

Preliminary investigation of multiple resistance in goosegrass (*Eleusine indica*) to premix of diuron and MSMA, glyphosate, clethodim, quizalofop in Malaysia

Muhammad Izwan Azlan^a , Khairun Nisa Kamarudin^a , Tse-Seng Chuah^{c*} 

^a Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA, 02600 Arau, Perlis, Malaysia.

Abstract: Background: The primary challenge in Malaysian vegetable farms is the herbicide resistance in *E. indica*. **Objective:** This study aimed to assess herbicide resistance in *E. indica* biotypes on a cucumber farm in Penang, Malaysia. **Methods:** Seedlings of the putative resistant and susceptible biotypes were propagated and transferred into seedling trays containing peat moss. The seedlings were subjected to premix of diuron and MSMA, glufosinate, glyphosate, oxyfluorfen, napropamide, quizalofop and clethodim treatments at the three-to-four-leaf stage.

Results: Dose-response tests showed that the resistant biotype was 15-fold, 12-fold, 80-fold, and 2-fold more resistant to glyphosate, clethodim, quizalofop, and the diuron plus MSMA premix, respectively, compared to the susceptible biotype based on herbicide rates which caused 50% reduction in shoot dry weight. However, both biotypes were susceptible to glufosinate, oxyfluorfen and napropamide treatments. **Conclusions:** The findings have confirmed multiple herbicide resistance in goosegrass to glyphosate, clethodim, quizalofop, and premix of diuron and MSMA.

Keywords: *E. indica*; Diuron; Glufosinate; MSMA; Napropamide; Oxyfluorfen

Journal Information:

ISSN - 2675-9462

Website: <http://awsjournal.org>

Journal of the Brazilian Weed Science Society

How to cite: Azlan MI, Kamarudin KN, Chuah TS. Preliminary investigation of multiple resistance in goosegrass (*Eleusine indica*) to premix of diuron and MSMA, glyphosate, clethodim, quizalofop in Malaysia. Adv Weed Sci. 2025;43:e020250119.

<https://doi.org/10.51694/AdvWeedSci/2025/43:00016>

Approved by:

Editor in Chief: Anderson Nunes Gabardo

Associate Editor: Asad Asaduzzaman

Conflict of Interest: The authors declare that there is no conflict of interest regarding the publication of this manuscript.

Received: December 30, 2024

Approved: April 22, 2025

* Corresponding author:

<chuahts@uitm.edu.my>



This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided that the original author and source are credited.

Copyright: 2022

1. Introduction

Eleusine indica (L.) Gaertn. or commonly known as goosegrass is a monocot weed from the Poaceae family, recognized as a significant C4 grassy weed that reproduces solely through seeds and is characterized by its rapid growth and tiller development (Takano et al., 2016). It is widely distributed across tropical and subtropical regions, particularly in Asia (Ma et al., 2019). As an annual grassy weed, it primarily propagates through seed propagation. Individual plants have been observed to produce up to 135,000 seeds (Holm et al., 1977), with an average seed count potentially reaching 40,000 per plant. The weed has been shown to germinate under alternating temperatures at 35/25 and 30/20 °C and moisture stress conditions ranging from -0.2 to -0.8 MPa (Hooda et al., 2023). Germination of *E. indica* primarily occurs within the top 2, 5 cm of soil, with seedlings rarely emerging from depths greater than 8 cm (Hooda et al., 2023; Ma et al., 2019).

In Malaysia, vegetable crops are extensively cultivated to meet local consumer demands. Smallholder farmers across Malaysia have significantly intensified vegetable production. *Eleusine indica* poses a substantial threat as a noxious weed in vegetable farms, often becoming one of the predominant weed species and leading to considerable losses ranging from 20–70% (Mennan et al., 2020). In vegetable farms, *E. indica* is one of weed species present in higher numbers (Majrashi et al., 2022). The weed is particularly problematic in annual row-crops such as vegetable crops, where they can establish rapidly before sufficient shading from the crop occurs. Field experiments have been carried out to assess the impact of glyphosate-susceptible and glyphosate-resistant *E. indica* biotypes on the yield of Chinese flowering cabbage at densities ranging from 0 to 40 plants m⁻² in Malaysia. The observed yield losses ranged from 32% to 67% for the susceptible biotype and 29% to 67% for the resistant biotype (Ismail, Goh, 2004). In other Asian countries, *E. indica* infestation has been associated with substantial yield losses. In India, onion (*Allium cepa*) bulb yield reductions of 40–80% have been reported, depending on the intensity and duration of weed competition (Ramalingam et al., 2013). Similarly, cabbage yields have been shown to decline by 45–80% due to *E. indica* interference (Akshatha et al., 2018). *E. indica* control in vegetables is especially critical early in the season, as weed competition during this period can substantially diminish vigor, uniformity, and overall yield. Most vegetables are slow-growing during the initial stage of development, making them vulnerable

to weed competition, which negatively impacts quantity and quality parameters. Vegetable production necessitates highly cultivated soil receiving regular irrigation, tilling, and fertilizer amendments. However, these agronomic practices may inadvertently enhance weed germination and growth (Robinson, 2015).

Herbicide application has become increasingly prevalent in agriculture due to its ease of use, rapid effects, and effectiveness in controlling weeds. Out of all the nations in Southeast Asia, Malaysia recorded the second highest herbicide use after Indonesia from 2020 to 2022. For example, Malaysia used 25,608 tonnes of pesticides, with herbicides accounting for 62.6%, followed by insecticides (16.9%), fungicides and bactericides (14.2%), and others (6.3%) in 2022 (Food and Agriculture Organization, 2024). However, a number of weeds have evolved resistance to herbicides in Malaysia due to heavy reliance upon herbicide for weed control (Heap, 2024). For instance, sethoxydim resistance has emerged in *E. indica* populations infesting oil palm plantations without evolving cross-resistance to fluazifop herbicide (Chuah et al., 2023). Further studies by Cha et al. (2014) have revealed the occurrence of glyphosate-resistant *E. indica* populations across several states in Malaysia. Jalaludin et al. (2010) and Chuah et al. (2010) have reported multiple resistant *E. indica* biotypes across glufosinate and glyphosate or paraquat in oil palm nurseries and bitter melon farms, respectively. A recent survey by Chuah et al. (2024) identified *E. indica* as the most problematic weed, persisting despite multiple rounds of herbicide spraying in oil palm plantations. Weeds undergo an evolutionary process of continuous adaptation, evolution, and the development of new resistance mechanisms as selection pressure increases. For example, most glyphosate-resistant *E. indica* plants carry the homozygous resistant allele S106, while heterozygous P/S-106 mutants and TIPS mutations are also commonly found in resistant populations in Malaysia. The TIPS mutation, which confers a high level of glyphosate resistance, results from a reduced binding affinity between glyphosate and the EPSPS binding site. Notably, a novel P381L mutation, when combined with T102I, P106S, or TIPS mutations, can further weaken glyphosate binding affinity (Franci et al., 2020).

In Malaysia, several herbicides are commonly employed for weed control in vegetable crops including napropamide, oxyfluorfen, glufosinate, glyphosate, clethodim, and quizalofop (Department of Agriculture Malaysia, 2024). Additionally, a premix of diuron and MSMA is also applied as a knockdown herbicide after harvest to manage weeds around vegetable farms. However, in 2024, a cucumber farmer in Tasek Gelugor, Penang, reported poor control of *E. indica* despite using glyphosate, quizalofop, clethodim, and premix of diuron plus MSMA. This prompted a study to confirm the first documented case of multiple resistance in *E. indica* to glyphosate, quizalofop, clethodim, and diuron combined with MSMA. The study also sought to quantify the resistance level of the resistant biotype in comparison

to a susceptible biotype and to evaluate the potential of using other alternative herbicides with different modes of action to control the resistant *E. indica* biotype.

2. Material and Methods

2.1 On-site field experiment

A field trial was conducted at a cucumber farm heavily infested with *E. indica* in Tasek Gelugor, Penang, Malaysia (5°27'50.9"N100°30'44.1"E), to evaluate the efficacy of glyphosate, clethodim, quizalofop, and a premix of diuron+MSMA against *E. indica*. The herbicides were applied at their recommended rates: glyphosate at 0.61 kg a.i. ha⁻¹, clethodim at 0.14 kg a.i. ha⁻¹, quizalofop at 0.2 kg a.i. ha⁻¹, and diuron+MSMA at 3.6 kg a.i. ha⁻¹, on 1 m × 2 m plots with less than 30% shade. The trial employed a completely randomized block design with three replications for each treatment. Herbicides were applied using a compression sprayer operating at 200 kPa with a flat-fan nozzle, delivering a spray volume of 450 L ha⁻¹. At the start of the trial, the target weed population consisted of vigorously growing young *E. indica* plants (average height 20 cm) and mature plants shedding seeds (average height 35 cm), with a density of four plants per m². Herbicide efficacy was assessed visually using a rating system based on the percentage of weed control, ranging from 0% (no visible symptoms) to 100% (complete plant death). Assessments were conducted at 7, 14 and 21 days after treatment (DAT) (Dear et al., 2003).

2.2 Rain shelter experiment

2.2.1 Seed sources

The putative resistant seeds from the plants that survived the herbicide treatment at the on-site field trial were collected and mixed together for further studies in the rain shelter, while the putative susceptible seeds were collected from a population near to roadside (100 m apart between putative resistant and susceptible biotypes) and has never been received herbicide application. The seeds were cleaned and the seed coats were scarified using sand paper to accelerate germination.

2.2.2 Herbicides

The response of plant samples to oxyfluorfen, napropamide, premix of diuron and MSMA, glyphosate, glufosinate, clethodim and quizalofop was evaluated and the information of each herbicide is listed in Table 1.

2.3 Screening of herbicide resistance in *E. indica*

Eleusine indica seeds acquired from the cucumber farm in Tasek Gelugor, putative resistant and susceptible seeds was sown and grown in seedling trays containing commercial

Table 1 - List of herbicide common names, trade names, mode of action, and manufacturers

Common name	Trade name	Formulation type	Mode of action	Manufactures
Glufosinate	Basta 15	Soluble concentrate	Inhibition of glutamine synthetase enzyme	Bayer Co. (Malaysia) Sdn Bhd
Glyphosate	Ecomax	Soluble concentrate	Inhibition of enolpyruvyl shikimate phosphate synthase (EPSPS)	CP Manufacturing
Clethodim	Tekad 26 EC	Emulsified concentrate	Inhibition of acetyl CoA carboxylase (ACCase)	Hextar Chemicals Sdn. Bhd
Oxyfluorfen	Boxy	Emulsified concentrate	Inhibition of protoporphyrinogen oxidase (PPO)	Zeenex AgroScience Sdn. Bhd
Napropamide	Devrinol 2-E	Emulsified concentrate	Very long-chain fatty acid synthesis inhibitors	Agricultural Chemical (M) Sdn. Bhd
Quizalofop*	-	-	Inhibition of acetyl CoA carboxylase (ACCase)	-
Diuron + MSMA	Monex HC	Suspension concentrate	Inhibition of photosystem II + Unknown mode of action	Ancom Crop Care Sdn. Bhd

* Herbicide information is kept confidential as requested by the manufacturer

peat moss and then sprayed with four groups of herbicides such as glyphosate, clethodim, quizalofop and diuron plus MSMA respectively at manufacturer-recommended rate: glyphosate at 0.61 kg a.i. ha⁻¹, clethodim at 0.14 kg a.i. ha⁻¹, quizalofop at 0.2 kg a.i. ha⁻¹, and diuron+MSMA at 3.6 kg a.i. ha⁻¹ when reaching three to four-leaf stage. Each tray was placed under the rain shelter with a temperature of 29 ± 6 °C with a 12 h length of light period (photoperiod) and the light intensity of 1,200–1,600 µEm⁻² s⁻¹. A compression sprayer with a flat-fan nozzle, was used to apply glyphosate, clethodim, quizalofop and diuron plus MSMA to the seedlings of the putative resistant and susceptible biotypes to deliver a spraying volume of 450 L ha⁻¹. The application volume of 450 L ha⁻¹ was determined based the label on the herbicides. A total of 100 seedlings were sprayed for each herbicide. The susceptible biotype was assigned when survival rate of is less than 20% at recommended herbicide rate. On the other hand, the resistant biotype was designated when more than 20% of plants survived the recommended rate (Panozzo et al., 2015). Numbers of seedlings which survived and died were recorded at 21 DAT.

The survival rate is calculated using the formula:

$$\text{Survival Rate (\%)} = \frac{(\text{Number of surviving plants in treated trays})}{(\text{Number of surviving plants in control trays})} \times 100\% \quad (1)$$

2.4 Dose-response tests

A preliminary investigation was conducted before conducting the dose-response testing. In this investigation, the seedlings suspected to be resistant and susceptible to the herbicide were treated with glyphosate, followed by clethodim, quizalofop and diuron + MSMA at their respective recommended rates to confirm the presence of multiple resistances to herbicides in *E. indica*. Different rates of glyphosate (0, 0.61, 1.22, 2.44, 4.88 and 9.76 kg a.i. ha⁻¹ for glyphosate-resistant biotype; 0, 7.62 x 10⁻³, 1.25 x 10⁻²,

0.31, 0.61 and 1.22 kg a.i. ha⁻¹ for glyphosate-susceptible biotype), clethodim (0, 0.52 x 10⁻², 1.56 x 10⁻², 4.67 x 10⁻², 0.14, 0.42, 1.26 and 3.78 kg a.i. ha⁻¹ for clethodim-resistant biotype; 0, 0.58 x 10⁻³, 0.17 x 10⁻², 0.52 x 10⁻², 1.56 x 10⁻², 4.67 x 10⁻² and 0.14 kg a.i. ha⁻¹ for clethodim-susceptible biotype), quizalofop (0, 0.2, 0.6, 1.8, 5.4 and 16.2 kg a.i. ha⁻¹ for quizalofop-resistant biotype; 0, 0.003, 0.01, 0.05, 0.2, 0.8 kg a.i. ha⁻¹ for quizalofop-susceptible biotype) and diuron+MSMA (0, 0.45, 0.90, 1.80, 3.60 and 7.20 kg a.i. ha⁻¹ for both resistant and susceptible biotypes) were used for the dose-response tests. The seedlings from each biotype were randomly divided into six to seven treatment groups, including the control plants, with each treatment having ten plants.

2.5 Herbicide efficacy tests

The resistant and susceptible *E. indica* biotypes were treated with glufosinate (0.50 kg a.i. ha⁻¹), oxyfluorfen (0.14 kg a.i. ha⁻¹) and napropamide (2.51 kg a.i. ha⁻¹) at their recommended field rates (Table 3). Oxyfluorfen and napropamide were used as pre-emergence treatments (0 to 1-leaf stage), while glufosinate and oxyfluorfen were applied post-emergence (3 to 4-leaf stage). A total of 100 seedlings were sprayed for each herbicide. Untreated controls were also included. Planting, herbicide application, and maintenance were conducted following previously established protocols. Plant survival was assessed three weeks after treatment.

2.6 Statistical Analysis

For on-site field trial in section 2.1, the treatments were arranged in a randomized completely block design with three replications. Data were analyzed using one-way repeated measured ANOVA, and mean differences were determined using Tukey's test at a 5% significance level.

For herbicide-resistant screening test in section 2.3, the treatments were arranged in a randomized completely design. A chi square test was conducted to analyze and compare the frequency of *E. indica* seedlings which survived after being treated with selected herbicides at 5% of significance level.

The experimental design used in section 2.4 was factorial in completely randomised block design with five replications, where factor one is biotype whereas second factor is herbicide rate. The level of plant survival and the shoot dry weight were recorded 3 weeks after the herbicide treatment. The plants were recorded as killed when they had no new growth or active tiller formation. The dry weights of the shoots were expressed as percentage of respective controls. The percentage data of shoot dry weight and plant survival were analysed using the three-parameter logistic curve model as described in the study conducted by Kuk et al. (2002):

$$y = \frac{a}{1 + \left(\frac{x}{x_0}\right)^b} \quad (2)$$

Where y = the percentage of shoot dry weight / plant survival, a = the coefficients corresponding to the upper asymptotes, b = the slope of the line, x_0 = the herbicide rate required to inhibit the shoot growth (GR_{50}) or provide plant survival (LD_{50}) by 50%, and x = the herbicide rate.

There were several pilot trials prior to final herbicide dose-response experiments. Each dose-response experiment was repeated at least twice with similar results, and therefore, only results from a single experiment were presented for each dose-response. The resistance level was calculated as the GR_{50} and LD_{50} of the R biotype divided by the GR_{50} and LD_{50} of the S biotype, respectively.

The treatments in section 2.6 were arranged in a randomized completely design. A chi square test was conducted to analyze and compare the frequency of *E. indica* seedlings which survived after being treated with selected herbicides at 5% of significance level.

3. Results and Discussion

3.1 Confirmation of multiple herbicide-resistant *E. indica*

The on-site trial conducted at a cucumber farm in Tasek Gelugor, Penang, Malaysia, validated the farmer's observation that *E. indica* had developed resistance to glyphosate, clethodim, quizalofop, and diuron + MSMA. At the recommended application rates, glyphosate, clethodim, and quizalofop achieved less than 20% control of *E. indica*, while diuron + MSMA provided approximately 70% control at 7 DAT (Figure 1). By 21 DAT, the control efficacy of glyphosate, clethodim, quizalofop, and diuron + MSMA had further declined by 13, 7, 5, and 28%, respectively, compared to 7 DAT, indicating plant recovery.

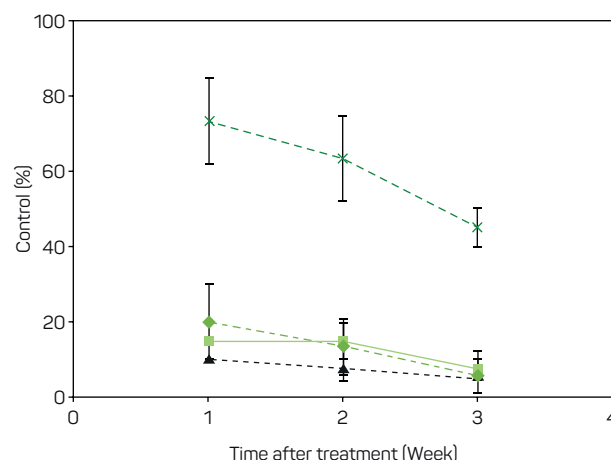


Figure 1 - Control of *E. indica* plants on a cucumber farm in Tasek Gelugor, Penang, Malaysia, with glyphosate (◆), clethodim (■), quizalofop (▲) and diuron + MSMA (×) at 0.61, 0.14, 0.2 and 3.6 kg a.i. ha⁻¹ (the recommended rate), respectively, throughout the period of 21 days after herbicide treatment. The vertical bars represent the standard error of the mean

The Chi-square test revealed a significant difference ($p < 0.05$) in the survival frequency of putative resistant and susceptible *E. indica* biotypes when treated with glyphosate, clethodim, quizalofop, and diuron + MSMA. The screening test confirmed that all putative resistant seedlings survived each herbicide treatment, whereas putative susceptible seedlings exhibited 90–100% control. These results indicate that all putative resistant *E. indica* seedlings were resistant to the tested herbicides, while the putative susceptible seedlings remained susceptible.

Dose-response tests confirmed that the resistant (R) biotype exhibited resistance to all four herbicides. Although survival rates for both R and susceptible (S) biotypes decreased as herbicide application rates increased, the S biotype showed a more pronounced decline in survival (Figures 2, 3, 4, and 5). At the different rates of glyphosate (0, 0.61, 1.22, 2.44, 4.88 and 9.76 kg a.i. ha⁻¹ for glyphosate-resistant biotype; 0, 7.62 × 10⁻³, 1.25 × 10⁻², 0.31, 0.61 and 1.22 kg a.i. ha⁻¹ for glyphosate-susceptible biotype), clethodim (0, 0.52 × 10⁻², 1.56 × 10⁻², 4.67 × 10⁻², 0.14, 0.42, 1.26 and 3.78 kg a.i. ha⁻¹ for clethodim-resistant biotype; 0, 0.58 × 10⁻³, 0.17 × 10⁻², 0.52 × 10⁻², 1.56 × 10⁻², 4.67 × 10⁻² and 0.14 kg a.i. ha⁻¹ for clethodim-susceptible biotype), quizalofop (0, 0.2, 0.6, 1.8, 5.4 and 16.2 kg a.i. ha⁻¹ for quizalofop-resistant biotype; 0, 0.003, 0.01, 0.05, 0.2, 0.8 kg a.i. ha⁻¹ for quizalofop-susceptible biotype) and diuron+MSMA (0, 0.45, 0.90, 1.80, 3.60 and 7.20 kg a.i. ha⁻¹ —the S biotype was 90–100% controlled, while the R biotype showed only 0–20% control).

The GR_{50} and LD_{50} values for R and S biotypes (Tables 2 and 3) indicated significant resistance in the R biotype. The logistic regression model fitted the data very well with adjusted R² value being in the range of 0.93 to 0.99. Based on shoot dry weight reduction, the R biotype exhibited

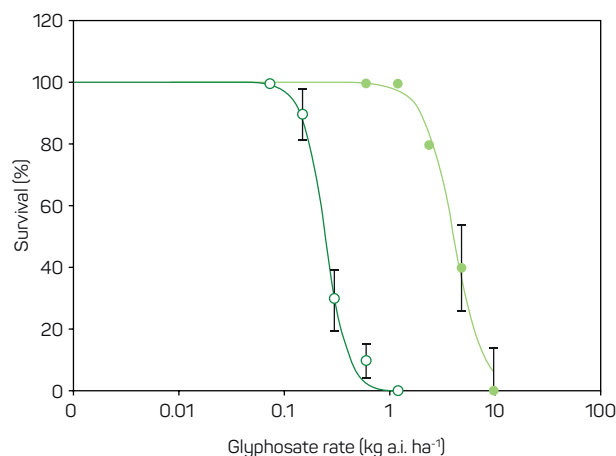


Figure 2 - Survival of the susceptible (O) and resistant (●) biotypes of *E. indica*, as affected by glyphosate in the whole-plant bioassay 21 days after treatment under rain shelter conditions. Every point is a mean of five replicates, each containing 10 plants. The vertical bars represent the standard error of the mean

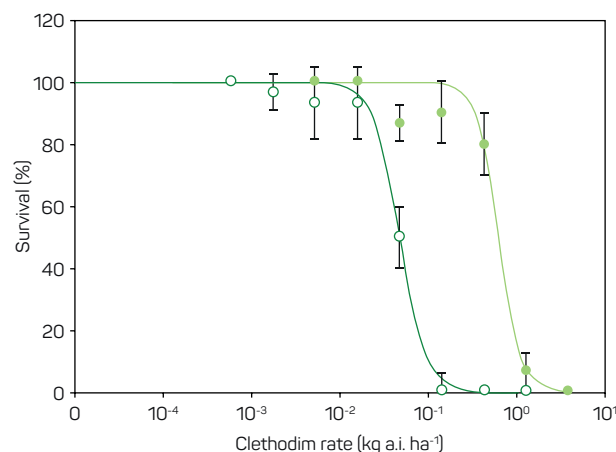


Figure 4 - Survival of the susceptible (O) and resistant (●) biotypes of *E. indica*, as affected by clethodim in the whole-plant bioassay 21 days after treatment under rain shelter conditions. Every point is a mean of five replicates, each containing 10 plants. The vertical bars represent the standard deviation of the mean

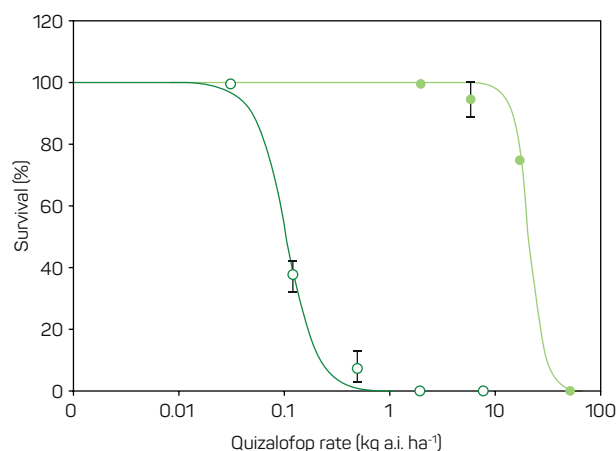


Figure 3 - Survival of the susceptible (O) and resistant (●) biotypes of *E. indica*, as affected by quizalofop in the whole-plant bioassay 21 days after treatment under rain shelter conditions. Every point is a mean of five replicates, each containing 10 plants. The vertical bars represent the standard deviation of the mean

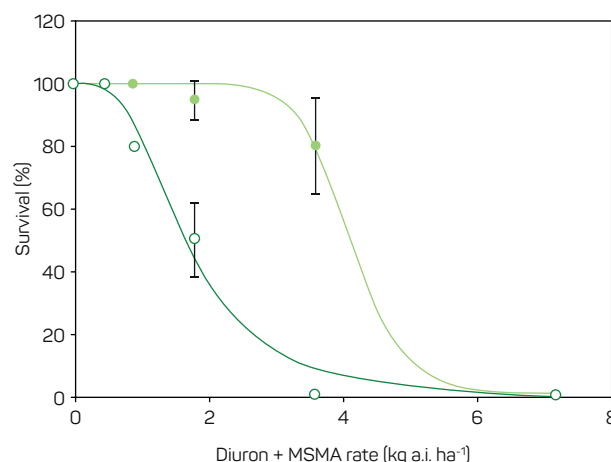


Figure 5 - Survival of the susceptible (O) and resistant (●) biotypes of *E. indica*, as affected by diuron+MSMA in the whole-plant bioassay 21 days after treatment under rain shelter conditions. Every point is a mean of five replicates, each containing 10 plants. The vertical bars represent the standard deviation of the mean

resistance levels of 15-fold (glyphosate), 12-fold (clethodim), 80-fold (quizalofop), and 2-fold (diuron + MSMA) compared to the S biotype. Survival analysis showed resistance levels of 16-fold, 14-fold, 218-fold, and 3-fold for glyphosate, clethodim, quizalofop, and diuron + MSMA, respectively, in the R biotype relative to the S biotype.

3.2 Efficacy of herbicides with other modes of action

Multiple-resistant *E. indica* biotypes can reduce the effectiveness of herbicide applications, highlighting the

need for integrating additional chemical control strategies to manage infestations. This study demonstrated that alternative herbicides, including glufosinate, oxyfluorfen, and napropamide, achieved 100% control for both biotypes. To date, *E. indica* has not been reported to develop resistance to napropamide or oxyfluorfen, resistance to glufosinate, however, has been documented (Heap, 2024). This suggests that oxyfluorfen and napropamide are promising options for herbicide rotation and mixtures to manage resistant *E. indica* biotypes effectively. Likewise, *E. indica* can also be controlled effectively by fenoxaprop, foramsulfuron, and topramezone (Shekoofa et al., 2020). Furthermore,

Table 2 - GR₅₀ values and resistance levels of the *E. indica* biotypes in relation to glyphosate, clethodim, quizalofop and diuron + MSMA

Herbicide	†GR ₅₀ (kg a.i. ha ⁻¹)		Resistance level
	R biotype	S biotype	
Glyphosate	1.80 (0.28)	0.12 (0.01)	15
Clethodim	0.12 (0.03)	0.01 (0.007)	12
Quizalofop	0.32 (0.09)	0.004 (0.001)	80
Diuron + MSMA	1.23 (0.23)	0.54 (0.1)	2

† GR₅₀ is the herbicide rate required to reduce the shoot dry weight by 50%. The values in parentheses are the standard error of the mean

Table 3 - LD₅₀ values and resistance levels of the *E. indica* biotypes in relation to glyphosate, clethodim, quizalofop and diuron + MSMA

Herbicide	†LD ₅₀ (kg a.i. ha ⁻¹)		Resistance level
	R biotype	S biotype	
Glyphosate	4.10 (0.24)	0.25 (0.02)	16
Clethodim	0.62 (0.08)	0.05 (0.002)	12
Quizalofop	2.18 (0.35)	0.01 (0.001)	218
Diuron + MSMA	4.12 (0.71)	1.67 (0.17)	3

† LD₅₀ is the herbicide rate required to reduce plant survival by 50%. The values in parentheses are the standard error of the mean

using oil palm residues in combination with oxyfluorfen (Dilipkumar et al., 2020) or S-metolachlor (Chuah et al., 2018) as mulch could be an alternative strategy for suppressing *E. indica*.

This study confirmed the occurrence of multiple resistance in a Malaysian *E. indica* population to two selective herbicides (clethodim and quizalofop) and three non-selective herbicides (glyphosate, diuron and MSMA). The results clearly indicate that the *E. indica* population with clethodim resistance also developed cross-resistance to quizalofop since both herbicides are ACCase inhibitors. The evolution of resistance to herbicides with four distinct modes of action in this resistance-prone species is alarming. It undermines the efficacy of glyphosate—the world's most widely used herbicide—and its alternatives (clethodim, quizalofop, diuron and MSMA), significantly reducing herbicide options for growers. This phenomenon is a direct consequence of selection pressures resulting from the over-reliance on herbicides for weed control.

To address this challenge, herbicides should be employed judiciously, incorporating rotations or mixtures with other modes of action and complementing chemical control with non-chemical strategies. Integrated weed management practices are strongly recommended to effectively manage this problematic weed species. The findings of this study provide an early warning to farmers, growers, and plantation managers worldwide about the risks of multiple herbicide resistance in *E. indica* following repeated applications of glyphosate, clethodim, quizalofop, and diuron + MSMA.

While this study focused on confirming resistance, it is hypothesized that multiple resistance mechanisms may be at play in *E. indica*. Possible resistance mechanisms in *E. indica*, including reduced absorption and translocation

(Ulguim et al., 2017), point mutations (Franci et al., 2020), gene amplification (Chen et al., 2023) and enhanced metabolism (Deng et al., 2023) have been documented depending on the herbicide. Further research is needed to investigate these mechanisms to better understand the evolution of resistance. Instances of multiple resistance in *E. indica* have been reported previously. For example, a *E. indica* biotype in Brazil exhibited low-level resistance to glyphosate and high cross-resistance level to clethodim and haloxyfop (Nunes et al., 2022). Other cases of resistance to four herbicide groups have been reported, including EPSPS inhibitors (glyphosate), photosystem I inhibitors (paraquat), glutamine synthase inhibitors (glufosinate), and ACCase inhibitors (fluzifop and butoxydim), as documented in a Malaysian *E. indica* biotype (Jalaluddin et al., 2014). A selected glufosinate-resistant subpopulation exhibited high-level resistance to glyphosate, with GR₅₀ and LD₅₀ R/S ratios showing 12- and 144-fold increases, respectively. This subpopulation also demonstrated low-level resistance to paraquat and ACCase-inhibiting herbicides such as fluzifop-P-butyl, haloxyfop-P-methyl, and butoxydim (Jalaluddin et al., 2014). However, the present study is the first to report resistance in *E. indica* to four herbicide groups with distinct modes of action—photosystem II inhibitors (diuron), unknown inhibitors (MSMA), EPSPS inhibitors (glyphosate), and ACCase inhibitors (quizalofop and clethodim)—within a single population in Malaysia.

4. Conclusions

In summary, this study confirmed multiple herbicide resistance in *E. indica* to glyphosate, clethodim, quizalofop,

and premix of diuron and MSMA. The resistant biotype showed significantly lower control rates and higher GR₅₀ (2 to 80-fold) and LD₅₀ (3 to 218-fold) values compared to the susceptible biotype. The findings highlight the urgent need for integrated weed management to address resistance caused by over-reliance on herbicides. Diversified strategies, including herbicide rotation, mixing, and non-chemical methods, are essential to mitigate resistance. This research serves as a warning to adopt sustainable practices and underscores the importance of further studies into resistance mechanisms for effective long-term weed control.

References

- Akshatha V, Prameela KP, Narayankutty C, Menon MV. Weed management in cabbage (*Brassica oleracea* var. *capitata* L.). J Trop Agric. 2018;56(2):187-90.
- Cha ST, Najihah MG, Sahid IB, Chuah TS. Molecular basis for resistance to ACCase-inhibiting fluzafop in *Eleusine indica* from Malaysia. Pestic Biochem Physiol. 2014;111:7-13. Available from: <https://doi.org/10.1016/j.pestbp.2014.04.011>
- Chen J, Li Z, Cui H, Yu H, Li X. Gene amplification of EPSPS with a mutation in conserved region: the evolved glyphosate resistance mechanism in *Eleusine indica*. Agronomy. 2023;13(3):1-11. Available from: <https://doi.org/10.3390/agronomy13030699>
- Chuah TS, Kent LW, Ismail BS. Potential of oil palm frond residues in combination with S-metolachlor for the inhibition of selected herbicide-resistant biotypes of goosegrass emergence and seedling growth. Sains Malays. 2018;47(4):671-82. Available from: <https://doi.org/10.17576/jsm-2018-4704-04>
- Chuah TS, Low VL, Cha TS, Ismail BS. Initial report of glufosinate and paraquat multiple resistance that evolved in a biotype of goosegrass (*Eleusine indica*) in Malaysia. Weed Biol Manag. 2010;10(4):229-33. Available from: <https://doi.org/10.1111/j.1445-6664.2010.00388.x>
- Chuah TS, Nam HC, Norzaidi I, Giap SGE. Seed germination characteristics as affected by interaction of moisture stress and temperature in sethoxydim-resistant biotype of goosegrass (*Eleusine indica* (L.) Gaertn.) from Malaysia. Sains Malays. 2023;52(7):1985-97. Available from: <https://doi.org/10.17576/jsm-2023-5207-08>
- Dear BS, Sandral GA, Spencer D, Khan MR, Higgins TJV. The tolerance of three transgenic subterranean clover (*Trifolium subterraneum* L.) lines with the bxn gene to herbicides containing bromoxynil. Aust J Agric Res. 2003;54(2):203-10. Available from: <https://doi.org/10.1071/AR02134>
- Deng W, Li Y, Yao S, Duan Z, Yang Q, Yuan S. ACCase gene mutations and P450-mediated metabolism contribute to cyhalofop-butyl resistance in *Eleusine indica* biotypes from direct-seeding paddy fields. Pestic Biochem Physiol. 2023;194. Available from: <https://doi.org/10.1016/j.pestbp.2023.105530>
- Department of Agriculture Malaysia. [SISMARP: pesticide information system]. Kuala Lumpur: Department of Agriculture Malaysia; 2024 [access October 15, 2024]. Malay. Available from: <http://www.portal.doa.gov.my/sismarp/>
- Dilipkumar M, Muhammad Amirul N, Zain NM, Ismail S, Chuah TS. Phytotoxic activity of oil palm frond mulch in combination with selected pre-emergence herbicide. Sains Malays. 2020;49(10):2403-10. Available from: <https://doi.org/10.17576/jsm-2020-4910-06>
- Food and Agriculture Organization – FAO. Faostat: food and agriculture data. Rome: Food and Agriculture Organization; 2024 [access March 5, 2024]. Available from: <https://www.fao.org/faostat/>
- Franci J, Lam KW, Chuah TS, Cha TS. Genetic diversity and in silico evidence of target-site mutation in the EPSPS gene in endowing glyphosate resistance in *Eleusine indica* (L.) from Malaysia. Pestic Biochem Physiol. 2020;165. Available from: <https://doi.org/10.1016/j.pestbp.2020.104556>
- Heap I. The international herbicide-resistant weed database. Weedsience. 2024 [access January 20, 2024]. Available from: <http://www.weedsience.org>
- Holm LG. The world's worst weeds: distribution and biology. Honolulu: University of Michigan East-West Center; 1977 [access April 10, 2024]. Available from: <https://www.cabidigitallibrary.org/doi/full/10.5555/19782319336>
- Hooda VS, Chauhan BS. Herbicide response and germination behavior of two goosegrass (*Eleusine indica*) populations in the Australian environment. Weed Sci. 2023;71(6):584-93. Available from: <https://doi.org/10.1017/wsc.2023.51>
- Ismail BS, Goh SS. [Interference caused by glyphosate-susceptible and resistant goosegrass (*Eleusine indica* (L.) Gaertn.) on chinese flowering cabbage (*Brassica chinensis* var. *parachinensis*)]. Malays Appl Biol. 2004;33(2):35-9. Malay.
- Jalaludin A, Ngim J, Bakar BH, Alias Z. Preliminary findings of potentially resistant goosegrass (*Eleusine indica*) to glufosinate-ammonium in Malaysia. Weed Biol Manag. 2010;10(4):256-60. Available from: <https://doi.org/10.1111/j.1445-6664.2010.00392.x>

Author's contributions

All authors read and agreed to the published version of the manuscript. TSC, KNK, and MIA: conceptualization of the manuscript and development of the methodology. MIA: data collection and curation. MIA and TSC: data analysis. MIA, and TSC: data interpretation. TSC, and KNK: supervision. MIA: writing the original draft of the manuscript. TSC, KNK, and MIA: writing, review and editing.

Acknowledgements

We thank Universiti Teknologi MARA which provided the facility to conduct the experiments.

- Jalaludin A, Yu Q, Powles SB. Multiple resistance across glufosinate, glyphosate, paraquat and ACC ase-inhibiting herbicides in an *Eleusine indica* population. *Weed Res.* 2014;55(1):82-9. Available from: <https://doi.org/10.1111/wre.12118>
- Kuk IY, Kwon OO, Jung HI, Burgos NR, Guh OJ. Cross-resistance pattern and alternative herbicides for *Rotala indica* resistant to imazosulfuron in Korea. *Pestic Biochem Physiol.* 2002;74(3):129-38. Available from: [https://doi.org/10.1016/S0048-3575\(02\)00155-4](https://doi.org/10.1016/S0048-3575(02)00155-4)
- Ma X, Ma Y, Wu H, Ren X, Jiang W, Ma Y. Emergence timing affects growth and reproduction of goosegrass (*Eleusine indica*). *Weed Technol.* 2019;33(6):833-39. Available from: <https://doi.org/10.1017/wet.2019.61>
- Majrashi AA. Preliminary assessment of weed population in vegetable and fruit farms of Taif, Saudi Arabia. *Braz J Biol.* 2022;82:1-9. Available from: <https://doi.org/10.1590/1519-6984.255816>
- Mennan H, Jabran K, Zandstra BH, Pala F. Non-chemical weed management in vegetables by using cover crops: a review. *Agronomy.* 2020;10(2):1-16. Available from: <https://doi.org/10.3390/agronomy10020257>
- Nunes JJ, Werle R, Freitas MAD, Cunha PCD. Multiple resistance in goosegrass to clethodim, haloxyfop-methyl and glyphosate. *Adv Weed Sci.* 2022;40:1-9. Available from: <https://doi.org/10.51694/AdvWeedSci/2022;40:00001>
- Panozzo S, Scarabel L, Collavo A, Sattin M. Protocols for robust herbicide resistance testing in different weed species. *J Vis Exp.* 2015;(101). Available from: <https://doi.org/10.3791/52923>
- Ramalingam SP, Chinnappagounder C, Perumal M, Palanisamy MA. Evaluation of new formulation of oxyfluorfen (23.5% EC) for weed control efficacy and bulb yield in onion. *Am J Plant Sci.* 2013;4(4):890-95. Available from: <https://doi.org/10.4236/ajps.2013.44109>
- Robinson R. Weed management in native grasslands: land of sweeping plains—managing and restoring the native grassland of South-Eastern Australia. Clayton: Csiro; 2015[access February 11, 2024]. Available from: <https://books.google.com.my/books>
- Schwartz-Lazaro LM, Gage KL, Chauhan BS. Weed biology and ecology in agroecosystems. *Front Agron.* 2012;3:1-4. Available from: <https://doi.org/10.3389/fagro.2021.730074>
- Chuah TS, Yusop N, Pauzi SA, NKCM, Aani SNA, Sahal MSAM et al. Survey of herbicide resistant weed management in oil palm estates from peninsular Malaysia and Indonesia. *Adv Weed Sci.* 2024;42:1-8. Available from: <https://doi.org/10.51694/AdvWeedSci/2024;42:00020>
- Shekoofa A, Brosnan JT, Vargas JJ, Tuck DP, Elmore MT. Environmental effects on efficacy of herbicides for postemergence goosegrass (*Eleusine indica*) control. *Sci Rep.* 2020;10(1):1-9. Available from: <https://doi.org/10.1038/s41598-020-77570-5>
- Takano HK, Oliveira Jr RS, Constantin J, Braz GBP, Padovese JC. Growth, development and seed production of goosegrass. *Planta Daninha.* 2016;34(2):249-58. Available from: <https://doi.org/10.1590/S0100-83582016340200006>
- Ulguim AR, Franco JJ, Silva JDG, Agostinetto D, Vargas L. Evaluation of the mechanism responsible for the low-level resistance to glyphosate in goosegrass. *Planta Daninha.* 2017;35:1-11. Available from: <https://doi.org/10.1590/S0100-83582017350100005>