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RESEARCH ARTICLE

Tank mixture of propanil and quinclorac for inhibiting a bispyribac-sodium-resistant barnyardgrass (*Echinochloa crus-galli*) biotype in Malaysia

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Abstract: Background: Herbicide-resistant *E. crus-galli* poses a significant threat to rice production in direct-seeded rice systems.

Objective: This study aimed to confirm the presence of bispyribac-sodium-resistant (R) *E. crus-galli* and determine the optimal mixture ratio of propanil and quinclorac to control the R biotype.

Methods: *E. crus-galli* seeds from putative resistant and susceptible biotypes were collected from rice fields located 10 km apart in Alor Setar, Kedah, Malaysia, and screened with bispyribac-sodium, propanil, and quinclorac. Dose-response tests were conducted to confirm herbicide resistance. Propanil and quinclorac were tank-mixed at ratios of 20:80, 40:60, 60:40, and 80:20 and their inhibitory effects were evaluated under glasshouse conditions.

Results: Dose-response tests showed a 128-fold increase in the resistant biotype (R) compared to the susceptible (S) biotype based on the dosage corresponding to 50% inhibition of shoot growth, and a 70-fold increase based on the dosage that caused 50% mortality. The mixture ratios of propanil and quinclorac exhibited synergistic action except for the 80:20 ratio, which was antagonistic. The 40:60 mixture ratio outperformed the others, allowing the rates of propanil and quinclorac to be reduced by 2.2 and 4.7-fold, respectively, compared to their single applications to achieve 95% mortality.

Conclusions: Dose-response tests revealed a high resistance level in the R biotype to bispyribac-sodium. Propanil and quinclorac at recommended rates were effective in controlling the R biotype. This study demonstrates that the ratio of propanil in combination with quinclorac is crucial in enhancing the herbicidal activity.

Keywords: *E. crus-galli*; Herbicide-Resistant; Tank-Mixture; Bispyribac-Sodium; Propanil; Quinclorac

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1. Introduction

In Malaysia, direct-seeded rice (DSR) was introduced in the 1970s, as an alternative to the time and water consuming traditional transplanting method. Nonetheless, during the initial establishment of rice seedlings, direct-seeded fields experience aerobic conditions which are more conducive for weed growth (Juraimi et al., 2011) and cause a shift in weed species populations (Kaur, Singh, 2017). By the 1990s, grass species such as *Echinochloa crus-galli*, *Leptochloa chinensis*, and *Ischaemum rugosum* became prevalent (Kaur, Singh, 2017). Weeds that emerge simultaneously with DSR seedlings are highly competitive, and without effective control, can lead to substantial yield losses. Weeds cause 5-85% rice yield losses in Malaysia, depending on factors such as field location, season, planting method, dominant weed flora, infestation duration, and management practices (Dilipkumar et al., 2017). Herbicides have been extensively used to control weeds. Continuous use of selective post-emergence herbicides for broadleaf weeds and sedges has led to the dominance of grassy weeds, while extensive use of herbicides for grassy weeds has increased the prevalence of broadleaf weeds and sedges (Juraimi et al., 2013).

Echinochloa crus-galli (L.) P. Beauv or commonly known as barnyardgrass is a self-pollinating, annual grass weed and one of the 10 most serious arable land weed species, found in more than 60 countries, particularly in rice fields (Wang, Wan, 2020; Chen et al., 2016; Holm, 1969). *E. crus-galli* caused a 40% rice yield reduction in DSR in Malaysia (Tasrif et al., 2004). In China, a density of six *E. crus-galli* plants per square meter resulted in a 10.8-25.3% reduction in rice yield (Zhang et al., 2014), while nine plants per square meter could reduce rice yield by 57% (Chauhan, Johnson, 2011). Barnyardgrass competes with rice plants, absorbing 80% of the available nitrogen from the soil through its fibrous roots (Dilipkumar et al., 2012). Generally, *Echinochloa* species become problematic due to their abundant seed production, seed dormancy, rapid growth and development, and environmental adaptability (Shabbir et al., 2019).

Various methods have been employed to control *E. crus-galli*. Herbicides from different chemical groups have been used, including bispyribac-sodium, propanil, and quinclorac. Bispyribac-sodium is a selective herbicide commonly used in rice cultivation for post-emergence control, functioning by inhibiting the acetolactate synthase (ALS) enzyme, also known as acetohydroxyacid synthase (AHAS). Propanil is a synthetic herbicide that inhibits photosystem II (PSII) in plants. Pratap and Rekha (2018) suggested that a post-emergence application of propanil at a dosage of 3,000 g ha^{-1} could increase grain yield and significantly reduce the density of weeds, including *E. crus-galli*, in DSR cultivation. Quinclorac is an auxin-mimicking herbicide that has had a significant impact on commercial agriculture (Grossmann, 2009).

However, the intensive and extensive use of the same herbicide over long periods leads to the evolution of resistance (Dilipkumar et al., 2017). Herbicide resistance in *E. crus-galli* poses a severe risk to sustainable crop production on a global scale (Bajwa et al., 2015). The first case of *E. crus-galli* resistance to propanil was reported in rice fields in Greece (Giannopolitis, Vassiliou, 1989). To date, there are 52 reported cases of *E. crus-galli* resistance to nine sites of action in various crops globally (Heap, 2024). In some instances, *E. crus-galli* has evolved multiple resistance. A study by Rahman et al. (2010) confirmed *E. crus-galli* resistance to propanil, quinclorac, and cyhalofop-butyl in Malaysian rice fields.

As resistance to many single active-ingredient herbicides has evolved, herbicide mixtures have been proposed as an alternative to slow the development of herbicide resistance (Wrubel, Gressel, 1994). Herbicide mixtures are considered one of the best strategies for weed control because they can target a broad range of weed species with multiple active ingredients. A synergistic mixture can enhance weed control efficiency. The efficiency of an herbicide mixture can be maximized through the proper selection of herbicides, mixing ratios and rates, and application timing. A combination of propanil and quinclorac showed additive and antagonistic effects in inhibiting barnyardgrass (Guh et al., 1993). However, their experiment was conducted on a random biotype of barnyardgrass with no herbicide resistance and used 1/16, 1/8, 1/4, and 1/2 of the recommended rates for mixtures. Studies on the effects of different mixing ratios on the efficacy of propanil and quinclorac against *E. crus-galli* are still very limited. Recently, farmers in Alor Setar, Kedah, Malaysia, have reported reduced effectiveness of bispyribac-sodium in controlling *E. crus-galli*. Therefore, the objective of the present research is to confirm the evolution of bispyribac-sodium-resistant (R) *E. crus-galli* biotype and to identify the optimal mixture ratio of propanil and quinclorac for inhibiting the R biotype.

2. Material and Methods

2.1 Seed Collection and Germination

Freshly matured seeds of putative resistant *E. crus-galli* were collected from a rice field in Anak Bukit, Kota Setar, Kedah, Malaysia (GPS coordinate: 6°12'32.0" N, 100°21'39.0" E), following complaints from farmers. The putative susceptible biotype was collected from a rice field within 10 km radius (GPS coordinates: 6°09'37.0" N, 100°24'05.0" E). Weed maturation usually aligns just before crop maturation, providing a short window to collect mature inflorescences before harvesting (Burgos et al., 2013). Therefore, the seeds were collected one week before the rice harvest. Seed heads were collected from more than 30 *E. crus-galli* plants from each location. The collected seeds were stored in a chiller at 5° C until further use. The *E. crus-galli* seeds were soaked in concentrated sulfuric acid for 12-15 minutes as a scarification method to break dormancy (Paudyal et al., 2021). After scarification, the seeds were germinated in a growth chamber set to 35/20° C day/night temperatures with a 12-hour photoperiod. Five days after germination, the 10 seedlings were transplanted into plastic pots (19 cm diameter x 26 cm height) containing 1.6 kg of moist sandy clay loam field soil per pot (58% sand, 20% silt, and 22% clay, pH 4.8, organic carbon 5.9%) for subsequent screening and dose-response tests.

2.2 Herbicide-Resistance Screening Test

The screening test was conducted to determine the occurrence of resistant biotype (R) and susceptible (S) biotypes. A total of 100 seedlings were sprayed for each herbicide—bispyribac-sodium, propanil, and quinclorac —at twice the recommended rates using a compression sprayer equipped with flat-fan nozzles, calibrated to deliver a volume of 450 L ha^{-1} at 200 kPa in the rain shelter at 29 ± 6° C with a 12 h photoperiod at a light intensity of 1,000–1,500 mEm^{-2} s^{-1}. Each seedling serves as a replicate. The recommended rates of bispyribac-sodium, propanil and quinclorac are 9.7, 3060, and 263 g ai ha^{-1}, respectively (Table 1). Following the label of the herbicides, bispyribac-sodium and propanil were applied to seedlings at the 3-4-leaf stage, while quinclorac was applied at the 1-2-leaf stage. The experiment was conducted in a completely randomized design (CRD) and the experiment was repeated. The pots were irrigated as needed throughout the experiment. The survival rate for each herbicide was recorded 14 days after treatment (DAT).

The survival rate is calculated using the formula:

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

2.3 Dose-Response Test for R and S Biotypes with Bispyribac-Sodium

All seedlings from putative resistant biotype survived after being treated with bispyribac-sodium. The survived plants from putative resistant biotype and putative susceptible plants from controlled variable were taken to maturity and the seeds harvested for the

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

Common name	Trade name	Mode of action	Formulation	Manufacturer
Bispyribac-sodium	Nominee® 100 SC	ALS enzyme inhibitor	Suspension concentrate	Agricultural Chemicals Sdn. Bhd.
Propanil	Stamweed	PSII inhibitor	Emulsifiable concentrate	Hextar Chemical Sdn. Bhd.
Quinclorac	Synapse 21.9SC	Auxin mimics	Suspension concentrate	Hextar Chemicals Sdn. Bhd.

dose-response test. A dose-response test was conducted with a series of herbicide rates. Ten seedlings were transplanted into each pot and treated with the herbicide at the 3 to 4-leaf stage. For the R biotype, the rates were 0, 1.2, 4.9, 19.4, 77.6, 310.4, and 1241.6 g ai ha^{-1}. For the S biotype, the rates were 0, 0.6, 1.2, 2.4, 4.9, 9.7, and 19.4 g ai ha^{-1}. The experiment was laid out in a completely randomized block design (CRBD) with three replicates and was repeated twice. The survival rate was recorded based on the number of seedlings that survived 14 DAT. The above-ground tissue of the surviving plants was harvested, dried in an oven at 70° C for three days and weighed. All data were expressed as percentages of their respective controls.

2.4 Inhibition of R Biotype with Tank-Mix Combination of Propanil and Quinclorac

All seedlings died following propanil and quinclorac treatments. Consequently, both herbicides were used in a tank mixture experiment. A dose-response test for the single application of propanil and quinclorac was conducted on seedlings at the 3-4 leaf stage. The rates of propanil applied were 0, 160, 240, 350, 530, 800, and 1,800 g ha^{-1}, while the rates of quinclorac were 0, 0.032, 0.13, 0.5, 2.0, 8.0, and 33.0 g ha^{-1}. The experiment was arranged in a CRBD with three replicates and was repeated. *E. crus-galli* survivors were counted at 14 DAT. Herbicide rates causing 20, 40, 60, and 80% mortality were estimated from the dose-response curves. These estimated herbicide rates were then sprayed on 3-4 leaf stage seedlings in tank mixtures. The tank mixture experiment involved propanil and quinclorac at ratios of 20:80, 40:60, 60:40, and 80:20, with each ratio having a series of five dosages. The experiment was arranged as a CRBD with three replicates and was repeated. The survival rate of the seedlings was recorded at 14 DAT, and the shoot dry weight was measured after drying the plants in the oven as previously described.

2.5 Statistical Analysis

For herbicide-resistant screening test, a chi square test was conducted to analyze and compare the frequency of *E. crus-galli* seedlings which survived after being treated with selected herbicides at 5% of significance level.

For dose-response test of R and S biotypes treated with bispyribac-sodium, the shoot dry weight and survival data were fitted to a log-logistic regression as follows:

$$Y = d/(1 + [x/x_0]^b)$$

Where Y is percentage of shoot dry weight or plant survival relative their respective controls, d is the upper limit where the dose is zero, x is the herbicide dose, x_0 is the herbicide dose required to reducing shoot dry weight or plant survival by half and b is proportional to the slopes of the curves around x_0. The resistance level (RL) was calculated as the x_0 of the R biotype divided by the x_0 of the S biotype.

For the tank mixture experiments, the shoot dry weight and survival data of *E. crus-galli* were expressed as percentages of their respective controls and fitted to the log-logistic regression as described previously. The doses that cause 95% mortality (LD_{95}) and 95% growth inhibition (GR_{95}) were estimated from the regression analysis.

3. Results and Discussion

3.1 Confirmation of Bispyribac-Sodium-Resistant Barnyardgrass

The Chi-square test revealed a significant difference ($p < 0.05$) in the survival frequency of *E. crus-galli* seedlings when treated with bispyribac-sodium, quinclorac, or propanil. The results indicated that all putative resistant *E. crus-galli* seedlings were resistant to bispyribac-sodium, whereas the seedlings died following quinclorac or propanil treatments. Further dose-response tests showed that as the herbicide rate increased, both the shoot dry weight and survival of resistant (R) and susceptible (S) biotypes decreased (Figure 1 and 2). The S biotype exhibited 100% mortality at the recommended rate of bispyribac-sodium (9.7 g ha^{-1}), while the R biotype was only completely inhibited when bispyribac-sodium was applied at 128 times the recommended rate (1,241 g ha^{-1}) (Figure 2), demonstrating a high resistance level of 128-fold (Table 2). The rates causing 50% growth reduction for the R and S biotypes were 35 and 0.5 g ha^{-1}, respectively, indicating a resistance level of 70-fold.

Damalas and Koutroubas (2023) reported that barnyardgrass is the species with the most resistant cases among *Echinochloa* spp., particularly against ALS inhibitors. Recently, bispyribac-sodium resistance in *E. crus-galli* from rice fields in India was documented, marking the first case of ALS resistance in *E. crus-galli* in India with a resistance

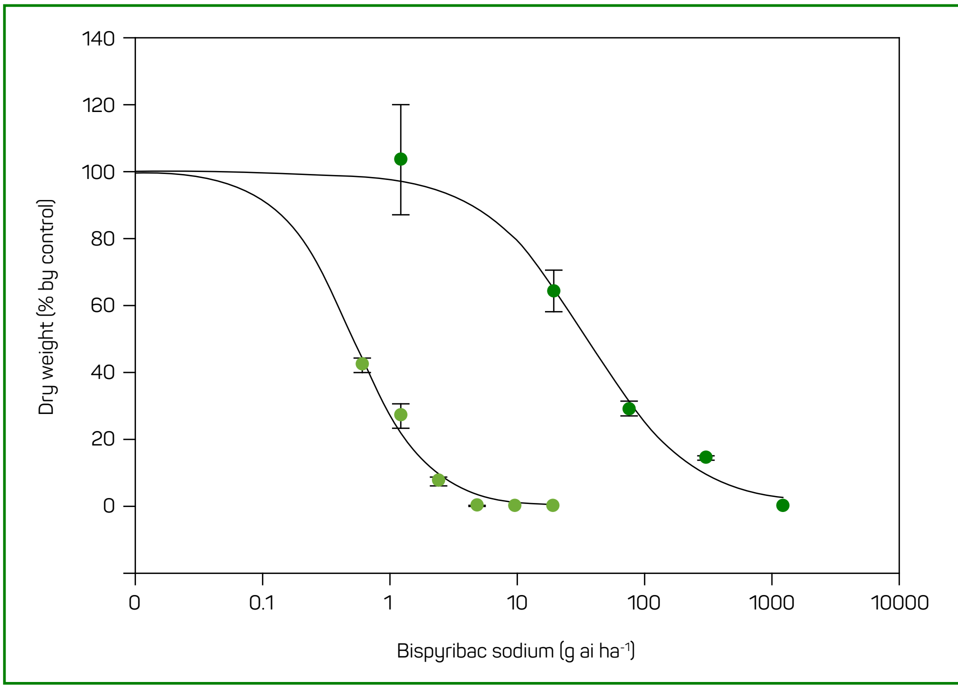

Figure 1 - Shoot dry weight of the susceptible (●) and resistant (●) biotypes of *E. crus-galli*, as affected by bispyribac sodium. Each value is mean of six replicates, with the vertical bars representing the standard deviation of the mean

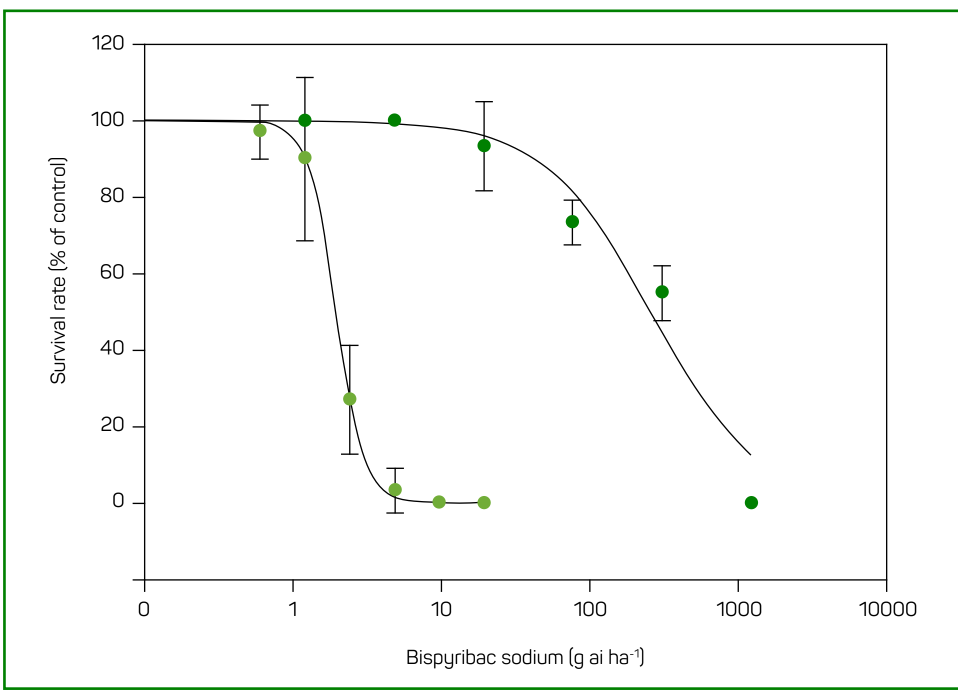

Figure 2 - Survival of the susceptible (●) and resistant (●) biotypes of *E. crus-galli*, as affected by bispyribac sodium. Each value is mean of six replicates, with the vertical bars representing the standard deviation of the mean

level of 2-4 fold (Choudhary et al., 2023). Riar et al. (2012) confirmed one of the biotypes of *E. crus-galli* from Arkansas was 15 times more resistant to bispyribac-sodium than the susceptible biotype. A study conducted on *E. crus-galli* from the rice fields of Iran showed cross-resistance to penoxsulam and bispyribac-sodium due to selection by ALS herbicides (Haghnama, Mennen, 2020). Although bispyribac-sodium is not a common ALS herbicide in Iranian rice fields, *E. crus-galli* exhibited resistance due to the presence of a resistant gene even before selection.

Table 2 - Estimated bispyribac-sodium LD_{50}, GR_{50} and resistance level (RL) values for putative resistant *E. crus-galli* (R) compared to susceptible *E. crus-galli* (S)

S	R	RL
LD_{50} (g ai ha^{-1})		128
2 (0.04)	256 (67)	
GR_{50} (g ai ha^{-1})		
0.5 (0.06)	35 (6)	70

[1] LD_{50} is the herbicide dose that causes 50% mortality. GR_{50} is the herbicide dose that causes 50% shoot growth reduction. The values in parentheses are the standard error of the mean

Table 3 - LD_{95} and GR_{95} values of *E. crus-galli* in relation to mixtures of propanil and quinclorac at different ratios

Mixture ratio	LD_{95} (g ai ha^{-1})		GR_{95} (g ai ha^{-1})	
	Propanil	Quinclorac	Propanil	Quinclorac
0:100	-	14.3 (1.8)	-	3.0 (0.6)
20:80	1,066 (36)	9.7 (0.3)	1,140 (111)	9.7 (0.9)
40:60	739 (67)	4.3 (0.3)	1,027 (4)	5.4 (0.03)
60:40	1,271 (215)	4.7 (0.9)	985 (33)	3.1 (0.1)
80:20	2,185 (224)	4.7 (0.7)	1,513 (40)	3.3 (0.1)
100:0	1,631 (116)	-	759 (9)	-

[1] LD_{95} is the herbicide dose that causes 95% mortality. GR_{95} is the herbicide dose that causes 95% shoot growth inhibition. The values in parentheses are the standard error of the mean

Bispyribac-sodium resistance in *E. crus-galli* can result from both target-site and non-target-site resistance mechanisms. For example, ALS-cross-resistant *E. crus-galli* biotypes have evolved resistance through target-site mechanisms, with ALS gene sequencing revealing substitutions of Ala122 to Val and Val to Ala122 (Riar et al., 2013). Additionally, reduced translocation of imazamox and bispyribac-sodium has been observed in resistant biotypes (Riar et al., 2013). Cusaro et al. (2022) presented the first study on the capacity of miRNA to regulate gene expression involved in the detoxification of bispyribac-sodium in *E. crus-galli* resistant biotypes. Among the five miRNAs studied, four showed very high expression in the resistant biotypes, and reduced target gene expression was observed in these four miRNAs.

3.2 Tank-Mixture of Propanil and Quinclorac

Based on the lethal dose causing 95% mortality (LD_{95}), the mixture ratios of propanil and quinclorac at 20:80, 40:60, and 60:40 resulted in a synergistic effect on *E. crus-galli* (Table 3). The LD_{95} values for *E. crus-galli* treated with either propanil or quinclorac alone were reduced by 1.3 to 3.3-fold when the herbicides were combined in these ratios. Conversely, the mixture ratio of propanil and quinclorac at 80:20 exhibited an antagonistic action. Although the rate of quinclorac could be reduced by 3-fold, the propanil rate needed to be increased by 1.3-fold to achieve LD_{95} at this ratio. By contrast, when considering the herbicide dose that causes 95% inhibition of shoot growth (GR_{95}), antagonistic effects were observed across all mixture ratios. Nevertheless, the most effective synergistic action was observed at a 40:60 mixture ratio, where the rates of propanil and quinclorac could be reduced by 2.2 and 3.3-fold, respectively, compared to their single applications to achieve LD_{95}. Notably, the LD_{95} value of propanil at this ratio was 739 g ai ha^{-1}, which is close to GR_{95} value of propanil applied singly at 759 g ai ha^{-1}. At 759 g ai ha^{-1}, propanil alone resulted in only 63% mortality (Figure 3). Combining propanil at 739 g ai ha^{-1} with quinclorac at 4.3 g ai ha^{-1} achieved 95% mortality and 90% growth inhibition (Figure 4). On the other hand, Guh et al. (1993) found that quinclorac at 300 g ha^{-1} in combination with propanil at 1,050 g ha^{-1} acted additively and resulted in 97% weed control.

The findings of present study suggest that the tank mixture of propanil and quinclorac can produce both synergistic and antagonistic effects depending on the mixture ratios. The observed compatibility between propanil and quinclorac may be attributed to their different modes of action. For example, quinclorac is quickly absorbed through plant roots and binds to auxin-binding proteins (ABP1), causing abnormal root and shoot growth (Fipke, Vidal, 2016). In contrast, propanil is primarily absorbed through the leaves and disrupts the electron transport chain by inhibiting photosystem II, (Kanawi et al., 2015). Nevertheless, a high proportion of propanil in the mixture might result in antagonistic effects, as the contact action of propanil can inhibit the herbicidal activity of quinclorac by reducing its translocation (Koo et al., 2000).

4. Conclusions

In summary, the bispyribac-sodium-resistant *E. crus-galli* biotype was confirmed to have a high resistance level, with a 128-fold increase for LD_{50} and a 70-fold increase for GR_{50}. It was

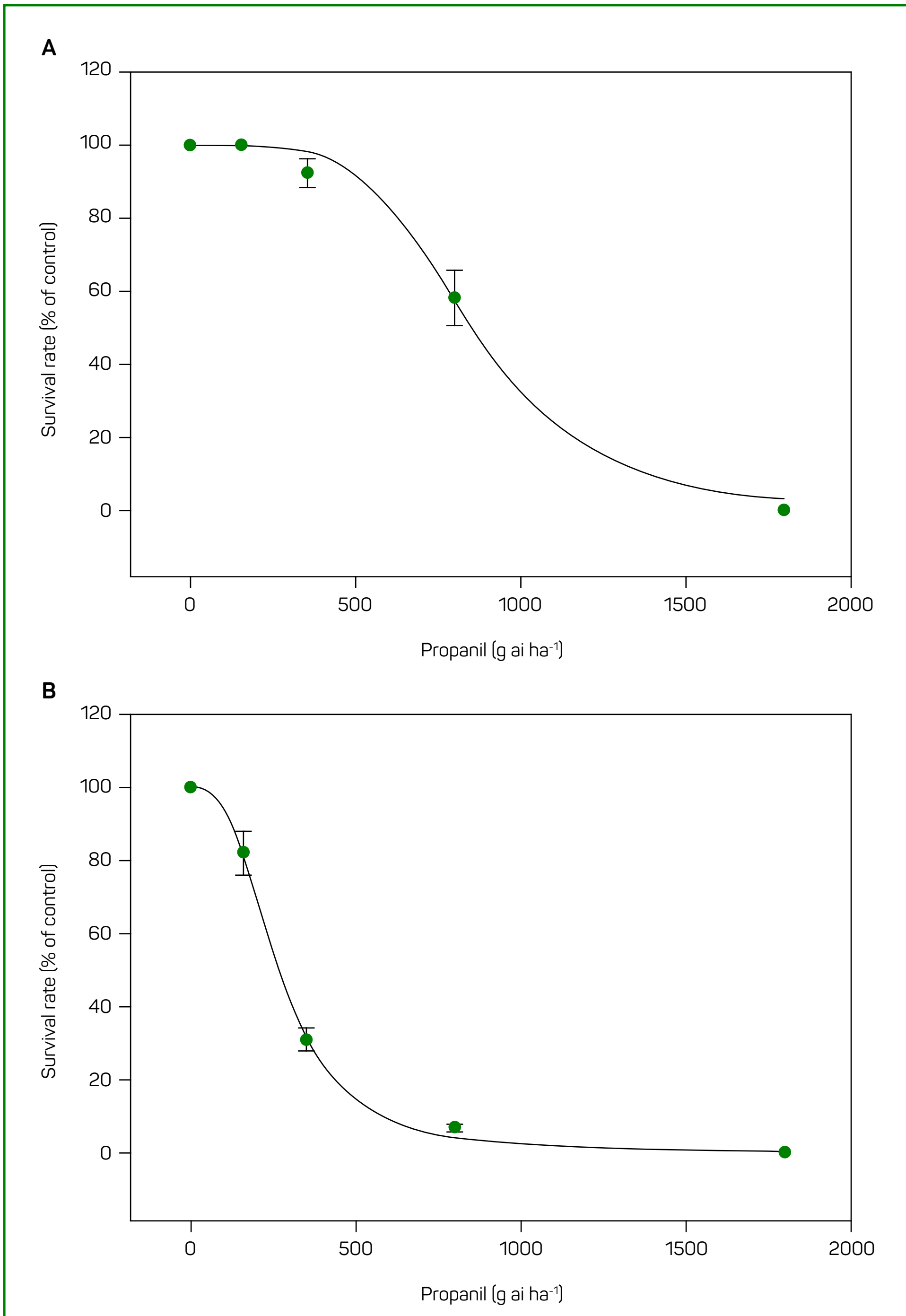

Figure 3 - Survival (a) and shoot dry weight (b) of *E. crus-galli* as affected by single application of propanil. Each value is mean of six replicates, with the vertical bars representing the standard deviation of the mean

also evident that the tank mixture ratios play a deciding factor for either synergistic or antagonistic effects between propanil and quinclorac. The most effective synergistic activity on the *E. crus-galli* biotype was observed with a 40:60 mixture ratio of propanil to quinclorac. To further validate the effectiveness of this mixture ratio, additional field studies are needed. Conducting field trials, considering all the biotic and abiotic factors will provide insights into the performance and sustainability of the propanil and quinclorac tank mixture for controlling the bispyribac-sodium-resistant *E. crus-galli* biotype in DSR systems.

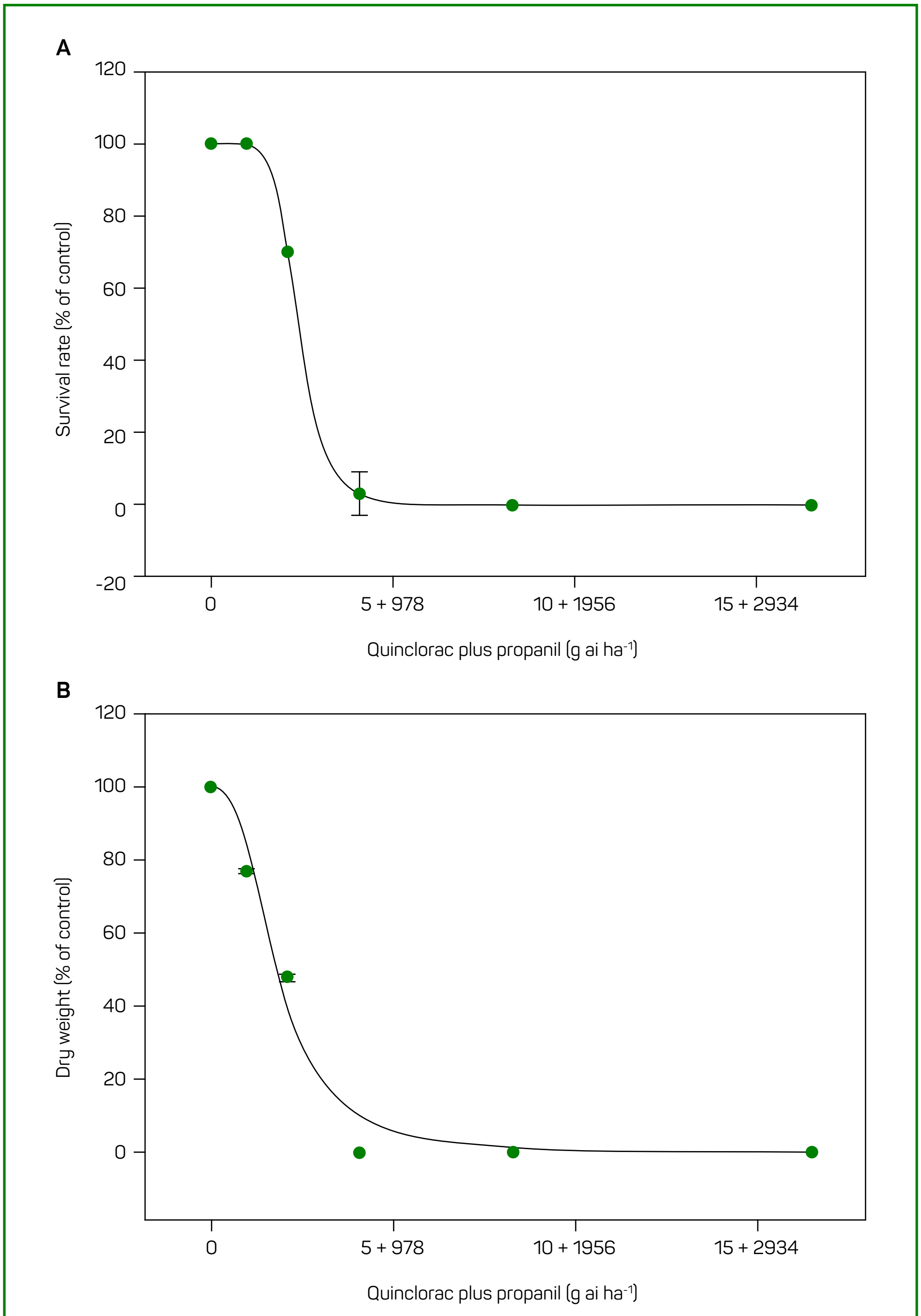

Figure 4 - Survival (a) and shoot dry weight (b) of *E. crus-galli* as affected by propanil and quinclorac at mixture ratio of 40:60. Each value is mean of six replicates, with the vertical bars representing the standard deviation of the mean

Author's contributions

All authors read and agreed to the published version of the manuscript. TSC, MD, and KG: conceptualization of the manuscript and development of the methodology. KG: data collection and curation. TSC, and KG: data analysis. KG, and TSC: data interpretation. MD: funding acquisition and resources. KG: vproject administration. TSC, and MSA: supervision. KG: writing the original draft of the manuscript. KG, TSC, MAS, MD, and NK: writing, review and editing.

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