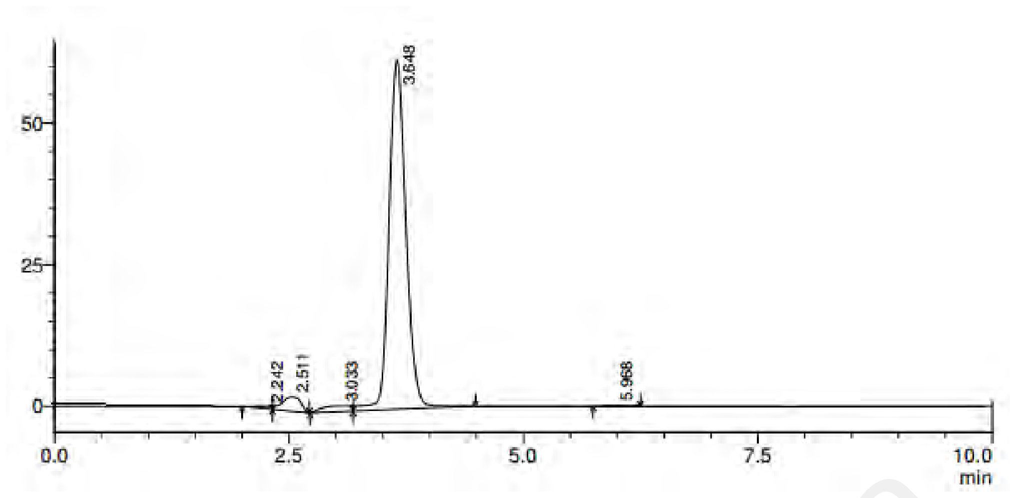

Figure 4.18: Fortification imazapic 0.1µg/mL.

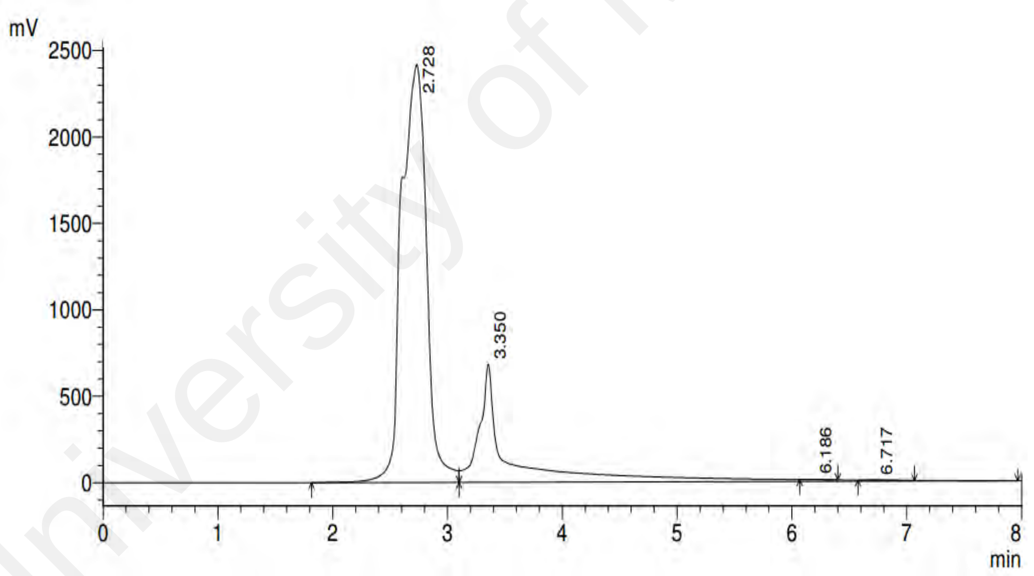

Figure 4.19: Fortification imazapic 0.5µg/mL.

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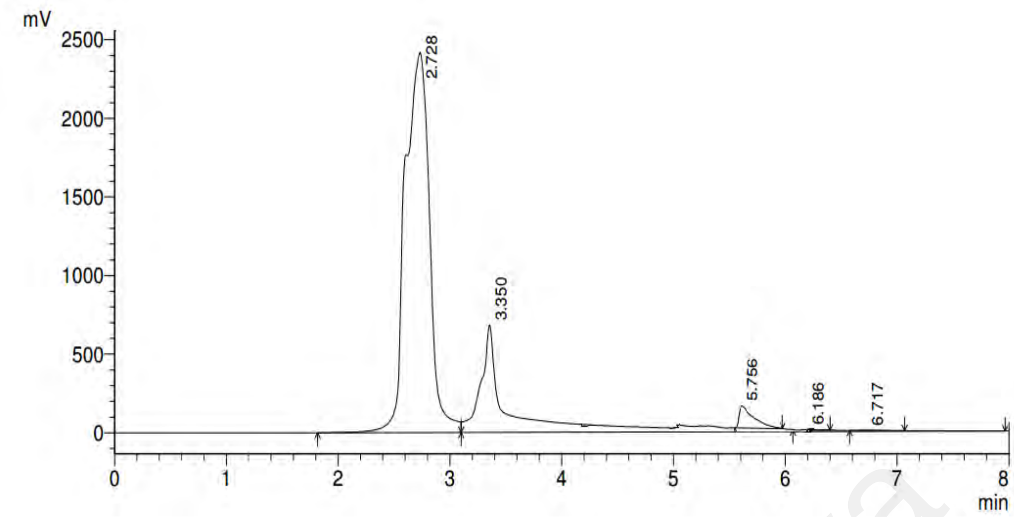

Figure 4.20: Fortification imazapic 0.5µg/mL.

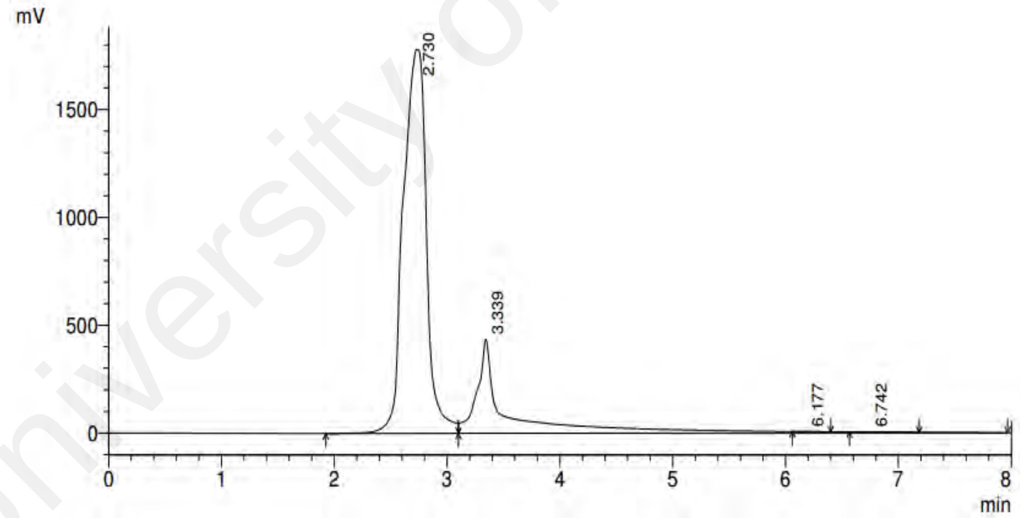

Figure 4.21: Fortification imazapyr0.5µg/mL.

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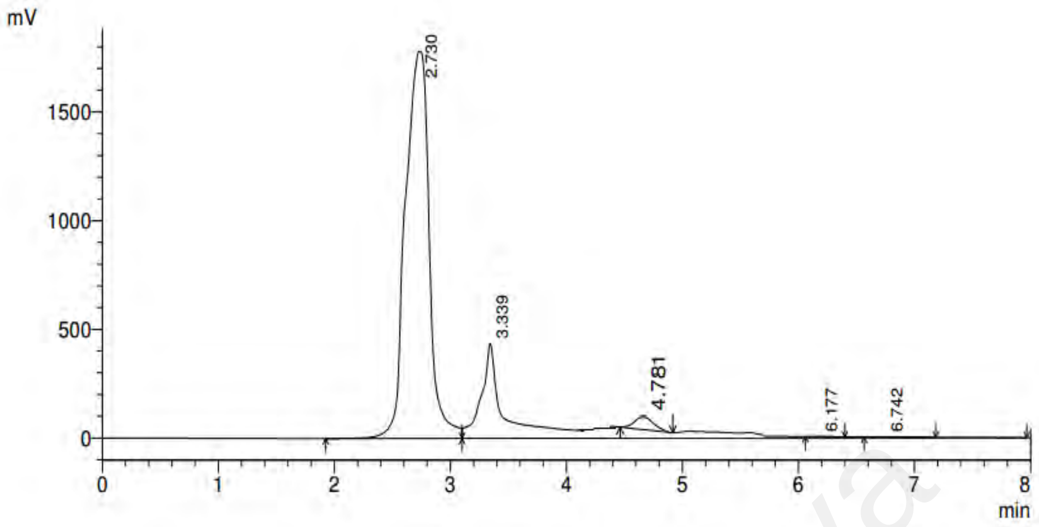

Figure 4.22: Fortification imazapyr0.5µg/mL.

Lastly, the parameters for HPLC method was controlled on specific parameters as Table 4.13.

Table 4.13: Chromatographic system parameters.

Mobile phase	(A) acetonitrile (100%) and mobile (B) purified water acidified with 10% acetic acid (pH adjusted to 2.8)
Column	C_{18} column (4.6 × 250 mm, 5 µm)
Flow rate	1.0 mL/min
Injection volume	17µL
Column Temperature	Between 30-35 °C
Wavelength	251nm
Run time	About 8 minutes

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4.3 Leaching Potential and Residues Activity of Imidazolinone Herbicide in Clearfield Rice Soil Using High-Performance Liquid Chromatography

20 Samples were collected from three fields sites of in Sawah Sempadan-Tanjung Karang district and analyzed using the aforementioned procedure. The following data were presented in Table 4.14, Figure 4.24 and Figure 4.25

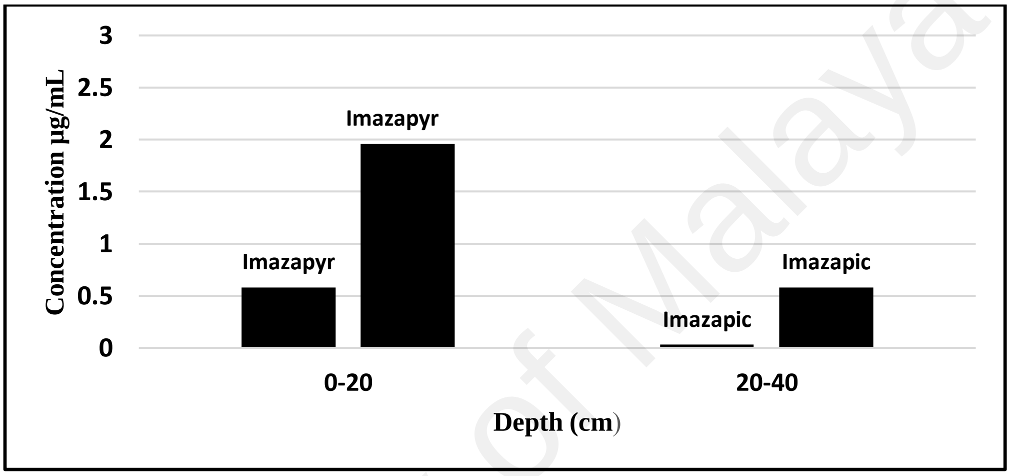

Figure 4.23: Actual Concentration of imazapic and imazapyr of soil sampling in plot 1.

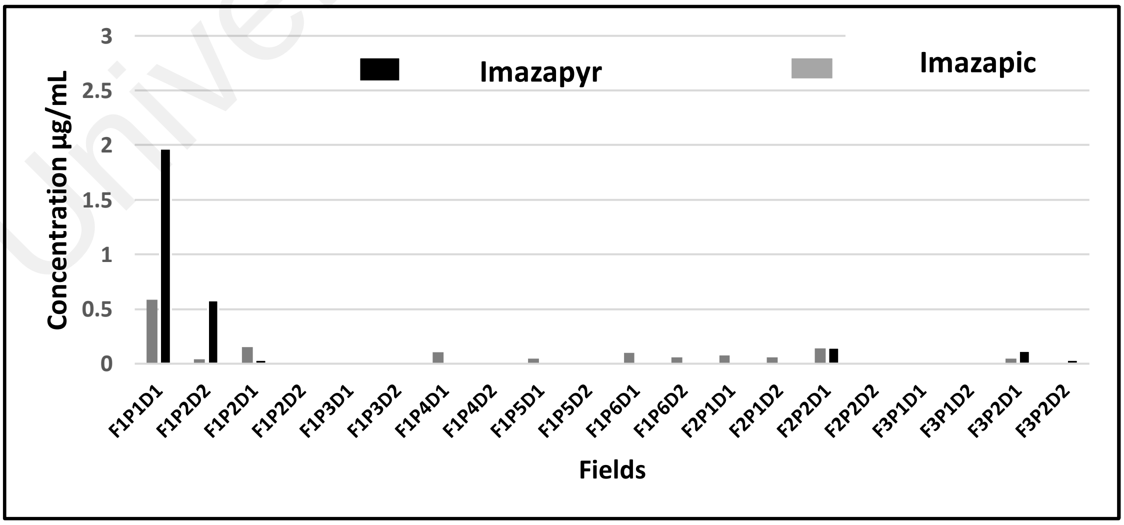

Figure 4.24: Actual concentration of imazapic and imazapyr (µg/ml) in the soil samples

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Table 4.14: Terminal residues of the Imazapic and Imazapyr (µg/mL) at various locations for the different samples.

Fields	Plot	Sample	[a] F. C	Depth (cm)	[b] R.T for Imazapic	[c] Area for Imazapic	Actual. Concentrationfor Imazapic	[b] R.T for Imazapyr	[c] Area for Imazapyr	Actual. Concentrationfor Imazapyr
Field 1	1	A	F1P1D1	0-20	5.76	64276	<LOQ	4.64	84945	1.96
		B	F1P1D2	20-40	5.92	1091	<LOQ	4.6	21381	0.58
	2	A	F1P2D1	0-20	5.65	16879	<LOQ	4.65	1079	<LOQ
		B	F1P2D2	20-40	n. d	n. d	n. d	n. d	n. d	n. d
	3	A	F1P3D1	0-20	n. d	n. d	n. d	n. d	n. d	n. d
		B	F1P3D2	20-40	n. d	n. d	n. d	n. d	n. d	n. d
	4	A	F1P4D1	0-20	5.71	5026	<LOQ	n. d	n. d	n. d
		B	F1P4D2	20-40	n. d	n. d	n. d	n. d	n. d	n. d
	5	A	F1P5D1	0-20	5.8	1608	<LOQ	n. d	n. d	n. d
		B	F1P5D2	20-40	n. d	n. d	n. d	n. d	n. d	n. d
	6	A	F1P6D1	0-20	5.75	4871	<LOQ	n. d	n. d	n. d
		B	F1P6D2	20-40	5.86	2318	<LOQ	n. d	n. d	n. d
Field 2	1	A	F2P1D1	0-20	5.72	3353	<LOQ	n. d	n. d	n. d
		B	F2P1D2	20-40	5.63	2214	<LOQ	n. d	n. d	n. d
	2	A	F2P2D1	0-20	5.8	7541	<LOQ	4.62	5421	<LOQ
		B	F2P2D2	20-40	n. d	n. d	n. d	n. d	n. d	n. d
Field 3	1	A	F3P1D1	0-20	n. d	n. d	n. d	n. d	n. d	n. d
		B	F3P1D2	20-40	n. d	n. d	n. d	n. d	n. d	n. d
	2	A	F3P2D1	0-20	5.96	1562	<LOQ	4.52	56983	<LOQ
		B	F3P2D2	20-40	n. d	n. d	n. d	4.78	1326	<LOQ

F.C: Field Code; [b] R. T: Retention time and [c] Average peak area represent the average peak areas value of same plot soil samples. Limit of quantitation (LOQ) for imazapicand imazapyr (3.09 μg/ mL) and (0.51 μg/ mL) respectively. The fields which did not contain IMI residues were removed.

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CHAPTER 5: DISCUSSION

It is high likely that several weedy rice biotypes in Malaysia have already 'evolved' to be resistant to imidazolinone herbicide (OnDuty™) possibly from consequential conferment of resistant genes from Clearfield® rice to weedy rice (Shivrain et al., 2008; Jaafar et al., 2014; Dilipkumar et al., 2018). Different weedy rice biotypes from this study showed a wide variation of IMI herbicide responses to the weed during pre-emergence (Figures 1, 2) and post-emergence (Figures 3,4) herbicide applications at various dosage. This confirmed that sampled weedy rice biotypes have various degrees of resistant towards IMI herbicide. This variation might be caused by accidental and/or voluntary hybridization between IMI tolerant cultivated rice with weedy rice in the Clearfield® rice fields.

Engku et al., (2016) reported the potential gene flow between Malaysian Clearfield® rice (MR220CL1 and MR220CL2) to various weedy rice biotypes producing resistant progenies in the F1 population. The hybridization introduces gene flow and subsequently increases genetic diversity and heterogeneity of the hybrid weedy rice populations (Chang, 2003), initiating hybridization-differentiation cycles of next generations (Gu et al., 2004; Mispan et al., 2013). This increases genotypic selection for adaptive/survival traits (Mispan et al., 2013; Zhang et al., 2017; Qiu et al., 2017) which later expand the probability of survival potential from selection pressure due to continuous IMI herbicide application (Kuk et al., 2008; Dilipkumar et al., 2018).

Viability percentage of the seedlings for herbicide treatment as a pre-emergence application at half-dose (21.4±19.6%), commercial dose (13.9±13.8%) and double-dose (7.3±4.1%) rate (Figure 3) shows application of OnDuty™ as pre-emergence herbicide

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might help in managing weedy rice at the early stage. Imidazolinone herbicide reportedly to have a slight effect on the percentage of seed germination in chickpea but a significant shift down in the speed of germination (Hoseiny-Rad & Jagannath, 2011). This is in line with the stewardship guideline for the Clearfield® Production System (CPS) to apply the OnDuty™ herbicide only between 0 to 7d after sowing (DAS). High percentage of imazapic (52.5%) one of active ingredients in OnDuty™ - acting as pre-emergence herbicide might contribute to this action (Dilipkumar et al., 2018).

However, this study showed that MR220CL2 (imidazolinone tolerance variety) seeds also affected by the pre-emergence application (Figure 5) if the seeds were directly sowed before pre-germination. The usage of OnDuty™ as pre-emergence herbicide could be more effective in transplanting method especially with an increased dosage from the commercial rate. However, the environmental impact of regular usage and high dosage of this herbicide need to be properly assessed because the current CPS practice showed potential herbicide leachate and carryover in the rice field soil (Bzour et al., 2019).

The application of OnDuty™ herbicide as a post-emergence herbicide can increase the potential of weedy rice to escape the CPS. Only 23.5% of weedy rice sampled populations can be fully controlled (susceptible) by commercial dosage and additional 35.3% were controlled by 2-dose. The low formulation rate of imazapyr (17.5% or equivalent of 38.5 g a.i. ha-1) as a post-emergence herbicide in the OnDuty™ will increase the probability for diverse weedy rice populations to survive. Dilipkumar et al., (2018) reported that imazapyr can control resistant biotype of weedy rice at 4,995 g a.i. ha-1. This wide margin creates ample window for weedy rice to adapt in the CPS

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environment and consequently become resistant to the herbicide through spontaneous mutation over time (Tan et al., 2005; Sales et al., 2008; Kuk et al., 2008).

Unfortunately, disobedience of some farmers to follow the CPS guidelines and stewardships has been reported (Dilipkumar et al., 2018; Harun et al., 2018) and personally observed especially on practicing CPS for more than three consecutive seasons in the same field, late application of OnDuty™ at 10 to 15 DAS, and reducing the herbicide dosage to cut input cost. Previous experience in Malaysia already reported that continuous use of phenoxy herbicides since late 1980s has caused the weed species shift to graminaceous species including weedy rice in Malaysia rice granaries (Baki, 2006; Mispan et al., 2019). Malaysia will face ecological risks of continuous weedy rice mutation in favor to its survival if no stringent ecological risk valuation including the screening and mitigation strategies to break selection of IMI resistant weedy rice in the CPS system (Sudianto et al., 2013; Mispan et al., 2019).

Studies revealed how much of IMI- herbicides run-off into soil are important due to their potentially deleterious effects on the environment (Schreiber et al., 2017). Improving methods for extraction from the environment are very important, because these residues of these herbicides remain present in Swedish soil after 8 hours (Börjesson et al., 2004b). Using HPLC-UV method is common in literature, and it was used by many researchers because it provides more realistic results (Helling & Doherty, 1995; Laganà et al., 1998; Pace et al., 1999).

IMI herbicides (imazapic/imazapyr) were widely used in Clearfield® rice soils. To date, only a few studies are available discussing on the residues of these herbicides, especially in the context of Malaysian soil. Therefore, for this purpose, high

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performance liquid chromatography (HPLC) with UV detection was developed using a Zorbax stable bond C18 (4.6 × 250 mm, 5 μm) column, with two mobile phases. The average percentage recovery for imazapyr and imazapic varied from 76%-107% and 71-77%, with 0.1-5 μg/ml fortification level, respectively. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 1.05 and 4.09 for imazapic and 0.171 and 0.511 μg/ml for imazapyr respectively, in the top 15 cm. In the extracted soil sample, it was 0.19 μg/ml for imazapic and 0.04 μg/ml for imazapyr, respectively. Based on this study, a pre-harvest period of 40-60 day is suggested for rice crops after IMI application.

Using this HPLC-UV method, Calibration curves from different known concentrations of imazapic and imazapyr herbicides (0.1, 0.5, 1.5, 10, 20 μg/mL) were constructed. The equations of analytical calibration graphs obtained by plotting peak areas against concentrations of the imazapic and imazapyr herbicides. The linear regression equations were y = 64,086x + 6626.7, with R = 0.9978 for imazapic, and y = 35078X + 3189.9, with R of 0.9998 for imazapyr respectively.

Previous researches reported that these herbicides are slow to degrade in soil under normal environmental conditions (Bajrai et al., 2017). Imazapyr has a half-life of 90-120 days, while imazapic has a half-life of 3 months. The K_{oc} (soil organic partition coefficient) for both herbicides were137 and 100ml g^{-1}, respectively, which means low adsorption and high mobility, and eventually the high level of leaching. Nevertheless, these herbicidal residues persist for extended periods of the times, thus representing a high risk of environmental contamination of soil, surface, and groundwater, especially imazapic (Souza et al., 2016).

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The LOD and LOQ were found to be 1.04 and 3.09 µg/ mL for imazapic, and 0.17 and 0.51 µg/mL for imazapyr, respectively as found from our previous study (Bzour et al., 2017). From Tables 4.14 and Figure 4.24, imazapic and imazapyr were found at depths more than 20 cm. The residues were at 0.58, 0.03 on the first 20 cm and 1.96, 0.58 at the 20-40cm depth, which agrees with (Neto et al., 2017; Refatti et al., 2017), who reported that imazapyr and imazapic can leach up to more than 25 cm. The presence of both IMI residues at 20-40cm depth in field 1 (plot-1) may be due to the soil sample location at the edge of the field (on the corner of the field), and it is the first sample collected (Figure 4.24).

The management practices and procedures of the farmers are instrumental towards the presence of these herbicides. Some plots were not as cultivated, and seldom ploughed, which may result in reduced sunlight, and accumulation of IMI residues throughout the seasons. Table 4.14 shows that the imazapic residues were present in most samples, especially at depths of more than 20 cm, in contrast to imazapyr residues, which were only found in the field 1 -plot 1 and field 3 -plot 2. This could be attributed to the concentration of imazapyr and imazapic in the whole compound (Onduty® compound were 0.58 and 0.19 g/L, respectively). Therefore, the concentration of imazapic is tripled, which could explain the accumulation and translocation of imazapic more than imazapyr. Vizantinopoulos and Lolos (1994) pointed out that imazapyr has low persistence, and can move and leach into deep layers, reaching more than 45 cm. The residues of imazapic in the plots decreased from soil depths of 20 – 40 cm. The residues in plot 1 were 0.58, 0.03, plot. 2: 0.03, (-), plot 3: (-), plot 4: 0.10, plot 5: 0.04, (-) and Plot 6 were 0.09, 0.05 µg/mL, respectively. The reason for the shift down of the peak areas could be due to translocation involving the movement of soil forming materials throughout the soil's profile and the leaching of herbicides into deeper layers.

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The adsorption of herbicides decreased due to increasing heavy rain and temperatures. The higher solubility in water, pH, high temperatures, and high rainfall in Malaysia are some of the main factors that play an important role in the transition of residual particles of herbicides through the pores or movement to deeper layers, as per (Börjesson et al., 2004a; Castillo et al., 1997; Fish et al., 2015; Grey et al., 2012).

Malaysia has almost daily high-intensity rainfall and medium daily temperatures. Studies reported that temperatures between 35 – 45 °C and increased soil moisture enhances both chemical and microbial degradation of herbicides, as well as their respective mobilities (Jourdan et al., 1998; Laabs et al., 2000; Neto et al., 2017). Therefore, different factors can affect the leaching of these types of herbicides into the depth of the soils, including the pH, concentration of herbicides, and type of the soil. At pH values greater than 6, the IMI herbicides are weakly adsorbed into the soil (Ozcan et al., 2017). Another important factor that affects the residual concentration is the type of the soil. IMI sorption increases alongside soil clay content, due to increased bindings of the herbicide to soil particles (Gianelli et al., 2014). In this research, the type of the soil was clay loamy, which means that the percentage of adsorption increase and IMI herbicides dissipation decrease (Sondhia et al., 2015). Sondhia (2013) reported that IMI-herbicides could leach into clay loam soil up to a depth of 70 cm.

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CHAPTER 6: CONCLUSION AND FUTURE RECOMMENDATIONS

6.1 Research Conclusion

The Clearfield® Rice Production System (CPS) technology was successfully managing weedy rice infestation and increasing crop production in Malaysia. However, there were concerns on the recurring presence of weedy rice in the CPS fields in recent years, suspecting the weedy rice has become resistance to imidazolinone herbicide (OnDuty™). This study showed different dosages of imidazolinone herbicide only reduced ~30% of germination rate but significantly decreased the weedy rice seedlings viability. Low viability rate for commercial (13.9±13.8%) and double (7.3±4.1%) herbicide dose indicated application of OnDuty™ as pre-emergence herbicide was effective to control weedy rice. The application of OnDuty™ at later stage after the recommended period increased weedy rice escape potential in the CPS fields by 64.7% to 76.5% for one- and two-dose applications, respectively. Therefore, there is a possibility that weedy rice in Malaysia has developed certain levels of resistance towards imidazolinone herbicide. Stringent ecological risk valuation of CPS is needed to mitigate the development of herbicide resistant weedy rice in the future.

A simple analytical method based on HPLC-UV was developed and validated to determine the IMI residues in the Clearfield® rice soils in this study. It is necessary to monitor the presence of herbicides residues in soils and waters and develop methods for reliable analysis, as important tools of regulatory programs to protect the environment. A gradient of mobile phase A (acetonitrile (100%)) and mobile B (purified water acidified with 10% acetic acid (pH adjusted to 2.8)) yields excellent separation and resolution, in a short analysis time, for the two herbicides (less than 7 min), with retention time for imazapyr and imazapic at ~4.6 and 5.9 min, respectively. Excellent

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linearity in the range of injected standard concentrations with a high degree of precision and accuracy could be achieved. Therefore, the proposed analytical method could be useful for detecting the imidazolinone family in agricultural soil and water in the future. Results of this study suggests the need for an extensive research to determine factors affecting the half-life of these herbicides and their contribution to their persistence. Also, further studies are needed on the laboratory level and plant bioassay to evaluate if these residues can indeed cause injuries to other crops.

The residual activity of herbicides may be detrimental to the environment, requiring analysis of the persistent residues in the soil and water. Since, a reliable method for the identification of IMI herbicides method was used to determine IMI-herbicide residues in the rice field soils. Residues of imazapic and imazapyr were found to fall within 0.03–0.58 μg/mL and 0.03–1.96 μg/mL, respectively, in three locations. IMI herbicides are persistent in the soil, and their residues remain for up to 85 days after application.

A pre-harvest study was suggested for these herbicides on water, which will provide a clearer indicator on the use of IMI in Clearfield® rice fields. The LOD and LOQ were found to be 1.04 and 4.09 for imazapic and 0.17 and 0.51 μg/mL for imazapyr, respectively. The results showed that residual herbicides were present in the soil in certain plots, reaching 20–40 cm. It was observed that high mobility herbicides can leach into deeper layers of the soil, which could threaten deep aquifers.

This study elucidated the environmental properties of IMI herbicides that are commonly used in major results also confirmed the need for more in-depth studies at different times of application, to precisely evaluate the actual leaching depth of these herbicides and its mechanisms.

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6.2 Future Work

Despite the progress of researches on weedy rice in Malaysia, information of its genetic diversity in crop fields and IMI- herbicide resistance weedy rice is still insufficient. Therefore, this limited information about weedy rice restrain the drawing of effective techniques tools and methods for weedy rice management. Therefore, to close the gaps, it is important to carry out national-wide study about the weedy rice resistant in rice crop cultivation fields in Malaysia. Some suggestion research in the future as low seed shattering weedy rice and shift up the susceptibility of these weedy rice to IMI-herbicides in the fields. This will help us to more understand hidden secrets related genetic diversity between weedy rice populations in the regions and try to put surely effective tools for mitigation strategies. The study of resistance in weedy rice specifically for imazapyr or imazapic is also needed. Consequently, future research should focus on these goals to save our crop from the carry over residues and water contamination.

General observations also indicated that weedy rice studies in Malaysia may develop a strong interest towards the 'omics' research to overcome such issues including weed management. The genomics studies in weedy rice for instance may arise in the country due to their rapid evolution dynamics in Malaysian rice agro-ecosystem (Mispan et al*.,* 2015). Weedy rice also can become a model plant for weed ecological genetic studies to elucidate genetic and evolutionary mechanism of weed adaptation and competitiveness in agro-ecosystems using combinative approaches of ecology, genetics and genomics to provide fundamental knowledge to improve or devise new weedy rice management strategies (Mispanet al*.,* 2013; Mispan 2014). The biotechnology techniques and technologies especially in the, metabolomics studies to illuminate useful chemical

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compounds (Saiman, 2014) can be transferred to weedy rice systems for further understanding on herbicide mechanisms and actions to weedy rice (Ruzmi et al., 2017).

More in-depth study should be conducted at different times of application to precisely evaluate the actual leaching depth of these herbicides. Secondly, the proposed analytical steps could be useful for detecting the IMI family in agricultural soil and water in the future. Thirdly, further investigation is needed to assess the impact of herbicides residues in water and human health.

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REFERENCES

Abdul Hamed, B., Nasir, MA., Mohd Asrore, K., Idrus, AH. (1994). *Weedy rice status and management in selangor north-west project*. (*Article in Malay).* Paper presented at Padi Angin Workshop. 18 May 1994, Penang, Seberang Prai. MARDI.

Abraham, C., & Jose, N. (2015). Weedy rice invasion and its management. *Indian Journal of Weed Science, 47*(3), 216-223.

Agriculture, U. S. D. O. (2017). World rice production 2017/2018. Retrieved on June 10, 2018 from https://www.worldriceproduction.com.

Ahmed, Q., Kamaruzaman, M., & Othman, A. (2012). Vegetative and reproductive growth of weedy rice in selangor, Malaysia: A comparative study with commercial rice varieties. *Malaysian Applied Biology, 41*(1), 29-35.

Ahuja, S. (1989). *Selectivity and detectability optimizations in hplc* (Vol. *104*): John Wiley & Sons, New York.

Akiyama, Y., Matsuoka, T., & Mitsuhashi, T. (2009). Multi-residue screening method of acidic pesticides in agricultural products by liquid chromatography/time of flight mass spectrometry. *Journal of Pesticide Science, 34*(4), 265-272.

Ali, U., Syed, J. H., Malik, R. N., Katsoyiannis, A., Li, J., Zhang, G., & Jones, K. C. (2014). Organochlorine pesticides (ocps) in south asian region: A review. *Science of the Total Environment, 476*, 705-717.

Alias, E. F., Mohamed Arshad, F., Mohd Noh, K., & Tasrif, M. (2011). *Food security: Self sufficiency of rice in Malaysia. Paper presented at National Conference on the Tenth Malaysia Plan*. 2nd December 2011, Seri Pacific Hotel, Kuala Lumpur.

Alister, C., & Kogan, M. (2005). Efficacy of imidazolinone herbicides applied to imidazolinone-resistant maize and their carryover effect on rotational crops. *Crop Protection, 24*(4), 375-379.

Anastassiades, M., Lehotay, S. J., Štajnbaher, D., & Schenck, F. J. (2003). Fast and easy multiresidue method employing acetonitrile extraction/partitioning and “dispersive solid-phase extraction” for the determination of pesticide residues in produce. *Journal of AOAC International, 86*(2), 412-431.

Andres, A., Fogliatto, S., Ferrero, A., & Vidotto, F. (2014). Susceptibility to imazamox in italian weedy rice populations and clearfield® rice varieties. *Weed Research, 54*(5), 492-500.

Andreu, V., & Picó, Y. (2004). Determination of pesticides and their degradation products in soil: Critical review and comparison of methods. *TrAC Trends in Analytical Chemistry, 23*(10), 772-789.

Area, R. G. (2010). Morphological study of the relationships between weedy rice accessions (Oryza sativa complex) and commercial rice varieties in pulau pinang. *Tropical Life Science Research, 21(2), 27-40.*

University of Malaya

Arnold, R. N., Mcurray, M. W., Gregory, E. J., & Smeal, D. (1993). Weed control in pinto beans (phaseolus vulgaris) with imazethapyr combinations. *Weed Technology*, *7*(2), *361-364.*

Arrieta-Espinoza, G., Sánchez, E., Vargas, S., Lobo, J., Quesada, T., & Espinoza, A. M. (2005). The weedy rice complex in costa rica. I. Morphological study of relationships between commercial rice varieties, wild Oryza relatives and weedy types. *Genetic Resources and Crop Evolution, 52*(5), 575-587.

Assalin, M. R., Queiroz, S. C., Ferracini, V. L., Oliveira, T., Vilhena, E., & Mattos, M. L. T. (2014). A method for determination of imazapic and imazethapyr residues in soil using an ultrasonic assisted extraction and lc-ms/ms. *Bulletin of Environmental Contamination and Ttoxicology, 93*(3), 360-364.

Avila, L. A., Lee, D. J., Senseman, S. A., McCauley, G. N., Chandler, J. M., & Cothren, J. T. (2005). Assessment of acetolactate synthase (als) tolerance to imazethapyr in red rice ecotypes (oryza spp) and imidazolinone tolerant/resistant rice (Oryza sativa) varieties. *Pest Management Science, 61*(2), 171-178.

Azman Hamzah, M. T. A., Asnawi, S., Yahya, S., & Rosniyana, A. (2017). Rice production and consumption in Malaysia. Retrieved on June 12, 2018 from http://ap.fftc.agnet.org/ap_db.php?id=735&print on 20/9/2018.

Azmi, M. (2002). *Impact of continuous direct seeding culture on weed species diversity in the Malaysian rice ecosystem.* Paper presented at the Proceedings of Regional Symposium on Environment and Natural Resources. 10-11th April 2002, Kuala Lumpur, Malaysia.

Azmi, M., Abdullah, M., Mislamah, B., & Baki, B. (2000). Management of weedy rice (Oryza sativa l.): The Malaysian experience. *Wild and Weedy Rice in Rice Ecosystems in Asia-A Review*, 91-96.

Azmi, M., Abdullah, MZ., & Muhamad, H. (2005a). Weedy rice (padi angin): A real threat to rice industry and farmers. Proceedings of 4th National Seed Symposium of the Malaysian Association of Seed Technologists, 5th June 2005, Putrajaya, Malaysia.

Azmi, M., Azlan, S., Yim, K., George, T., & Chew, S. (2012). Control of weedy rice in direct-seeded rice using the clearfield production system in Malaysia. *Pak J. Weed Science Research, 18*, 49-53.

Azmi, M. & Baki, B.B. (2003). *Weed species diversity and management practices in the Sekinchan Farm Block, Selangor's South West Project: A highly productive rice area in Malaysia*. Presented at the 19th Asian-Pacific Weed Science Society Conference, 17th November 2003, Manila, the Philippines.

Azmi, M., Baki, B., & Johnson, D. (2007). *Weed species of rice over the last decade in the muda granary of Malaysia.* Paper presented at the Proceedings of the 21st Asian Pacific Weed Science Society (APWSS) Conference, 2-6th October 2007, Colombo, Sri Lanka.

University of Malaya

Azmi, M., Muhammad, H. and Johnson, DE. (2005b). Impact of Weedy Rice Infestation on Rice Yield and Influence of Crop Establishment Technique. Proceedings of 20th Asian-Pacific Weed Science Society Conference. 15th October 2005, Ho Chi Minh City, Vietnam.

Azmi, M., Hadzim, K., Habibuddin, H., Saad, A., Lim, W., & George, V. (2008). Malaysian rice varieties tolerant to the herbicide imidazolinone for control of weedy rice infestation. *MARDI Kepala Batas, 39*.

Azmi, M., & Karim, S. (2008). Weedy rice-biology, ecology and management. *Kuala Lumpur, Malaysia: Malaysian Agricultural Research and Development Institute (MARDI)*.

Baek, J.-S., & Chung, N.-J. J. R. (2012). Seed wintering and deterioration characteristics between weedy and cultivated rice. *Rice*, *5*(1), Article#21.

Bahm, M. A., Barnes, T. G., & Jensen, K. C. (2011). Herbicide and fire effects on smooth brome (bromus inermis) and kentucky bluegrass (poa pratensis) in invaded prairie remnants. *Invasive Plant Science and Management, 4*(2), 189-197.

Bailey, W. A., & Wilcut, J. W. (2003). Tolerance of imidazolinone-resistant corn (zea mays) to diclosulam 1. *Weed Technology, 17*(1), 60-64.

Bajrai, F., Ismail, B., Mardiana-Jansar, K., & Omar, R. (2017). Persistence of imazapic and imazapyr in paddy soil and water. *Advances in Agrigultural and Environmental Engine (IJAAEE), 4,* 12-15.

Bakar, B. (2004). Invasive weed species in Malaysian agro-ecosystems: Species, impacts and management. *Malaysian Journal of Science, 23*(1), 1-42.

Bakar, B., Bakar, M., & Man, A. (2000). Weedy rice (Oryza sativa l.) in peninsular Malaysia. *Wild and weedy rice in rice ecosystems in Asia—a review. IRRI, Los Banos*, 51-54.

Bakar, B. H. (2006). *Weed science in the service of humanity*: Jabatan Penerbitan, Universiti Malaya.

Bakar, F., Ismail, B., & Bajrai, F. (2016). *Application of bioassay technique to determine onduty herbicide resistance in soil.* Paper presented at the AIP Conference Proceedings. 17th November 2016, Selangor, Malaysia.

Baki, B. (2006). *Weed ecology and management in rice ecosystems*: University of Malaya Press, Kuala Lumpur.

Baki, B. (2007). Utilization of weeds and their relatives in Malaysia. *Kyungpook National University Press*.

Baki, B., Chin, D., & Mortimer, M. (2000). *Wild and weedy rice in rice ecosystems in asia—a review.* Paper presented at the Limited proceedings. 2nd November 2000, Phillippines.

University of Malaya

Baki, B. B., & Shakirin, M. M. (2010). Spatio-temporal distribution pattern of new biotypes of weedy rice (Oryza sativa L.) in Selangor North-West Project, Malaysia. *Korean Journal of Weed Science*, *30*, 68-83.

Baki, H. B. (2006). Weed science in the service of humanity. *Jabatan Penerbitan, Universiti Malaya*. PP(1-148).

Battaglin, W., Furlong, E., Burkhardt, M., & Peter, C. (2000). Occurrence of sulfonylurea, sulfonamide, imidazolinone, and other herbicides in rivers, reservoirs and ground water in the midwestern united states, 1998. *Science of the Total Environment, 248*(2), 123-133.

Bayer, D. (1991). *Pesticide risk assessment in rice paddies: Theory and practice* (E. Capri Ed. First edition 2008 ed.): Elsevier B. V.

Begum, G. (2004). Carbofuran insecticide induced biochemical alterations in liver and muscle tissues of the fish clarias batrachus (linn) and recovery response. *Aquatic Toxicology, 66*(1), 83-92.

Bhullar, M. S., & Chauhan, B. S. (2015). Seed bank dynamics and emergence pattern of weeds as affected by tillage systems in dry direct-seeded rice. *Crop Protection, 67*, 168-177.

Bhushan, L., Ladha, J. K., Gupta, R. K., Singh, S., Tirol-Padre, A., Saharawat, Y., . . . Pathak, H. (2007). Saving of water and labor in a rice–wheat system with no-tillage and direct seeding technologies. *Agronomy Journal, 99*(5), 1288-1296.

Blouin, D. C., Webster, E. P., & Bond, J. A. (2010). On a method of analysis for synergistic and antagonistic joint-action effects with fenoxaprop mixtures in rice (Oryza sativa). *Weed Technology, 24*(4), 583-589.

Bond, J. A., & Walker, T. W. (2011). Differential tolerance of clearfield rice cultivars to imazamox. *Weed Technology, 25*(2), 192-197.

Börjesson, E., Torstensson, L., & Stenström, J. (2004a). The fate of imazapyr in a swedish railway embankment. *Pest Management Science, 60*(6), 544-549.

Börjesson, E., Torstensson, L., & Stenström, J. (2004b). The fate of imazapyr in a swedish railway embankment. *PS Pest Management Science, 60*(6), 544-549.

Bouman, B., Peng, S., Castaneda, A., & Visperas, R. (2005). Yield and water use of irrigated tropical aerobic rice systems. *Agricultural Water Management, 74*(2), 87-105.

Buhler, D. D. (2002). 50th anniversary-invited article: Challenges and opportunities for integrated weed management. *Weed Science, 50*(3), 273-280.

Burgos, N. R., Norman, R. J., Gealy, D. R., & Black, H. (2006). Competitive in uptake between rice and weedy rice. *Field Crops Research, 99*(2), 96-105.

University of Malaya

Burgos, N. R., Norsworthy, J. K., Scott, R. C., & Smith, K. L. (2008). Red rice (Oryza sativa) status after 5 years of imidazolinone-resistant rice technology in Arkansas. *Weed Technology, 22*(1), 200-208.

Burgos, N. R., Singh, V., Tseng, T. M., Black, H., Young, N. D., Huang, Z., & Caicedo, A. L. (2014). The impact of herbicide-resistant rice technology on phenotypic diversity and population structure of United States weedy rice. *Plant Physiology, 166*(3), 1208-1220.

Burke, I. C., Price, A. J., Wilcut, J. W., Jordan, D. L., Culpepper, A. S., & Tredaway-Ducar, J. (2004). Annual grass control in peanut (Arachis hypogaea) with clethodim and imazapic 1. *Weed Technology, 18*(1), 88-92.

Burnside, O., Fenster, C., & Wicks, G. (1963). Dissipation and leaching of monuron, simazine, and atrazine in Nebraska soils. *Weeds*, *11*(3), 209-213.

Busconi, M., Rossi, D., Lorenzoni, C., Baldi, G., & Fogher, C. (2012). Spread of herbicide-resistant weedy rice (red rice, Oryza sativa l.) after 5 years of clearfield rice cultivation in italy. *Plant Biology, 14*(5), 751-759.

Busi, R., Nguyen, N. K., Chauhan, B. S., Vidotto, F., Tabacchi, M., & Powles, S. B. (2017). Can herbicide safeners allow selective control of weedy rice infesting rice crops? *Pest Management Science, 73*(1), 71-77.

Campbell, M., & Swain, F. (1973). Factors causing losses during the establishment of surface-sown pastures. *Journal of Range Management*, *26*(5), 355-359.

Cao, Q., Lu, B.-R., Xia, H., Rong, J., Sala, F., Spada, A., & Grassi, F. (2006). Genetic diversity and origin of weedy rice (Oryza sativa f. Spontanea) populations found in north-eastern china revealed by simple sequence repeat (ssr) markers. *Annals of Botany, 98*(6), 1241-1252.

Cassol, G. V., Avila, L. A. d., Zemolin, C. R., Piveta, A., Agostinetto, D., & Merotto Júnior, A. (2015). Sensitivity of imidazolinone-resistant red rice (Oryza sativa l.) to glyphosate and glufosinate. *Ciência Rural, 45*(9), 1557-1563.

Castillo, L. E., de la Cruz, E., & Ruepert, C. (1997). Ecotoxicology and pesticides in tropical aquatic ecosystems of central america. *Environmental Toxicology and Chemistry, 16*(1), 41-51.

Chauhan, Singh, R. G., & Mahajan, G. (2012). Ecology and management of weeds under conservation agriculture: A review. *Crop Protection, 38*, 57-65.

Chauhan, B., Migo, T., Westerman, P., & Johnson, D. (2010). Post-dispersal predation of weed seeds in rice fields. *Weed Research, 50*(6), 553-560.

Chauhan, B. S. (2012). Weed ecology and weed management strategies for dry-seeded rice in Asia. *Weed Technology, 26*(1), 1-13.

Chauhan, B. S. (2013a). *Management strategies for weedy rice in asia*: Int. Rice Res. Inst.

Chauhan, B. S. (2013b). Strategies to manage weedy rice in Asia. *Crop Protection, 48*, 51-56.

Chauhan, B. S., Abeysekera, A. S., Wickramarathe, M. S., Kulatunga, S. D., & Wickrama, U. B. (2014). Effect of rice establishment methods on weedy rice (Oryza sativa l.) infestation and grain yield of cultivated rice (Oryza. sativa l.) in sri lanka. *Crop Protection, 55*, 42-49.

Chauhan, B. S., & Abugho, S. B. (2013). Weed management in mechanized-sown, zero-till dry-seeded rice. *Weed Technology, 27*(1), 28-33.

Chauhan, B. S., & Johnson, D. E. (2009). Influence of tillage systems on weed seedling emergence pattern in rainfed rice. *Soil and Tillage Research, 106*(1), 15-21.

Chauhan, B. S., & Johnson, D. E. (2010). Weedy rice (Oryza sativa) i. Grain characteristics and growth response to competition of weedy rice variants from five Asian countries. *Weed Science, 58*(4), 374-380.

Chen, L. J., Lee, D. S., Song, Z. P., Suh, H. S., & LU, B. R. (2004). Gene flow from cultivated rice (Oryza sativa) to its weedy and wild relatives. *Annals of Botany, 93*(1), 67-73.

Chi, T. T. N., Mai, T. T. N., Tiyen, T., Paris, T. R., & Heong, K. (2002). Beliefs and management practices of weedy rices: A comparison among male and female rice farmers in the mekong delta, south vietnam. *OmonRice, 10*, 101-106.

Chin, D. V. (2001). Biology and management of barnyardgrass, red sprangletop and weedy rice. *Weed Biology and Management, 1*(1), 37-41.

Croughan, T., Utomo, H., Sanders, D., & Braverman, M. (1996). Herbicide-resistant rice offers potential solution to red rice problem. *Louisiana Agriculture (USA)*.

Croughan, T. P. (2003). Clearfield rice: It's not a gmo. *Louisiana Agriculture, 46*(4), 24-26.

Da Costa Marinho, M. I., Costa, A. I. G., Vieira, N. M., Paiva, M. C. G., de Freitas, F. C. L., da Silva, A. A. J. E., & safety, e. (2019). Validation and application of a quechers based method for estimation of half-life of imidazolinone herbicides in soils by lc-esi-ms/ms. *Ecotoxicology and Environmental Safety*, *167*, 212-217.

D'Ascenzo, G., Gentili, A., Marchese, S., & Perret, D. (1998). Development of a method based on liquid chromatography–electrospray mass spectrometry for analyzing imidazolinone herbicides in environmental water at part-per-trillion levels. *Journal of Chromatography A, 800*(1), 109-119.

Dass, A., Shekhawat, K., Choudhary, A. K., Sepat, S., Rathore, S. S., Mahajan, G., & Chauhan, B. S. (2016). Weed management in rice using crop competition-a review. *Crop Protection*, *95*, 45-52.

Dauer, J., Hulting, A., Carlson, D., Mankin, L., Harden, J., & Mallory-Smith, C. (2017). Gene flow from single and stacked herbicide-resistant rice (Oryza sativa): Modeling occurrence of multiple herbicide-resistant weedy rice. *Pest Management Science*, *24*(2), 348-355.

University of Malaya

De Datta, S. (1995). *Weed management perspectives for sustainable agriculture in rice–based systems.* Paper presented at the Proc. I (A) 15th Asian–Pacific Weed Science. Soc. Conf., 24-28 July 1995, Tsukuba (Japan).

de Oliveira Arias, J. L., Rombaldi, C., Caldas, S. S., & Primel, E. G. (2014). Alternative sorbents for the dispersive solid-phase extraction step in quick, easy, cheap, effective, rugged and safe method for extraction of pesticides from rice paddy soils with determination by liquid chromatography tandem mass spectrometry. *Journal of Chromatography A, 1360*, 66-75.

Debnath, M., Karan, T., Pandey, J., & Biswas, M. (2010). Comparative phytochemical and biological evaluation of different extracts obtained from the leaves of saraca asoka. *Pharmacognosy Journal, 2*(12), 476-480.

Delouche, J. C., & Labrada, R. (2007). *Weedy rices: Origin, biology, ecology and control* (Vol. 188): Food & Agriculture Org.

Demoliner, A., Caldas, S. S., Costa, F. P., Gonçalves, F. F., Clementin, R. M., Milani, M. R., & Primel, E. G. (2010). Development and validation of a method using spe and lc-esi-ms-ms for the determination of multiple classes of pesticides and metabolites in water samples. *Journal of the Brazilian Chemical Society, 21*(8), 1424-1433.

Diarra, A., Smith Jr, R. J., & Talbert, R. E. (1985). Growth and morphological characteristics of red rice (Oryza sativa) biotypes. *Weed Science*, *33*(3), 310-314.

Dilipkumar, M., Burgos, N., Chuah, T., & Ismail, S. (2018). Cross-resistance to imazapic and imazapyr in a weedy rice (Oryza sativa) biotype found in Malaysia. *Planta Daninha*, *36*, 1806-9681.

Ducar, J. T., Wilcut, J. W., & Richburg III, J. S. (2004). Weed management in imidazolinone-resistant corn with imazapic 1. *Weed Technology, 18*(4), 1018-1022.

Ehler, L. E. (2006). Integrated pest management (ipm): Definition, historical development and implementation, and the other ipm. *Pest Management Science, 62*(9), 787-789.

Engku, A., Norida, M., Juraimi, A., Rafii, M., Abdullah, S., & Alam, M. (2016). Gene flow from clearfield® rice to weedy rice under field conditions. *Plant, Soil and Environment, 62*(1), 16-22.

Estorninos Jr, L. E., Gealy, D. R., Talbert, R. E., & Gbur, E. E. (2005). Rice and red rice interference. I. Response of red rice (Oryza sativa) to sowing rates of tropical japonica and indica rice cultivars. *Weed Science, 53*(5), 676-682.

Estorninos, L. E., Gealy, D. R., Gbur, E. E., Talbert, R. E., & McClelland, M. R. J. W. S. (2005). Rice and red rice interference. II. Rice response to population densities of three red rice (Oryza sativa) ecotypes. *Weed Science, 53*(5), 683-689.

University of Malaya

Fariss, M. W., & Reed, D. J. (1987). High-performance liquid chromatography of thiols and disulfides: Dinitrophenol derivatives. *Methods in Enzymology, 143*, 101-109.

Farooq, M., Siddique, K. H., Rehman, H., Aziz, T., Lee, D.-J., & Wahid, A. (2011). Rice direct seeding: Experiences, challenges and opportunities. *Soil and Tillage Research, 111*(2), 87-98.

Ferrer, C., Lozano, A., Agüera, A., Girón, A. J., & Fernández-Alba, A. (2011). Overcoming matrix effects using the dilution approach in multiresidue methods for fruits and vegetables. *Journal of Chromatography A, 1218*(42), 7634-7639.

Ferrero, A. (2003). Weedy rice, biological features and control. *FAO Plant Production and Protection Paper (FAO)*.

Ferrero, A., & Finassi, A. (1995). Viability and soil distribution of red rice (Oryza sativa var. Silvatica) seeds [wild rice, a weed in most rice growing areas in the world]. *Mededelingen-Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen Universiteit Gent (Belgium)*.

Ferrero, A., & Vidotto, F. (1998). *Germinability after flowering, shattering ability and longevity of red rice seeds.* Paper presented at the Proceeding of the 6th European Weed Research Society Mediterranean Symposium, 15 May 1998. Montpellier, France. Doorwerth, The Netherlands: European Weed Science Society.

Fish, J. C., Webster, E. P., Blouin, D. C., & Bond, J. A. (2015). Imazethapyr co-application interactions in imidazolinone-resistant rice. *Weed Technology, 29*(4), 689-696.

Fogliatto, S., Vidotto, F., & Ferrero, A. (2011). Germination of weedy rice in response to field conditions during winter. *Weed Technology, 25*(2), 252-261.

Fogliatto, S., Vidotto, F., & Ferrero, A. (2012). Morphological characterisation of italian weedy rice (Oryza sativa) populations. *Weed Research, 52*(1), 60-69.

Fredericks, L. (1981). Ecology, new technology and rural development: Impressions of kampung ulu tiram burok. *(Green revolution and rural development in Asia). 東南アジア研究, 18*(4), 587-605.

Gagne, J. A., Fischer, J. E., Sharma, R. K., Traul, K. A., Diehl, S. J., Hess, F. G., & Harris, J. E. (1991). Toxicology of the imidazolinone herbicides. *The Imidazolinone Herbicides*, 179-182.

Garrido, R. J. L.-B., & López-Bellido, L. (2001). Effects of crop rotation and nitrogen fertilization on soil nitrate and wheat yield under rainfed mediterranean conditions. *Agronomie, 21*(6-7), 509-516.

Carol, A., Mallory, S., & Donald, C. 1990. Identification of Sulfonylurea Herbicide-Resistant Prickly Lettuce (Lactuca serrola). *Weed Technology, 4*, 163-168.

Gealy, D. R., Agrama, H. A., & Eizenga, G. C. (2009). Exploring genetic and spatial structure of us weedy red rice (Oryza sativa) in relation to rice relatives worldwide. *Weed Science, 57*(6), 627-643.

Gealy, D. R., & Gressel, J. (2005). Gene movement between rice (Oryza sativa) and weedy rice (Oryza sativa)-a us temperate rice perspective. *Crop Ferality and Volunteerism*, 323-354.

Gealy, D. R., Mitten, D. H., & Rutger, J. N. (2003). Gene flow between red rice (Oryza sativa) and herbicide-resistant rice (Oryza. Sativa): Implications for weed management 1. *Weed Technology, 17*(3), 627-645.

Gealy, D. R., Saldain, N. E., & Talbert, R. E. (2000). Emergence of red rice (Oryza sativa) ecotypes under dry-seeded rice (Oryza sativa) culture 1. *Weed Technology, 14*(2), 406-412.

George, (2013). Methods of Soil, Plant, and Water Analysis. ICARDA (International Center for Agricultural Research in the Dry Areas) Beirut, Lebanon.

Gianelli, V. R., Bedmar, F., & Costa, J. L. (2014). Persistence and sorption of imazapyr in three argentinean soils. *Environmental Toxicology and Chemistry, 33*(1), 29-34.

Goetz, A. J., Wehtje, G., Walker, R. H., & Hajek, B. (1986). Soil solution and mobility characterization of imazaquin. *Weed Science*, 788-793.

Goulart, I., Matzenbacher, F., & Merotto, A. (2012). Differential germination pattern of rice cultivars resistant to imidazolinone herbicides carrying different acetolactate synthase gene mutations. *Weed Research, 52*(3), 224-232.

Graham, R., Pratley, J., Slater, P., Humphreys, E., Murray, E., Clampett, W., & Lewin, L. (1994). *Herbicide resistance in cyperus difformis, a weed of new south wales rice crops.* Paper presented at the Proceedings of Temperate Rice Conference—Achievements and Potential. 21-24 February 1994, Leeton, New South Wales, Australia. Griffith, Australia: Temperate Rice Conference Organising Committee, 433-435.

Green, J., Barker, J., Marshall, E., Froud-Williams, R., Peters, N., Arnold, G., . . . Karp, A. (2001). Microsatellite analysis of the inbreeding grass weed barren brome (anisantha sterilis) reveals genetic diversity at the within-and between-farm scales. *Molecular Ecology, 10*(4), 1035-1045.

Gressel, J., & Valverde, B. E. (2009). A strategy to provide long-term control of weedy rice while mitigating herbicide resistance transgene flow, and its potential use for other crops with related weeds. *Pest Management Science, 65*(7), 723-731.

Grey, T. L., Cutts III, G. S., & Johnson, J. (2012). Imidazolinone-resistant soft red winter wheat weed control and crop response to als-inhibiting herbicides. *Weed Technology, 26*(3), 405-409.

University of Malaya

Grimm, A., Fogliatto, S., Nick, P., Ferrero, A., & Vidotto, F. (2013). Microsatellite markers reveal multiple origins for italian weedy rice. *Ecology and Evolution, 3*(14), 4786-4798.

Grymes, C. F., Chandler, J. M., & Nester, P. R. (1995). Response of soybean (glycine max) and rice (Oryza sativa) in rotation to ac 263,222. *Weed Technology*, 504-511.

Gu, X.-Y., Chen, Z.-X., & Foley, M. E. (2003). Inheritance of seed dormancy in weedy rice. *Crop Science, 43*(3), 835-843.

Gu, X.-Y., Kianian, S. F., & Foley, M. E. (2005). Seed dormancy imposed by covering tissues interrelates to shattering and seed morphological characteristics in weedy rice. *Crop Science, 45*(3), 948-955.

Gunawardana, D. (2008). Harvesting serendipity. *Rice Today, 7*(4), 36-39.

Hakim, M., Juraimi, A. S., Hanafi, M., Ismail, M. R., Selamat, A., Rafii, M., & Latif, M. (2014). Biochemical and anatomical changes and yield reduction in rice (Oryza sativa l.) under varied salinity regimes. *BioMed Research International, 2014*, 1-11.

Hakim, M. A., Juraimi, A. S., Musa, M. H., Ismail, M. R., Rahman, M. M., & Selamat, A. (2013). Impacts of weed competition on plant characters and the critical period of weed control in rice under saline environment. *Australian Journal of Crop Science, 7*(8), 1141-1151.

Hamdani, M., Juraimi, AS., & Mazlan, N. (2015). *Herbicide resistant weeds in Malaysian rice fields: Will weedy rice become the next candidate.* 25th Asian-Pacific Weed Science Society Conference. 12th December 2015, Hyderabad, India.

He, Q., Kim, K. W., & Park, Y. J. (2017). Population genomics identifies the origin and signatures of selection of Korean weedy rice. *Plant Biotechnology Journal, 15*(3), 357-366.

Heap, I. (2014). Herbicide resistant weeds. In *Integrated pest management* (281-301): Springer.

Heap, I. (2015). The international survey of herbicide resistant weeds, 2012. *Retrived on October 1, 2018 from:* http://www. *weedscience. org*.

Helling, C. S., & Doherty, M. A. (1995). Improved method for the analysis of imazapyr in soil. *Pesticide Science, 45*(1), 21-26.

Hogendoorn, E. A. (2006). High-performance liquid chromatography methods in pesticide residue analysis. *Encyclopedia of Analytical Chemistry*.

Holdiness, M. R. (1985). Chromatographic analysis of antituberculosis drugs in biological samples. *Journal of Chromatography B: Biomedical Sciences and Applications, 340*, 321-359.

University of Malaya

Hollaway, K., Kookana, R. S., Noy, D., Smith, J., & Wilhelm, N. (2006). Persistence and leaching of sulfonylurea herbicides over a 4-year period in the highly alkaline soils of South-Eastern Australia. *Australian Journal of Experimental Agriculture, 46*(8), 1069-1076.

Hoseiny-Rad, M., & Jagannath, S. (2011). Effect of herbicide imazethapyr (pursuit™) on chickpea seed germination. *Archives of Phytopathology and Plant Protection, 44*(3), 224-230.

Ibrahim, M. B., Juraimi, A. S., Hakim, M., Aslani, F., Karim, S., & Alam, M. A. (2017). Residual effect of imidazolinone herbicide used in clearfield (r) rice on non-clearfield rice. *Bangladesh Journal of Botany, 46*(1), 335-341.

Itoh, K. and Matsunaka, S. (1990) Parapatric differentiation of paraquat resistant biotypes in some compositae species. In 'Biological approaches and evolutionary trends in plants', S. Kawano ed. Academic Press, 33 -49.

Itoh, K., & Wang, G. (1997). *An outbreak of sulfonylurea herbicide resistance in scrophulariaceae paddy weeds in Japan.* Paper presented at the Proceedings 16th Asian-Pacific Weed Science Society Conference. 8-12th September 1997, Kuala Lumpur, Malaysia.

Jaafar, N. F., Juraimi, A. S., Ahmad-Hamdani, M. S., UDDIN, M. K., & Man, A. (2014a). Distribution of weedy rice escape variants in clearfield rice production system. *Research on Crops, 15*(4), 754-762.

Jason K. Norsworthy, R. C. S. (2011). Weed management in furrow - irrigated imidazoline - resistant hybrids rice production system. *Weed Technology, 25*, 25-29.

Jia, S. (2002). *Studies on gene flow in china–a review.* Paper presented at the The 7th International Symposium on the Biosafety of Genetically Modified Organisms. 10th October 2002, Beijing, China.

Jourdan, S. W., Majek, B. A., & Ayeni, A. O. (1998). Imazethapyr bioactivity and movement in soil. *Weed Science*, 608-613.

Juraimi, A. S., Uddin, M. K., Anwar, M. P., Mohamed, M. T. M., Ismail, M. R., & Man, A. (2013). Sustainable weed management in direct seeded rice culture: A review. *Australian Journal of Crop Science, 7*(7), 989-1002.

Kaloumenos, N. S., Capote, N., Aguado, A., & Eleftherohorinos, I. G. (2013). Red rice (Oryza sativa) cross-resistance to imidazolinone herbicides used in resistant rice cultivars grown in northern Greece. *Pesticide Biochemistry and Physiology, 105*(3), 177-183.

Kane, N. C., & Baack, E. J. (2007). The origins of weedy rice. *Molecular Ecology, 16*(21), 4423-4425.

Karim, R. S., Man, A. B., & Sahid, I. B. (2004). Weed problems and their management in rice fields of Malaysia: An overview. *Weed Biology and Management, 4*(4), 177-186.

University of Malaya

Karunarathna KDK, S. S. a. W. S. (2017). Density dependent competitive interactionsbetween weedy rice (Oryza sativa f. Spontanea)and cultivated rice in Sri Lanka. *Agricultural Research and Technology Journal*. *4*(1), 1-9.

Kelly Vaughan, L., Ottis, B. V., Prazak-Havey, A. M., Bormans, C. A., Sneller, C., Chandler, J. M., & Park, W. D. (2001). Is all red rice found in commercial rice really Oryza sativa?. *Weed Science, 49*(4), 468-476.

Kemmerich, M., Bernardi, G., Adaime, M. B., Zanella, R., & Prestes, O. D. (2015). A simple and efficient method for imidazolinone herbicides determination in soil by ultra-high performance liquid chromatography–tandem mass spectrometry. *Journal of Chromatography A, 1412*, 82-89.

Khaliq, A., Matloob, A., Ahmad, N., Rasul, F., & Awan, I. (2012). Post emergence chemical weed control in direct seeded fine rice. *Animal and Plant Science, 22*(4), 1101-1106.

Khan, N., Khan, N., Khan, S., Khan, M., & Marwat, K. (2012). Combined effect of nitrogen fertilizers and herbicides upon maize production in Peshawar. *Journal of Animal and Plant Science, 2*, 12-18.

Kharkwal, M., & Shu, Q. (2009). The role of induced mutations in world food security. *Induced plant mutations in the genomics era. Food and Agriculture Organization of the United Nations, Rome*, 33-38.

Khush, G. (1993). Floral structure, pollination biology, breeding behaviour, transfer distance and isolation considerations. *World bank technical paper. Biotechnology Series*(1).

Kim, S.-C., & Ha, W.-G. (2005). Direct seeding and weed management in korea. *Rice Is Life: Scientific Perspectives for the 21st Century. Los Banos, Philippines: International Rice Research Institute, and Tsukuba, Japan: Japan International Research Center for Agricultural Sciences*, 181-184.

Klingman, T. E., King, C. A., & Oliver, L. R. (1992). Effect of application rate, weed species, and weed stage of growth on imazethapyr activity. *Weed Science*, 227-232.

Kohara, H. (1996). Resistance to sulfonylurea herbicides in monochoria korsakowii regel et maack. In hokkaido. *Weed Science and Technolology, 41*, 236-237.

Koutros, S., Silverman, D. T., Alavanja, M. C., Andreotti, G., Lerro, C. C., Heltshe, S., Beane Freeman, L. E. (2015). Occupational exposure to pesticides and bladder cancer risk. *International Journal of Epidemiology, 45*(3), 792-805.

Kraehmer, H. (2012). Innovation: Changing trends in herbicide discovery. *Outlooks on Pest Management, 23*(3), 115-118.

Krieger, R. (2001). *Handbook of pesticide toxicology, two-volume set: Principles and agents* (Vol. *1*): Academic press. New York.

Krynitsky, A. J., Stout, S. J., Nejad, H., & Cavalier, T. C. (1999). Multiresidue determination and confirmation of imidazolinone herbicides in soil by high-

University of Malaya

performance liquid chromatography/electrospray ionization mass spectrometry. *Journal-AOAC International, 82(4)*, 956-962.

Kumar, V., Bellinder, R., Gupta, R., Malik, R., & Brainard, D. (2008). Role of herbicide-resistant rice in promoting resource conservation technologies in rice–wheat cropping systems of india: A review. *Crop Protection, 27*(3), 290-301.

Kwon, S. L., Smith Jr, R. J., & Talbert, R. E. (1991). Interference durations of red rice (Oryza sativa) in rice (o. Sativa). *Weed Science*, 363-368.

Laabs, V., Amelung, W., Pinto, A., Altstaedt, A., & Zech, W. (2000). Leaching and degradation of corn and soybean pesticides in an oxisol of the brazilian cerrados. *Chemosphere, 41*(9), 1441-1449.

Labrada, R. (2002). Major weed problems in rice–red/weedy rice and the echinochloa complex. *FAO rice information, (11-17). Rome.*

Laganà, A., Fago, G., & Marino, A. (1998). Simultaneous determination of imidazolinone herbicides from soil and natural waters using soil column extraction and off-line solid-phase extraction followed by liquid chromatography with uv detection or liquid chromatography/electrospray mass spectroscopy. *Analytical Chemistry, 70*(1), 121-130.

Laganà, A., Fago, G., Marino, A., & Penazzi, V. M. (2000). Liquid chromatography mass spectrometry tandem for multiresidue determination of selected post-emergence herbicides after soil column extraction. *Analytica Chimica Acta, 415*(1), 41-56.

Langevin, S. A., Clay, K., & Grace, J. B. (1990). The incidence and effects of hybridization between cultivated rice and its related weed red rice (Oryza sativa l.). *Evolution*, 1000-1008.

Lao, W., & Gan, J. (2006). High-performance liquid chromatographic separation of imidazolinone herbicide enantiomers and *their* methyl derivatives on polysaccharide-coated chiral stationary phases. *Journal of Chromatography A, 1117*(2), 184-193.

León, L., Mozo-Mulero, C., Martín-Infante, F., Maraver, J., Carbajo, J., & Mozo, J. J. A. J. o. C. (2018). Inexpensive fia method to determine trace levels of imazapyr by uv-detection enhanced with electrochemical polarization. *Arabian Journal of Chemistry, 12*(7), 1042-1049.

Li, L.-F., Li, Y.-L., Jia, Y., Caicedo, A. L., & Olsen, K. M. (2017). Signatures of adaptation in the weedy rice genome. *Nature Genetics, 49*(5), 811-814.

Lode, O., & Meyer, K. (1999). Report 'leaching of pesticides'. *The Norwegian Crop Research Institute, 8*, 99.

Londo, J., & Schaal, B. (2007). Origins and population genetics of weedy red rice in the USA. *Molecular Ecology, 16*(21), 4523-4535.

Loux, M. M, and Reese, D. K. (1993). Effect of Soil Type and pH on Persistence and Carryover of Imidazolinone Herbicides. *Weed Science*. *7*(2), 452-458.

Lu, B.-R., & Snow, A. A. (2005). Gene flow from genetically modified rice and its environmental consequences. *Bioscience, 55*(8), 669-678.

Ma, J.-w., Chen, L., Wang, J.-f., Jiang, G., & Pan, C. (2007). Determination of imazethapyr residues in soil by spe-hplc. *Pesticides Shenyang, 46*(12), 843.

Mahajan, G., & Chauhan, B. (2015). Weed control in dry direct-seeded rice using tank mixtures of herbicides in south Asia. *Crop Protection, 72*, 90-96.

Mai, V., Chien, H., Suong, V., & Thiet, L. (1998). *Survey and analysis of farmers' seed contamination by weed and weedy rice seeds in south vietnam. 1997.* Paper presented at the International Symposium on Wild and Weedy Rices in Agroecosystems. 10-11 August 1998, Ho chi Minh City, Vietnam.

Mai, V., Chien, H., Van, V., Thi, V., Suong, T., & Thiet, L. (2000). *Rice seed contamination in vietnam.* Paper presented at the Wild and weedy rice in rice ecosystems in Asia: a review. Limited Proceedings. 5 January 2000, No. 2. International Rice Research Institute.

Maneechote, C., Jamjod, S., Rerkasem, B., & Rerkasem, K. (2005). *Farmers' participation in development of integrated methods of weedy rice control in thailand.* Paper presented at the Proceedings of 20th Asian Pacific Weed Science Society Conference, Ho Chi Minh City, Vietnam.

Mangels, G. (1991). Behavior of the imidazolinone herbicides in soil—a review of the literature. *The Imidazolinone Herbicides. Boca Raton, FL: CRC*, 191-209.

Mannion, A. M. (1995). Agriculture, environment and biotechnology. *Agriculture, Ecosystems & Environment, 53*(1), 31-45.

Mansor, M., Karim, S. R., & Abidin, Z. (2012). Effects of farmers cultural practices on the weedy rice infestation and rice production. *Scientific Research and Essays, 7*(5), 609-615.

Marambe, B. (2009). Weedy rice—evolution, threats and management. *Trop Agriculturist, 157*, 43-64.

Marchesan, E., Dos Santos, F. M., Grohs, M., De Avila, L. A., Machado, S. L., Senseman, S. A., & Sartori, G. M. (2010). Carryover of imazethapyr and imazapic to nontolerant rice. *Weed Technology, 24*(1), 6-10.

Marcia, e. a. (2014). Amethod for determination of imazapic and imazethapyr residues in soil using an ultrasonic assisted extraction. *Bull Environ Contam Toxical, 93*, 360-364.

Martins, G. L., Friggi, C. A., Prestes, O. D., Vicari, M. C., Friggi, D. A., Adaime, M. B., & Zanella, R. (2014). Simultaneous lc–ms/ms determination of imidazolinone herbicides together with other multiclass pesticide residues in soil. *CLEAN–Soil, Air, Water, 42*(10), 1441-1449.

Masson, J. A., & Webster, E. P. (2001). Use of imazethapyr in water-seeded imidazolinone-tolerant rice (Oryza sativa) 1. *Weed Technology, 15*(1), 103-106.

Mastan, J., Srinivas, B., Rao, T. N., & Apparao, K. (2016). Determination of imazamox and imazethapyr herbicide residues in soybean oil. *Deer Pharma Chemica, 8(8), 102-107.*

Mazlan, A. Z., Hussain, H., & Zawawi, M. A. M. (2016). Potential dermal exposure assessment of farmers to herbicide imazapic in an agriculture area. *Procedia-Social and Behavioral Sciences, 234*, 144-153.

McDonald, M. J. S. S. T. (1999). Seed deterioration: Physiology, repair and assessment. *Seed Science and Technolology, 27*, 177-237.

McWilliams, D. A. (1997). Southern weed science society's weeds of the united states, produced by information design (software). *Journal of Natural Resources and Life Sciences Education, 26*(1), 83-84.

Meins, K., Scott, R., Dillon, T., & Pearrow, N. (2003). Tolerance of clearfield rice to imazamox. *BR Wells Rice Research Studies*, 132-136.

Merotto, A., Goulart, I.G., Nunes, A., Kalsing, A., Markus, C., Menezes, V. & Wander, A. (2006). Evolutionary and social consequences of introgression of non - transgenic herbicide resistance from rice to weedy rice in Brazil. *Evolutionary Application*, *9*, 837-846.

Michael, J. L., & Neary, D. G. (1993). Herbicide dissipation studies in southern forest ecosystems. *Environmental Toxicology and Chemistry, 12*(3), 405-410.

Mispan. M. S. (2008). Descriptive analyses on the new biotype of weedy rices in selangor north-west project, Malaysia. (Unpublished masters thesis) *University of Malaya, Kuala Lumpur, Malaysia.*

Mispan, M. (2015). Descriptive analyses on the new biotype of weedy rices in selangor north-west project, Malaysia. (Unpublished master thesis) *University Malaya, Kuala Lumpur, Malaysia*.

Mispan, M. S. (2008). " Descriptive analyses on the new biotype of weedy rices in Selangor North-West project, Malaysia. (Unpublished master thesis) *University Malaya, Kuala Lumpur, Malaysia*.

Mispan, M. S., Zhang, L., Feng, J., & Gu, X.-Y. (2013). Quantitative trait locus and haplotype analyses of wild and crop-mimic traits in US. weedy rice. *G3: Genes Genomes Genetics. 3*, 1049-1059.

Miura, K., Lin, S., Yano, M., Nagamine, T. J. T., & Genetics, A. (2002). Mapping quantitative trait loci controlling seed longevity in rice (Oryza sativa l.) *Theoretical and Applied Genetics. 104*(6-7), 981-986.

Mohler, C., & Asdale, J. (1993). Response of weed emergence to rate of vicia villosa roth and secale cereale l. Residue. *Weed Research, 33*(6), 487-499.

University of Malaya

Moraes, B. S., Clasen, B., Loro, V. L., Pretto, A., Toni, C., de Avila, L. A., . . . Reimche, G. B. (2011). Toxicological responses of cyprinus carpio after exposure to a commercial herbicide containing imazethapyr and imazapic. *Ecotoxicology and Environmental Safety, 74*(3), 328-335.

Morishima, H., & Gadrinab, L. U. (1987). Are the asian common wild rices differentiated into the indica and japonica types. *Crop Exploration and Utilization of Genetic Resources*, 11-20.

Mortimer, A., Ulf-Hansen, P., & Putwain, P. (1992). Modelling herbicide resistance-a study of ecological fitness. In *Resistance'91: Achievements and developments in combating pesticide resistance* (pp. 148-164): Springer.

Mortimer, M., Pandey, S., & Piggin, C. (2000). Weedy rice: Approaches to ecological appraisal and implications for research priorities. *Proceedings of Wild and Weedy Rice in Rice Ecosystems in Asia. A review. Los Banos, Philippines: International Rice Research Institute*, 97-105.

Muker, H., & Sharma, H. (1991). Isolation distance for producing hybrid rice seed. *International Rice Research Notes, 16*(1), Article#6.

Mukherjee, P., Sarkar, A., & Maity, S. K. (2008). Critical period of crop-weed competition in transplanted and wet-seeded kharif rice (Oryza sativa l.) under terai conditions. *Indian Journal of Weed Science, 40*(3 and 4), 147-152.

Mukhopadhyay, S. (1995). *Current scenario and future lines of weed management in rice crop with particular references to India.* Paper presented at the Proc. 15th APWSS, conf. Tsukuba, Japan.

Najim, M., Lee, T., Haque, M. A., & Esham, M. (2007). Sustainability of rice production: A Malaysian perspective. *Journal of Agricultural Sciences, 3*(1), (1-12).

Naredo, M. E. B., Juliano, A. B., Lu, B.-R., & Jackson, M. T. (1998). Taxonomic status of Oryza glumaepatula steud. Ii. Hybridization between new world diploids and aa genome species from asia and australia. *Genetic Resources and Crop Evolution, 45*(3), 205-214.

Neto, M. D. d. C., Souza, M. d. F., Silva, D. V., Faria, A. T., da Silva, A. A., Pereira, G. A. M., & de Freitas, M. A. M. (2017). Leaching of imidazolinones in soils under a clearfield system. *Archives of Agronomy and Soil Science, 63*(7), 897-906.

Noldin, J. (1998). *Red rice situation and management in the Americas*. *In* "International Symposium on Wild and Weedy Rices in Agro-ecosystem. 10 August 1998, Asian Pacific Weed Science Society". 36-41.

Noldin, J., Chandler, J., & McCauley, G. (2006). Seed longevity of red rice ecotypes buried in soil. *Planta Daninha, 24*(4), 611-620.

Noldin, J. A. (2000). Red rice status and management in the americas. *Wild and Weedy Rice in Rice Ecosystems in Asia-A Review. International Rice Research Institute: Los Baños (Philippines)*, 21-24.

Norsworthy, J. K., Bond, J., & Scott, R. C. (2013). Weed management practices and needs in Arkansas and Mississippi rice. *Weed Technology, 27*(3), 623-630.

Novakova, O. (1994). Determination of imazethapyr and imazapyr residues in soil by coupled-column liquid chromatography. *Chromatographia, 39*(1), 62-66.

Oka, H.-I. (2012). *Origin of cultivated rice. National Inst. Genetic, Miasima 411, Japan*.

Olofsdotter, M., Valverde, B., & Madsen, K. H. (2000). Herbicide resistant rice (Oryza sativa l.): Global implications for weedy rice and weed management. *Annals of Applied Biology, 137*(3), 279-295.

Olsen, K. M., Caicedo, A. L., & Jia, Y. (2007). Evolutionary genomics of weedy rice in the USA. *Journal of Integrative Plant Biology, 49*(6), 811-816.

Ottis, B. V., Chandler, J. M., & Mccauley, G. N. (2003). Imazethapyr application methods and sequences for imidazolinone-tolerant rice (Oryza sativa) 1. *Weed Technology, 17*(3), 526-533.

Ottis, B. V., O'barr, J. H., Mccauley, G. N., & Chandler, J. M. (2004). Imazethapyr is safe and effective for imidazolinone-tolerant rice grown on coarse-textured soils 1. *Weed Technology, 18*(4), 1096-1100.

Ottis, B. V., Smith, K. L., Scott, R. C., & Talbert, R. E. (2005). Rice yield and quality as affected by cultivar and red rice (Oryza sativa) density. *Weed Science, 53*(4), 499-504.

Owen, M. D. (1997). Midwest experiences with herbicide resistant crops. Proceedings of the Western Society of Weed Science. 9-10.

Ozcan, C., Cebi, U. K., Gurbuz, M. A., & Ozer, S. (2017). Residue analysis and determination of imi herbicides in sunflower and soil by gc–ms. *Chromatographia*, 1-10.

Pacanoski, Z., & Glatkova, G. (2009). The use of herbicides for weed control in direct wet-seeded rice (Oryza sativa l.) in rice production regions in the republic of macedonia. *Plant Protect. Sci, 45*(3), 113-118.

Pace, P., Senseman, S., Ketchersid, M., & Cralle, H. (1999). Supercritical fluid extraction and solid-phase extraction of ac 263,222 and imazethapyr from three texas soils. *Archives of Environmental Contamination and Toxicology, 37*(4), 440-444.

Pandey, S., & Velasco, L. (2005). Trends in crop establishment methods in asia and research issues. *Rice Is Life: Scientific Perspectives for the 21st Century*, 178-181.

Parameswari, Y., & Srinivas, A. (2017). Weed management in rice–a review. *Internatinonal journal of Applied and Pure science and Agriculture*, *3(1),* 75-80

University of Malaya

Pellerin, K. J., Webster, E. P., Zhang, W., & Blouin, D. C. (2003). Herbicide mixtures in water-seeded imidazolinone-resistant rice (Oryza sativa) 1. *Weed Technology, 17*(4), 836-841.

Pellerin, K. J., Webster, E. P., Zhang, W., & Blouin, D. C. (2004). Potential use of imazethapyr mixtures in drill-seeded imidazolinone-resistant rice 1. *Weed Technology, 18*(4), 1037-1042.

Perera UIP, Ratnasekera WADPR, Senanayake SGJN (2010). Morphological diversity of weedy rice accessions collected in Ampara district. Proceedings of the 15th International Forestry and Environmental Symposium, 26-27 November 2010, University of Sri Jayawardhanapura, Sri Lanka. 190–194.

Power, J., & Follett, R. (1987). Monoculture. *Scientific American (USA). 256(3)*,78-87.

Pusadee, T., Schaal, B. A., Rerkasem, B., & Jamjod, S. (2013). Population structure of the primary gene pool of Oryza sativa in thailand. *Genetic Resources and Crop Evolution, 60*(1), 335-353.

Pyšek, P., & Prach, K. (2003). Research into plant invasions in a crossroads region: History and focus. *Biological Invasions, 5*(4), 337-348.

Qi, Y., Yan, B., Fu, G., Guan, X., Du, L., & Li, J. (2017). Germination of seeds and seedling growth of amaranthus retroflexus l. Following sublethal exposure of parent plants to herbicides. *Scientific Reports, 7*(157), 1-8.

Rainbolt, C. R., Thill, D. C., Yenish, J. P., & Ball, D. A. (2004). Herbicide-resistant grass weed development in imidazolinone-resistant wheat: Weed biology and herbicide rotation 1. *Weed Technology, 18*(3), 860-868.

Raj, S. K., & Syriac, E. K. (2017). Weed management in direct seeded rice: a review. *Agricultural Reviews, 38*(1), 41-50.

Rajamoorthy, Y., & Munusamy, S. (2015). Rice industry in Malaysia: Challenges, policies and implications. *Procedia Economics and Finance, 31*, 861-867.

Ramezani, M., Simpson, N., Oliver, D., Kookana, R., Gill, G., & Preston, C. (2009). Improved extraction and clean-up of imidazolinone herbicides from soil solutions using different solid-phase sorbents. *Journal of Chromatography A, 1216*(26), 5092-5100.

Rao, A., Johnson, D., Sivaprasad, B., Ladha, J., & Mortimer, A. (2007). Weed management in direct-seeded rice. *Advances in Agronomy, 93*, 153-255.

Rao, A. N., Brainard, D. C., Kumar, V., Ladha, J. K., & Johnson, D. E. (2017). Chapter two-preventive weed management in direct-seeded rice: Targeting the weed seedbank. *Advances in Agronomy, 144*, 45-142.

Rashid, M. H., Alam, M. M., Khan, M. A. H., & Ladha, J. (2009). Productivity and resource use of direct-(drum)-seeded and transplanted rice in puddled soils in rice–rice and rice–wheat ecosystems. *Field Crops Research, 113*(3), 274-281.

University of Malaya

Rathore, M., Singh, R., & Kumar, B. (2013). Weedy rice: An emerging threat to rice cultivation and options for its management. *Current Science, 105*(8), 1067-1072.

Rathore, M., Singh, R., Kumar, B., & Chauhan, B. (2016). Characterization of functional trait diversity among indian cultivated and weedy rice populations. *Scientific Reports, 6* (24167), 1-9.

Ratnasekera, D., Perera, U. I., He, Z., Senanayake, S. G., Wijesekara, G. A., Yang, X., & Lu, B. (2014). High level of variation among sri lankan weedy rice populations, as estimated by morphological characterization. *Weed Biology and Management, 14*(1), 68-75.

Reagon, M., Thurber, C. S., Gross, B. L., Olsen, K. M., Jia, Y., & Caicedo, A. L. (2010). Genomic patterns of nucleotide diversity in divergent populations of us weedy rice. *BMC Evolutionary Biology, 10*(1), 1471-2184.

Reagon, M., Thurber, C. S., Olsen, K. M., Jia, Y., & Caicedo, A. L. (2011). The long and the short of it: Sd1 polymorphism and the evolution of growth trait divergence in us weedy rice. *Molecular Ecology, 20*(18), 3743-3756.

Rebelo, A. M., Dolzan, M. D., Heller, M., Deschamps, F. C., Abate, G., Micke, G. A., & Grassi, M. T. (2016). Simultaneous determination of herbicides in rice by quechers and lc-ms/ms using matrix-matched calibration. *Journal of the Brazilian Chemical Society, 27*(1), 186-193.

Refatti, J. P., Avila, L. A. d., Noldin, J. A., Pacheco, I., & Pestana, R. R. (2017). Leaching and residual activity of imidazolinone herbicides in lowland soils. *Ciência Rural, 47*(5), 1678-4596.

Rekha, K. B., Raju, M., & Reddy, M. (2002). Effect of herbicides in transplanted rice. *Indian Journal of Weed Science, 34*(2), 123-125.

Richburg III, J. S., Wilcut, J. W., & Vencill, W. K. (1996). Imazethapyr systems for peanut (arachis hypogaea l.). *Peanut Science, 23*(1), 9-14.

Richburg III, J. S., Wilcut, J. W., & Wiley, G. L. (1995). Ac 263,222 and imazethapyr rates and mixtures for weed management in peanut (arachis hypogaea). *Weed Technology*, *1*, 801-806.

Roberts, E. J. A. o. B. (1961). The viability of rice seed in relation to temperature, moisture content, and gaseous environment. *Annals of Botany*, *25*(3), 381-390.

Roso, A., Merotto Jr, A., Delatorre, C., & Menezes, V. (2010). Regional scale distribution of imidazolinone herbicide-resistant alleles in red rice (Oryza sativa l.) determined through snp markers. *Field Crops Research, 119*(1), 175-182.

Sadohara, H., Watanabe, O., & Rich, G. (2000). Control of weedy rice. *Wild and Weedy Rice in Rice Ecosystems in Asia-A Review (Baki, BB, Chin, DV and Mortimer, M. eds.). IRRI, Manila, Philippines*, 97-105.

University of Malaya

Santos, L., Pinto, J., Piveta, L., Noldin, J., Galon, L., & Concenço, G. (2014). Carryover effect of imidazolinone herbicides for crops following rice. *American Journal of Plant Sciences, 5*(08), 1049-1058.

Sasaki, K., Fukuta, Y., & Sato, T. J. P. b. (2005). Mapping of quantitative trait loci controlling seed longevity of rice (Oryza sativa l.) after various periods of seed storage, *124*(4), 361-366.

Sasaki, S., & Kozlowski, T. (1968). Effects of herbicides on seed germination and early seedling development of pinus resinosa. *Botanical Gazette, 129*(3), 238-246.

Sattin, M., Berto, D., Zanin, G., & Tabacchi, M. (1999). *Resistance to als inhibitors in weeds of rice in north-western italy.* Paper presented at the Brighton Crop Protection Conference Weeds. 10th Sptember 1999, *3(5)*, 783-790.

Scarabel, L., Cenghialta, C., Manuello, D., & Sattin, M. (2012). Monitoring and management of imidazolinone-resistant red rice (Oryza sativa l., var. Sylvatica) in clearfield® italian paddy rice. *Agronomy, 2*(4), 371-383.

Schreiber, F., Scherner, A., Massey, J. H., Zanella, R., & Avila, L. A. (2017). Dissipation of clomazone, imazapyr, and imazapic herbicides in paddy water under two rice flood management regimes. *Weed Technology, 31*(2), 330-340.

Senseman, S. A. (2007). *Herbicide handbook* .US : 458 p; 9th ed.

Service, F. A. (2015). Southeast Asia: 2015/16 rice production outlook at record levels. Retrieved on June 15, 2018 from https://ipad.fas.usda.gov/highlights/2015/06/Southeast_Asia.

Shafiee, M., Boyce, A., Khandaker, M., Saad, J., Aziz, T. A., Mispan, M., Bakar, B. (2013). Pilot studies on rice. Yield enhancement with foliar application of sbaja in sungai besar, selangor, Malaysia. *Life Science Journal, 10*(1), 329-335.

Shaifuddin, S. N. M., Mazlan, A. Z., Hussain, H., Sameni, M., & Ishak, A. R. (2014). Analytical method development for imazapyr and imazapic herbicides using high performance liquid chromatography. International Conference on Chemical, Environment & Biological Sciences (CEBS-2014) Sept. 17-18, 2014 Kuala Lumpur (Malaysia).

Sharma, P. K., Ladha, J. K., & Bhushan, L. (2003). Soil physical effects of puddling in rice–wheat cropping systems. *Improving the Productivity and Sustainability of Rice–Wheat Systems: Issues and Impacts*(improvingthepro), *9*, 97-113.

Shaw, D. R., & Wixson, M. B. (1991). Postemergence combinations of imazaquin or imazethapyr with ac 263,222 for weed control in soybean (glycine max). *Weed Science*, *12*, 644-649.

Shivrain, V., Burgos, N., Agrama, H., LAWTON-RAUH, A., Lu, B., Sales, M., . . . Moldenhauer, K. (2010). Genetic diversity of weedy red rice (Oryza sativa) in arkansas, USA. *Weed Research, 50*(4), 289-302.

University of Malaya

Shivrain, V. K., Burgos, N. R., Anders, M. M., Rajguru, S. N., Moore, J., & Sales, M. A. (2007). Gene flow between clearfield™ rice and red rice. *Crop Protection, 26*(3), 349-356.

Shivrain, V. K., Burgos, N. R., Gealy, D. R., Sales, M. A., & Smith, K. L. (2009). Gene flow from weedy red rice (Oryza sativa l.) to cultivated rice and fitness of hybrids. *Pest Management Science, 65*(10), 1124-1129.

Shivrain, V. K., Burgos, N. R., Gealy, D. R., Smith, K. L., Scott, R. C., Mauromoustakos, A., & Black, H. (2009). Red rice (Oryza sativa) emergence characteristics and influence on rice yield at different planting dates. *Weed Science, 57*(1), 94-102.

Shivrain, V. K., Burgos, N. R., Moldenhauer, K. A., Mcnew, R. W., & Baldwin, T. L. (2006). Characterization of spontaneous crosses between clearfield rice (Oryza sativa) and red rice (Oryza sativa) 1. *Weed Technology, 20*(3), 576-584.

Shivrain, V. K., Burgos, N. R., Sales, M. A., Mauromoustakos, A., Gealy, D. R., Smith, K. L., . . . Jia, M. (2009). Factors affecting the outcrossing rate between clearfield™ rice and red rice (Oryza sativa). *Weed Science, 57*(4), 394-403.

Silva, D. R. O. d., Avila, L. A. d., Agostinetto, D., Dal Magro, T., Oliveira, E. d., Zanella, R., & Noldin, J. A. (2009). Pesticides monitoring in surface water of rice production areas in southern brazil. *Ciência Rural, 39*(9), 2383-2389.

Singh, R. (2013). Hplc method development and validation-an overview. *Journal of Pharmaceutical Education and Research, 4*(1), 26-33.

Singh, V., Singh, S., Black, H., Boyett, V., Basu, S., Gealy, D.,Caicedo, A. (2017). Introgression of clearfield™ rice crop traits into weedy red rice outcrosses. *Field Crops Research, 207*, 13-23.

Singh, V., Zhou, S., Ganie, Z., Valverde, B., Avila, L., Marchesan, E., Norsworthy, J. (2017). Rice production in the americas. In *Rice production worldwide* (pp. 137-168): Springer.

Siwar, C., Diana, N., Yasar, M., & Morshed, G. (2014). Issues and challenges facing rice production and food security in the granary areas in the east coast economic region (ecer), Malaysia. *Research Journal of Applied Sciences, Engineering and Technology, 7*(4), 711-722.

Smith Jr, R., & Seaman, D. (1973). Weeds and their control. *Rice in the United States: Varieties and Production. US Dep. Agr., Agr. Hand, 289*, 135-140.

Smith Jr, R., Talbert, R., & Baltazar, A. (1992). Control, biology and ecology of propanil-tolerant barnyardgrass. *Arkansas Rice Research Studies. University of Arkansas*, *1*, 46-50.

Snow, A., Pilson, D., Rieseberg, L. H., Paulsen, M., Pleskac, N., Reagon, M., . . . Selbo, S. (2003). A bt transgene reduces herbivory and enhances fecundity in wild sunflowers. *Ecological Applications, 13*(2), 279-286.

University of Malaya

Solomon, W. L., Kanter, D. G., Walker, T. W., Baird III, G. E., Lanford, L. S., Shaifer, S., & Fitts, P. W. (2012). Registration of 'cl162'long-grain rice. *Journal of Plant Registrations, 6*(1), 23-26.

Somaratne, S., Karunarathna, K. D., Weerakoon S. R., Abeysekera, A. S & Weeresena, O. (2014). Salient characters of Weedy rice (Oryza sativa f. spontanea) populations in highly infested areas in Sri Lanka, *Ruhuna Journal of Science*, *5*, 31-36.

Sondhia, S. (2013). Evaluation of imazethapyr leaching in soil under natural rainfall conditions. *Indian Journal of Weed Science, 45*(1), 58-61.

Sondhia, S., Khankhane, P. J., Singh, P. K., & Sharma, A. R. (2015). Determination of imazethapyr residues in soil and grains after its application to soybeans. *Journal of Pesticide Science, 40*(3), 106-110.

Song, B. K., Chuah, T. S., Tam, S. M., & Olsen, K. M. (2014). Malaysian weedy rice shows its true stripes: Wild Oryza and elite rice cultivars shape agricultural weed evolution in southeast asia. *Molecular Ecology, 23*(20), 5003-5017.

Song, D., Wu, G., Vrinten, P., & Qiu, X. (2017). Development of imidazolinone herbicide tolerant borage (borago officinalis l.). *Plant Science*, *1*, 262, 74-80.

Song, J., Feng, G., Tian, C., & Zhang, F. (2005). Strategies for adaptation of suaeda physophora, haloxylon ammodendron and haloxylon persicum to a saline environment during seed-germination stage. *Annals of Botany, 96*(3), 399-405.

Song, Z. P., Lu, B. R., Zhu, Y. G., & Chen, J. K. (2003). Gene flow from cultivated rice to the wild species Oryza rufipogon under experimental field conditions. *New Phytologist, 157*(3), 657-665.

Souza, M., Cneto, M., Marinho, M., Saraiva, D., Faria, A., Silva, A., & Silva, D. (2016). Persistence of imidazolinones in soils under a clearfield system of rice cultivation. *Planta Daninha, 34*(3), 589-596.

Statista, (2017).View trends, analysis and statistics. Retrieved on December 11, 2018 from https://www.statista.com/topics/1443/rice/.

Steele, G. L., Chandler, J. M., & McCAULEY, G. N. (2002). Control of red rice (Oryza sativa) in imidazolinone-tolerant rice (o. Sativa) 1. *Weed Technology, 16*(3), 627-630.

Stewart, G., & Pittman, D. W. (1931). Twenty years of rotation and manuring experiments at logan, Logan,Utah.

Sudianto, E., Beng-Kah, S., Ting-Xiang, N., Saldain, N. E., Scott, R. C., & Burgos, N. R. (2013). Clearfield® rice: Its development, success, and key challenges on a global perspective. *Crop Protection, 49*, 40-51.

Sudianto, E., Neik, T.-X., Tam, S. M., Chuah, T.-S., Idris, A. A., Olsen, K. M., & Song, B.-K. (2016). Morphology of Malaysian weedy rice (Oryza sativa): Diversity, origin and implications for weed management. *Weed Science, 64*(3), 501-512.

University of Malaya

Suh, J., Lee, C., Lee, J., Kim, J., Kim, S., Cho, Y., Jena, K. (2012). Identification of quantitative trait loci for seedling cold tolerance using rils derived from a cross between japonica and tropical japonica rice cultivars. *Euphytica, 184*(1), 101-108.

Suria, A. J., Juraimi, A. S., Rahman, M., Man, A. B., & Selamat, A. (2011). Efficacy and economics of different herbicides in aerobic rice system. *African Journal of Biotechnology, 10*(41), 8007-8022.

Süzer, S. & Büyük, H (2010). Residual effects of spraying imidazolinone-family herbicides on Clearfield®* sunflower production from the point of view of crop rotation. *Helia*, *52*, 25-36.

Sweeney, M., & McCouch, S. (2007). The complex history of the domestication of rice. *Annals of Botany, 100*(5), 951-957.

Szmigielska, A. M., & Schoenau, J. J. (1999). Analysis of imazethapyr in agricultural soils by ion exchange membranes and a canola bioassay. *Communications in Soil Science & Plant Analysis, 30*(13-14), 1831-1846.

Tan, S., Evans, R. R., Dahmer, M. L., Singh, B. K., & Shaner, D. L. (2005). Imidazolinone-tolerant crops: History, current status and future. *Pest Management Science, 61*(3), 246-257.

Tanveer, A., Nadeem, M. A., Ali, A., Tahir, M., & Zamir, M. S. (2009). Germination behaviour of seeds from herbicide treated plants of chenopodium album l. *Anais da Academia Brasileira De Ciências, 81*(4), 873-879.

Tariq, M. I., Afzal, S., Hussain, I., & Sultana, N. (2007). Pesticides exposure in pakistan: A review. *Environment International, 33*(8), 1107-1122.

Taylor, N., Chavarriaga, P., Raemakers, K., Siritunga, D., & Zhang, P. (2004). Development and application of transgenic technologies in cassava. *Plant Molecular Biology, 56*(4), 671-688.

Terano, R., Mohamed, Z., & Din, N. S. Z. (2016). Determinants of farmers' adoption of clearfield production system in Malaysia. *Agriculture and Agricultural Science Procedia, 9*, 103-107.

Thanh, N. C., Chin, D., Tai, N. T., & Son, T. (1999). Study on the dormancy characteristic of five popular weedy rice varieties in the mekong river delta. *Omon Rice, 7*, 155-157.

Timmer, C. (2010). The changing role of rice in asia's food security (adb sustainable development working paper series no. 15). *Metro Manila: Asian Development Bank. 0.10 0.30*.

Ulbrich, A. V., Souza, J. R. P., & Shaner, D. (2005). Persistence and carryover effect of imazapic and imazapyr in brazilian cropping systems 1. *Weed Technology, 19*(4), 986-991.

University of Malaya