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Joint Action of Selective Herbicides Used in Paddy Field on Control of *Lemna minor* L.

(Tindakan Bersama Racun Herbisid Terpilih Digunakan di Sawah Padi terhadap Kawalan *Lemna minor* L.)

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ABSTRACT

To minimise herbicide inputs, reduce environmental burdens, and delay the evolution of herbicide resistance, it is of the utmost significance to study the interaction between herbicides. This may be accomplished by selecting the optimal combination, which, through synergy, delivers more cost-effective weed control than a single herbicide. Under laboratory conditions, interactions between binary mixtures of bensulfuron, propanil, 2,4-D, or bentazon on chlorophyll reduction of *Lemna minor* were investigated using the Additive Dose Model. Mixtures of propanil/2,4-D resulted in low antagonism whereas strong antagonism was evident in bensulfuron-methyl/propanil. Combinations of bensulfuron/bentazon and bensulfuron/2,4-D led to moderate antagonism. By contrast, propanil in combination with bentazon demonstrated a modest degree of synergism. The findings of this study could provide good guideline and insights when mixing two herbicides for weed control in rice fields and other aquatic environment.

Keywords: Antagonism; joint-action; synergism; tank mixture

ABSTRAK

Kajian mengenai tindakan bersama racun herba adalah amat penting untuk mengurangkan kegunaan racun herba, mengurangkan masalah alam sekitar dan melambatkan evolusi kerintangan racun herba. Ini boleh dicapai dengan memilih campuran optimum melalui tindakan sinergistik yang memberikan kawalan rumpai yang lebih berkesan kos daripada racun herba tunggal. Penyelidikan ini dijalankan untuk menilai interaksi antara campuran binari bensulfuron, propanil, 2,4-D, atau bentazon terhadap pengurangan klorofil pada *Lemna minor* dengan menggunakan Model Dos Tambahan dalam keadaan makmal. Campuran bensulfuron/propanil, bensulfuron/bentazon, bensulfuron/2,4-D dan propanil/2,4-D menyebabkan antagonisme yang rendah hingga kuat. Namun, sinergisme yang sederhana diperoleh pada campuran propanil dan bentazon. Hasil kajian ini dapat memberi panduan dan kefahaman apabila menggabungkan dua racun herba untuk kawalan rumpai di sawah padi dan persekitaran akuatik yang lain.

Kata kunci: Antagonisme; sinergisme; tangki campuran; tindakan bersama

INTRODUCTION

Several issues are brought on by aquatic weeds like *Lemna minor*. The growth of macrophytes like *Lemna minor* may affect the biological, chemical, and physical components of an ecosystem (Gerardo & de Lima 2022). They significantly lessen the water flow in streams, channels, and irrigation and drainage systems. Water flow obstruction causes water levels to rise in canals and streams, which causes floods, seepage into nearby regions, cracks in canal banks, and insufficient irrigation water delivery to farms far from the main water supply (Rao 2002). Additionally, a slower flow rate results in greater siltation and a smaller carrying capacity, which calls for more frequent mechanical cleaning (Gerardo & de Lima 2022). The interference of *L. minor* with the pumping and flow of irrigation water in Portugal throughout the summer has been recorded by Gerardo and de Lima (2022).

The selective herbicides 2,4-D isopropylamine, bensulfuron-methyl, bentazon sodium, and propanil are often used in Malaysia to control weeds in paddy fields without harming rice plants. Combining herbicides to suppress weeds is a popular strategy that has increased the efficiency of the herbicides (Chitband et al. 2019). When two or more herbicides are given to target plants in a tank combination, they are sprayed simultaneously or mixed (Mahajan & Chauhan 2015). Several different mechanisms of action are involved in a single tank mixing herbicide application (Merritt et al. 2020). Farmers frequently combine two or more herbicides without understanding how these herbicides work together. Tank mixing herbicides with several modes of actions can slow down the development of herbicide-resistant weeds (Bianchi et al. 2020). As reference models for joint action, the Additive Dose Model (ADM) or the Multiplicative Survival Model (MSM) are frequently utilised. The ADM predicts that the relevant herbicides will work in a manner comparable to one another and will not interfere with the binding sites of one another. The MSM, however, believes that herbicides with various modes of action (Chitband et al. 2019). Synergism is dependent on species and occurs more often in broadleaf than in monocot weeds. However, there are few investigations on the synergistic effects of herbicide combinations on aquatic or semi-aquatic plants (Kaushik et al. 2007). Therefore, this study was undertaken to examine the interactions between bensulfuron, propanil, 2,4-D, and bentazon, and determine effective tank combinations with minimal herbicide use for *Lemna minor* control.

MATERIALS AND METHODS

PLANT MATERIALS

Lemna minor plants were collected from the freshwater hatchery at Universiti Malaysia Terengganu (UMT). Then, culture of *L. minor* was conducted in the glasshouse at a temperature of 37.0 °C, humidity of 57.3% and light intensity of 98.58 µmol. Each *L. minor* plant was measured by using a chroma meter CR400 to get homogeneous plants of *L. minor* which have green color with -a values in range of -9.50 ± 0.5 (about 5 mm in length) before being subjected to herbicidal treatment.

HERBICIDES

Four herbicides used in this study are bensulfuron-methyl, propanil, 2,4-D isopropylamine and bentazon sodium as shown in Table 1.

CULTURE OF *Lemna minor*

First of all, 250 g potting mixture of sandy loam soil, sand and peat (2:1:1, w/w/w) containing all necessary macro- and micro-nutrients was put into each tray (35 cm × 25 cm × 8 cm). After that, two litter of water was added slowly, followed by transplanting about 20 individual of *L. minor* into each tray. In order to ensure sufficient water for *L. minor* growth, adding water is required for every two days to avoid desiccation. About two weeks later, *L. minor* attained the standard degree of green color with -a values in the range of -9.50 ± 0.50 were used for subsequent experiment.

TABLE 1. Herbicide characteristics

Active ingredient	Mode of action	%w/w	Trade name	Formulation
Bensulfuron-methyl	Acetolactate synthase inhibitor	10.0	Londax 10WP	Wettable Powder
Propanil	Photosystem II inhibitor	35.0	Minconil 350	Emulsifiable Concentrate
2,4-D isopropylamine	Auxin mimic	45.0	Keris	Soluble Concentrate
Bentazone sodium	Photosystem II inhibitor	44.0	Basagran	Soluble Concentrate

DOSE-RESPONSE EXPERIMENT

Five binary combinations namely bensulfuron-methyl/propanil, propanil/2,4-D isopropylamine, 2,4-D isopropylamine/bensulfuron-methyl, bensulfuron-methyl/bentazon sodium and propanil/bentazon sodium were examined. The mixture ratios chosen for each combination mixture are 90:10%, 80:20%, 70:30%, 60:40%, 50:50%, 40:60%, 30:70%, 20:80% and 10:90%. The *L. minor* plants treated with distilled water were served as control. *Lemna minor* plants were dipped into the herbicide mixture and incubated in a growth chamber at alternate temperature of 25 °C/30 °C with 12 h photoperiod. The green color (a value) of the *L. minor* was measured by using a chroma meter (CR400) after two days of incubation (48 h).

STATISTICAL ANALYSIS

Regression test was used in order to analyze data. The experimental design of this study is a complete randomized design (CRD). In nine fixed-ratio mixtures, there are five concentrations for each binary mixture with four replicates for each concentration. Each replicate contains five plants of *L. minor*. The basis of the response surface model is the sigmoid log-logistic dose-response model as follows (Sørensen et al. 2007):

$$y_i = \frac{d}{1 + \exp\{b_i[\ln(x_i) - \ln(e_i)]\}} \qquad b_i > 0 \qquad (1)$$

where y_i denotes the response for the *i*th herbicide dose x_i (i = 1....,5), and d is the common upper limit of the response of all mixture ratios when the chemical concentration (x_i) is zero. The lower limit is assumed to be zero. The parameter e_i is the dose of herbicide mixture *ii* giving a response of 50% of d, and b_i is proportional to the slope around e_i. The regression fits were done in a sequential fashion. Firstly, the regressions with similar response curve, that is, those where slope b is the same for all curves, were tested against the regressions where slope b could vary freely. Subsequently, e_i was substituted with Equation (2) to test if η is significantly different for 1,0.

The straight line represents the ADM isoboles of predicted responses. The ED_{50} values obtained were plotted on the graph and compared to the ADM isoboles. Points above the isoboles indicate that the joint action of a mixture is lower than predicted by ADM while points below the isoboles indicate a joint action higher than predicted by ADM (Kudsk & Mathiassen 2003).

The Vølund model was used as the default isobole model, including two parameters (η_1 and η_2) to describe the isobole curvature. This model allows a larger degree of antagonism and can describe asymmetric isoboles (Vølund 1992):

$$\left(\frac{\chi_1}{\mathrm{ED}_1}\right)^{\eta_1}\left(\frac{\chi_1}{\mathrm{ED}_1}+\frac{\chi_2}{\mathrm{ED}_2}\right)^{1-\eta_1}+\left(\frac{\chi_2}{\mathrm{ED}_2}\right)^{\eta_2}\left(\frac{\chi_1}{\mathrm{ED}_1}+\frac{\chi_2}{\mathrm{ED}_2}\right)^{1-\eta_2}=1 \quad (2)$$

In this model, $\chi\chi$ is the concentration of a herbicide in a mixture at a predefined effect level, and ED is the concentration of the same herbicide giving that effect, when tested alone. The subscription 1 and 2 denote the two herbicides in the mixture. A η value larger than 1 indicate antagonism, whereas η values that are smaller than 1 indicate synergism. Different η values describe asymmetric isoboles.

The sum of toxic units (ΣTU) signifies the relative amount of herbicide in a mixture that will give a certain effect. This study works with a 50% effect level. If the ΣTU is 1, the mixture effect follows ADM. If it is 0.8, only 80% of the chemicals are needed to reduce the response to 50% compared with that expected from ADM. The ΣTU can be calculated for all mixture ratios. The ΣTU of the 50:50% effect ratio ($\Sigma TU_{50:50}$) is used as a measure of the size of synergism or antagonism across the two isobole models, as it is this mixture ratio at which the deviation from ADM is largest for symmetric isoboles. For the Vølund isobole model, the $\Sigma TU_{50:50}$ is calculated as $1/(2^{-\eta_1} + 2^{-\eta_2})$ (Sørensen et al. 2007). One-sample T test was used to determine deviation from ADM based on η_1 and η_2 values. Significant difference in $\Sigma TU_{50:50}$ between propanil/bentazone sodium and other combinations was analyzed by T test at 5% of significance level.

RESULTS AND DISCUSSION

The joint actions of bensulfuron-methyl/propanil, propanil/2,4-D isopropylamine, 2,4-D isopropylamine/bensulfuron-methyl, bensulfuron-methyl/bentazon sodium and propanil/bentazon sodium were tested using floating macrophyta, *L. minor* by employing Additive Dose Model (ADM). Based on the results, all combinations that involve bensulfuron-methyl, propanil/2,4-D isopropylamine exhibited antagonism, only propanil/bentazon sodium showed synergism. None of the combinations tested in this study were additive. To give an overview of the finding, these results are summarised in Table 2. Besides, one-sample T test was conducted to determine deviation from ADM based on η_1 and η_2 values.

Herbicide antagonism is defined as the reduction of control on certain weeds as the result of applying mixtures of two or more herbicides (Meyer, Norsworthy & Kruger 2020). Factors like target plant species, other pesticides, growth stage of the target plant, application method of herbicide and the environment can lead to herbicide antagonism (Merritt et al. 2020). Antagonism is observed when the effect of the combination of herbicides is less than the prediction made by referring to the mechanism of chemicals involved individually (Alebrahim, Kalkhoran & Tseng 2023). The uptake rate of one herbicide can be reduced by the presence of another herbicide (Barbieri et al. 2022), as can the metabolism rate, since some herbicides are known to reduce the activity of enzymes active in the metabolism of other herbicides (Ottis, Mattice & Talbert 2005). Also, the transport of systemic herbicides can be affected by quickly acting herbicides that lower the metabolism rate in the plant, and thereby slowing the transport processes (Scherder, Talbert & Lovelacc 2005). When a mixture of herbicides acts antagonistically, it may have to be reapplied which can elevate the production costs, reduce the potential of grain yield, and increase the environmental risks (Matzenbacher et al. 2015).

According to Hatzios and Penner (1985), antagonism interaction can be grouped into four categories, namely biochemical antagonism, competitive antagonism, physiological antagonism, and chemical antagonism. Biochemical antagonism occurred when one chemical decreases the amount of herbicide which reaches the site of action but either reducing penetration or transport or by enhancing metabolic inactivation or sequestering. When the antagonist binds at the active site and prevents the binding of the (more active) herbicide, this interaction is called competitive antagonism. In contrast, physiological antagonism happens when two herbicides have opposite biological effects and counteract each other. Last but not least, an interaction of two herbicides can be named as chemical antagonism when the herbicide reacts chemically with another chemical. Usually, more than one of these mechanisms may be involved and detailed studies are needed for a better understanding of the underlying control process.

Regardless the target plant species, joint action of herbicides were appeared to be antagonism three time more than synergism. This trend held no matter whether the interacting herbicides were absorbed by the same or different parts of the plant, had the same or different translocating abilities, had the same or different modes of action, and regardless of whether the target plants were annual or perennial plants, or crops or weeds. Antagonistic interactions occurred much more frequently when the target plants were monocot than dicot (Barbieri et al. 2022). Antagonism can be prevented by skipping the tank-mixing step and applying the herbicides separately (Merritt et al. 2020).

JOINT ACTION OF BENSULFURON-METHYL AND PROPANIL

Strong antagonism has been established with combinations of bensulfuron-methyl and propanil at all ratios (Figure 1). The effective dosage that causes 50% leaf discoloration (ED_{50}) for bensulfuron-methyl and propanil, respectively, is 886 ppm and 20 ppm. This herbicide combination has η_1 and η_2 values of 6.92 ± 0.55 and 3.07 ± 0.13, respectively. With a total of toxic units (TU50:50) of 7.83 ± 0.47, about 783% of the chemicals are required to provide a 50% reduction in reaction relative to that predicted from ADM.

TABLE 2. Parameters for the five binary herbicide combinations tested on *Lemna minor*

Combination	η_1	η_2	$\Sigma TU_{50:50}$
Bensulfuron-methyl/propanil	6.91 ± 0.55	3.07 ± 0.14	7.83 ± 0.47*
2,4-D isopropylamine/ Bensulfuron-methyl	1.75 ± 0.06	16.11 ± 0.12	3.37 ± 0.14*
Propanil/2,4-D isopropylamine	7.18 ± 0.05	0.61 ± 0.02	1.51 ± 0.02*
Bensulfuron-methyl/Bentazon sodium	9.11 ± 0.91	1.74 ± 0.16	3.32 ± 0.37*
Propanil/bentazon sodium	0.03 ± 0.001	0.31 ± 0.14	0.56 ± 0.01

η_1 and η_2 are two parameters to describe the isobole curvature of Vølund model

A η value larger than 1 indicate antagonism, whereas η values that are smaller than 1 indicate synergism

All mixtures deviated from ADM based on η_1 and η_2 values analyzed by one sample T test at 5% of significance level

The ΣTU of the 50:50% effect ratio ($\sum TU_{50:50}$) is a measure of the size of synergism or antagonism across the two isobole models

*denotes significant difference in $\Sigma TU_{50:50}$ between propanil/bentazone sodium and other combinations after being analyzed by T test at 5% of significance level

According to Lancaster, Jugulam and Jones (2021), herbicides such as bensulfuron and chlorsulfuron which inhibit acetolactate synthase (ALS) are mobile in both the xylem and phloem. Herbicide phloem mobility relates to sucrose transfer (Devine, Bestman & Vanden Born 1990). It has been demonstrated that ALS inhibitors reduced sucrose transport without affecting sucrose generation in the source leaves (Kim & Vanden Born 1997, 1996). Bentazon, a member of the same chemical family as propanil, inhibits the electron transport in PSII, resulting in a decrease in sucrose synthesis and translocation (Willingham et al. 2008). Sucrose flow from the propanil-treated leaf will decrease due to the suppression of sucrose biosynthesis by PSII. A PSII-inhibitor may thereby limit the translocation of phloem chlorophyll transportable herbicides such as ALS inhibitors. In other words, systemic herbicides such as bensulfuron-methyl may be neutralised by contact herbicides such as propanil, demonstrating physiological antagonism.

Reductions in herbicide absorption and translocation partially explain why the efficiency of graminicides is diminished when combined with ALS and photosystem II herbicides. When coupled with cyhalofop, propanil inhibited the translocation of cyhalofop from the treated leaf of barnyardgrass. Propanil is a broad-spectrum herbicide used in paddy fields that inhibits photosystem II reaction, resulting in chlorosis within a few days. Leaf chlorosis can ultimately result in leaf burn and membrane integrity loss (Barbieri et al. 2022). As a result, bensulfuron-methyl is rendered ineffective as an ALS inhibitor and systemic herbicide.

JOINT ACTION OF 2,4-D ISOPROPYLAMINE AND BENSULFURON-METHYL

The interaction between 2,4-D isopropylamine and bensulfuron-methyl was somewhat hostile. This dataset contains only four ratios with antagonistic impact; the remaining five ratios are omitted from study owing to a lack of equation fit (Figure 2). 480 ppm and 886 ppm are the ED_{50} values for 2,4-D isopropylamine and bensulfuron-methyl, respectively. The η_1 value for this herbicide mixture is 1.75 ± 0.06 and the η_2 value is 16.12 ± 0.12 The combination of 2,4-D isopropylamine

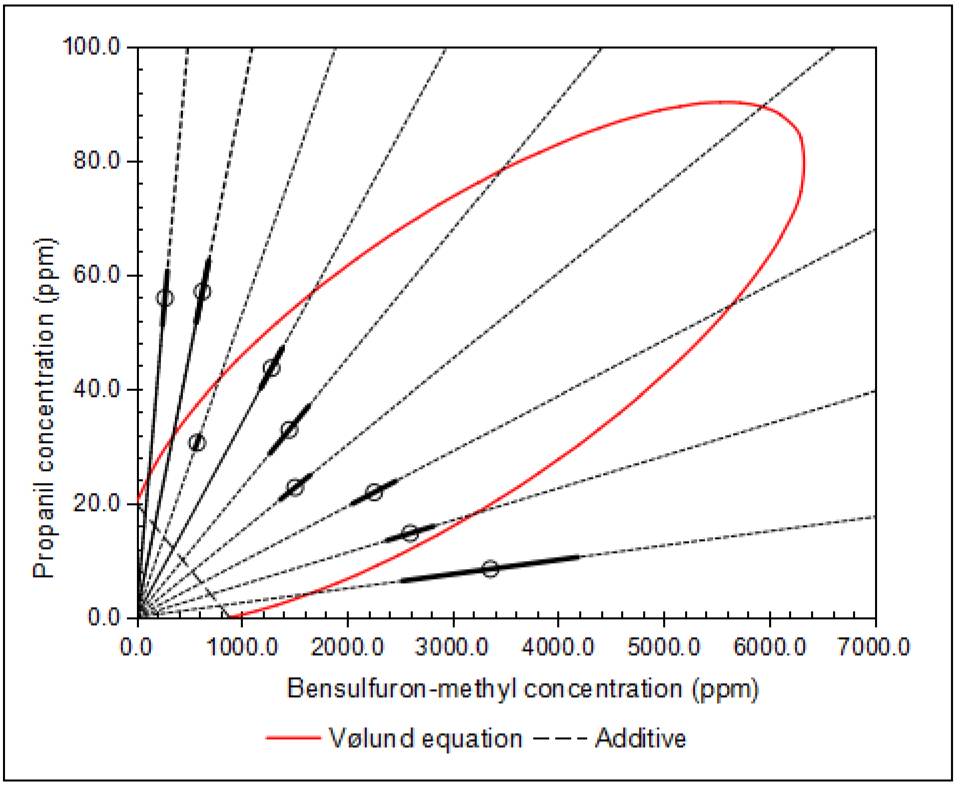

FIGURE 1. Isobologram of mixtures of bensulfuron-methyl and propanil

and bensulfuron-methyl has a TU50:50 value of 3.38 ± 0.14, indicating that about 337% more of the chemicals are required to achieve a 50% reduction in response compared to ADM.

Dicamba, which has the same mechanism of action as 2,4-D, is easily translocated throughout the plant via phloem and xylem and readily accumulates in meristematic tissues, where it inhibits nucleic acid and protein synthesis. 2,4-D is a synthetic herbicide that imitates auxin's characteristics (Martins et al. 2021). 2,4-D affects plants by altering the flexibility of the cell wall, regulating protein synthesis, and increasing ethylene production (Tiwari, Kharwar & Tiwari 2019). It is transported via the roots, stems, and leaves to the meristematic location of the plant (Song 2013).

In contrast, extremely low doses of bensulfuron-methyl impede cell proliferation by inhibiting acetolactate synthase (ALS), a critical enzyme in the production of branched-chain amino acids (valine, leucine, and isoleucine). Bensulfuron-methyl is absorbed from roots and shoots and transported from leaves to meristematic tissues via the xylem and phloem. Therefore, it may be assumed that 2,4-D reduces the inhibition of cell division caused by bensulfuron-methyl by stimulating cell division in meristematic zones.

JOINT ACTION OF PROPANIL AND 2,4-D ISOPROPYLAMINE

Propanil and 2,4-D isopropylamine mixes exhibited the lowest antagonism among all tested mixtures, even though all ratios in this combination exhibited antagonistic action apart from one ratio that did not match the equation (Figure 3). The ED$_{50}$ of propanil is 20 ppm, while that of 2,4-D isopropylamine is 480 ppm. Interaction parameters η_1 and η_2 are 7.18± 0.05, and 0.61 ±0.02, respectively. With a total of toxic units (TU50:50) of 1.51 ±0.02, about 151% of the chemicals are required to lower the reaction to 50%, compared to what would be predicted from ADM.

Propanil is a contact herbicide, which can inhibit the translocation of systemic herbicides and diminish the control of some weeds below expectations. For a few other weeds, combination propanil with other systemic herbicides has resulted in diminished effectiveness (Ottis, Mattice & Talbert 2005; Willingham et al. 2008). Propanil produced fast necrosis and defoliation of weeds, leading to a loss of membrane integrity (Scherder, Talbert & Lovelacc 2005). This may have hindered the translocation and efficacy of triclopyr, which has the same mechanism of action as 2,4-D isopropylamine, to other plant tissues. However, decreasing the concentration of

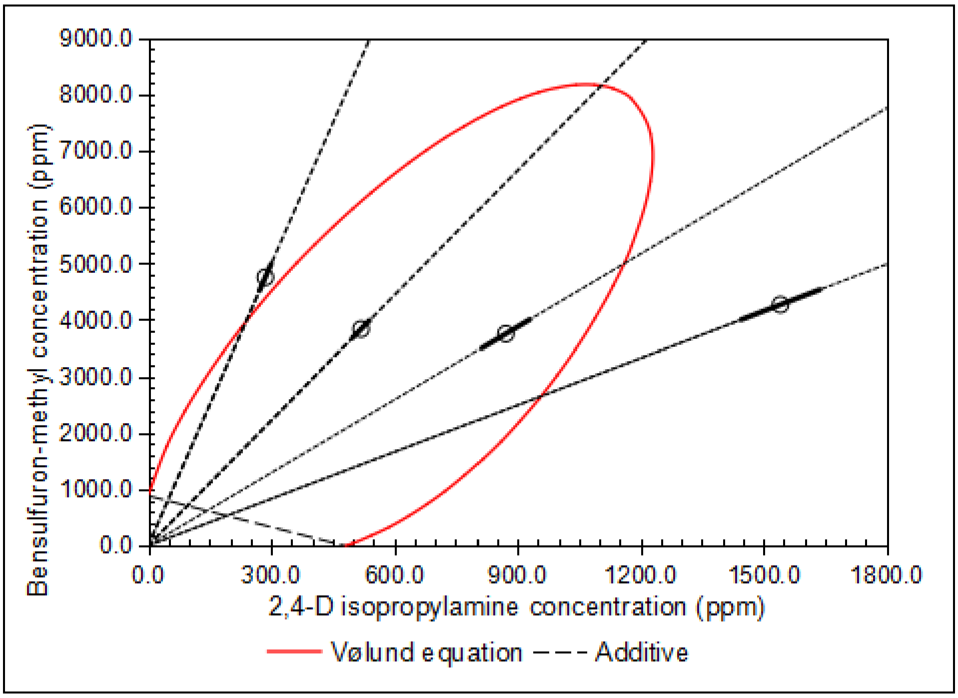

FIGURE 2. Isobologram of mixtures of 2,4-D isopropylamine and bensulfuron-methyl

propanil may lessen the fast necrosis produced by this herbicide, so improving its control.

Norsworthy et al. (2009) state that 2,4-D in combination with propanil is one of the most effective herbicides for controlling broadleaf weeds such as *Polygonum pensylvanicum*, *Sida spinosa*, and *Ipomoea wrightii* on levees. However, this contradicts the findings of the present investigation. Other study on the combination of propanil with the systemic herbicide cyhalofop showed that a considerable proportion of cyhalofop stayed in the treated leaf and was not translocated to other plant tissues. The reduced translocation may be due to caustic leaf burn caused by propanil, resulting in a loss of membrane integrity, which may have led to a decrease in translocation to other plant tissues (Barbieri et al. 2022). This shows that translocation of the systemic herbicide 2,4-D isopropylamine may be decreased due to the corrosive leaf burn induced by propanil, producing in antagonism in this combination. However, when the dosage of propanil is lowered, the antagonistic effect is diminished.

JOINT ACTION OF BENSULFURON-METHYL AND BENTAZON SODIUM

The combination of bensulfuron-methyl and bentazon sodium exhibited mild antagonism, with five ratios being antagonistic and four ratios being omitted from study due to an insufficient fit in the equation (Figure 4). The ED_{50} concentrations for bensulfuron-methyl and bentazon sodium are 886 ppm and 2,000 ppm, respectively. In this herbicide combination, η_1 equals 9.11±0.91 and and η_2 equals 1.74±0.16. The TU50:50 of bensulfuron-methyl/bentazon sodium is 3.32±0.37, which is significantly lower than the TU50:50 of 2,4-D isopropylamine/bensulfuron-methyl, indicating that around 332% more of the chemicals are required to reduce the reaction to 50% than would be predicted from ADM.

Bentazon inhibited foliar absorption and transport of [14C]-imazethapyr in redroot pigweed (*Amaranthus retroflexus* L.) and Olathe pinto bean *(Phaseolus vulgaris* L.), (Ohio State University Extension 2020). Imazethapyr, a member of the imidazolinones family, shares the same mode of action as the phloem-mobile herbicide bensulfuron-methyl. These authors also demonstrated that decreased foliar absorption could be compensated for by adding urea ammonium nitrate, but decreased translocation was unaffected, indicating that bentazon's inhibitory impact on imazethapyr translocation was physiological basis.

It appears that herbicides that inhibit PSII can impede the transport of phloem-mobile herbicides such

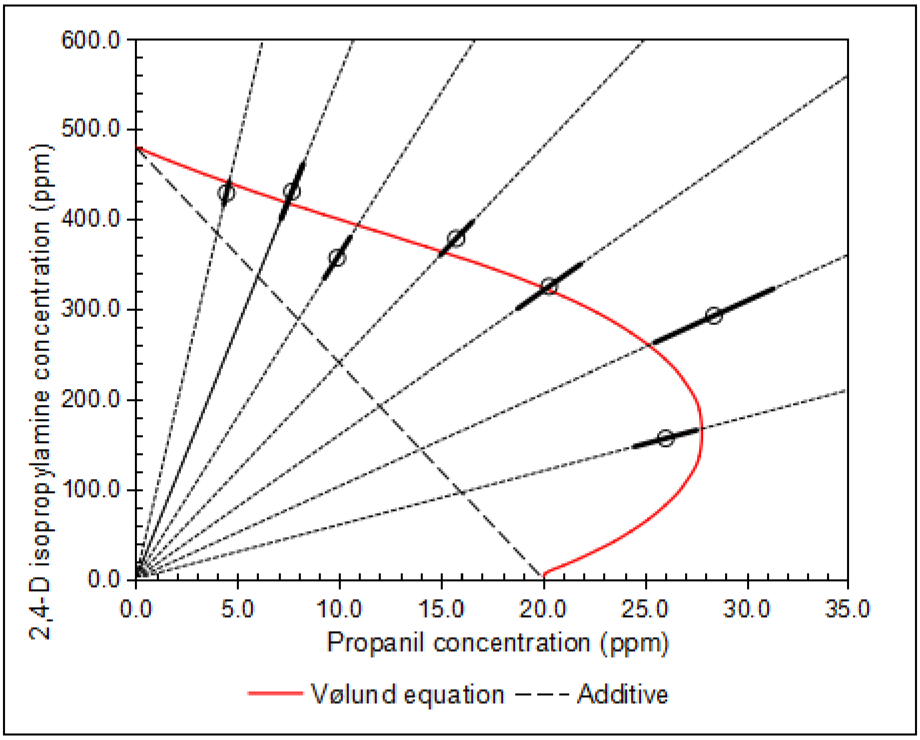

FIGURE 3. Isobologram of mixtures of propanil and 2,4-D isopropylamine

as ALS and ACCase inhibitors. Therefore, it is fair to believe that bentazon sodium, a PS II inhibitor and contact herbicide, decreases the phloem transport of the ALS-inhibitor bensulfuron-methyl, resulting in physiological antagonism (Barbieri et al. 2022).

JOINT ACTIONS OF PROPANIL AND BENTAZON SODIUM

Five ratios of propanil and bentazon sodium exhibited synergistic response, two ratios showed antagonistic reaction, and the remaining two ratios are removed from study owing to lack of fit in the equation (Figure 5). Propanil and bentazon sodium have ED_{50} concentrations of 20 and 2000 ppm, respectively. The η_1 and η_2 values for this combination of herbicides are 0.03 ± 0.001 and 0.33 ± 0.14, respectively. The TU50:50 for this combination is 0.56 ± 0.01, indicating that only approximately 56% of the chemicals are required to provide a 50% reduction in response relative to ADM.

Herbicides that impede photosynthetic activity suppress several broadleaf and some grassy weeds. These herbicides inhibit photosynthesis, the mechanism by which plants produce food. The herbicide has no effect on plants until after they emerge and starts photosynthesis. Bentazon, like propanil, is known to impede electron transport in photosystem II, hence reducing sucrose synthesis and translocation (Jugulam & Shyam 2019). Although both propanil and bentazon sodium inhibit photosynthesis, their binding sites are distinct.

Both bentazone and propanil are non-translocated contact herbicides (Herrera-Herrera et al. 2016). For effective weed control with these herbicides, complete covering of the foliage is necessary. Zhang, Hamill and Weaver (1995) argue that the combination of two herbicides, one of which is translocatable and the other of which is not, may lower the likelihood of antagonistic interactions during translocation. Therefore, it may be predicted that the likelihood of an antagonistic interaction between two contact herbicides will be extremely low, but the likelihood of a synergistic interaction will grow. Moreover, when two herbicides that limit photosynthesis are applied as a tank combination or sequentially, the target plants may be harmed to the same extent as when the two herbicides have separate biological actions.However, a large dosage of propanil causes antagonism.

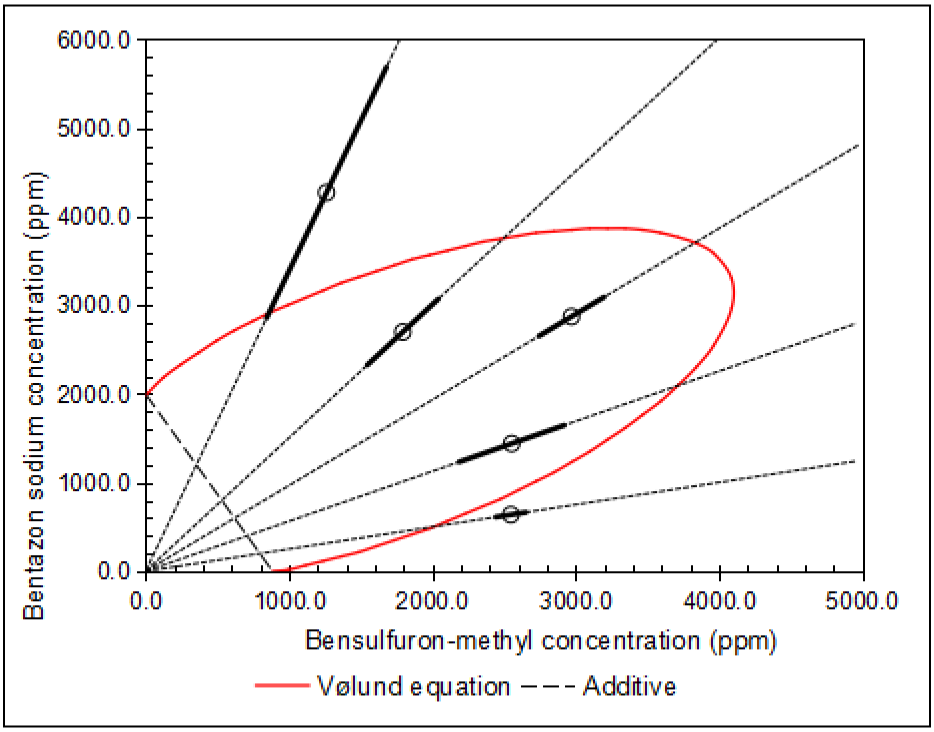

FIGURE 4. Isobologram of mixtures of bensulfuron-methyl and bentazon sodium

Tank mixing of two or more herbicides is a widely adopted strategy for controlling a broad range of weeds in key agronomic crops while minimizing costs. Joint action studies have shown that tank mixing a contact herbicide and a systemic herbicide is not recommended due to moderate to high antagonistic action. However, it is feasible to mix two contact herbicides at an appropriate ratio (Table 2 & Figure 5). This information is crucial for the development of new premix herbicide products at reduced rates for weed control in rice fields. For example, the combination of propanil and bentazon sodium in this tank mixture was found to be synergistic, although only five ratios showed combined synergism. To determine the most cost-effective combination ratio, the costs of each ratio were calculated. Among these five ratios, a 30% propanil and 70% bentazon sodium ratio was found to be the most economical, costing just RM1.29 per 300 L spray volume per hectare (data not shown). The results of this study offer valuable guidelines and insights for mixing two herbicides for weed control in rice fields and other aquatic environments.

CONCLUSION

Using the Additive Dose Model, five binary mixes of bensulfuron-methyl, propanil, 2, 4-D, or bentazon were tested on *Lemna minor*. The combination activity of bensulfuron /propanil, 2,4-D /bensulfuron, bensulfuron /bentazon, and propanil/2,4-D demonstrates the antagonistic properties. Only a propanil/bentazon sodium tank combination showed synergistic interaction. The most successful tank mixture for controlling *Lemna minor* with little herbicide application is 30% propanil and 70% bentazon sodium.

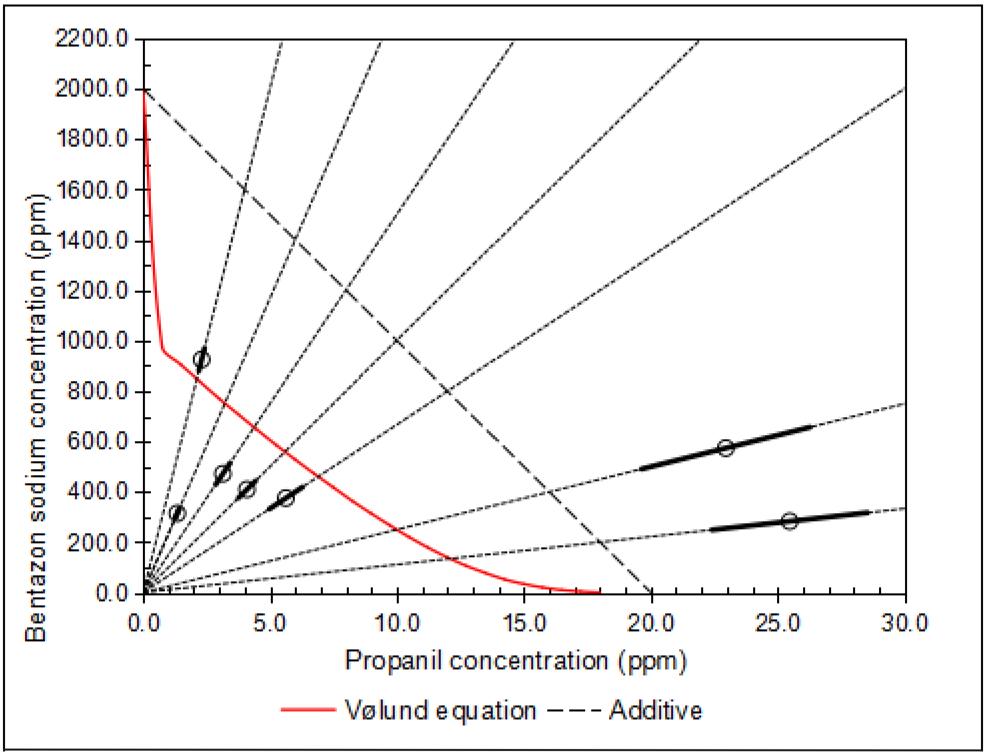

FIGURE 5. Isobologram of mixtures of propanil and bentazon sodium

REFERENCES

Alebrahim, M.T., Kalkhoran, E.S. & Tseng, T-M.P. 2023. Joint action of herbicides on weeds and their risk assessment on earthworm (*Eisenia fetida* L.). In *New Insights in Herbicide Science,* edited by Mendes, K.F. IntechOpen Publishing.

Barbieri, G.F., Young, B.G., Dayan, F.E., Streibig, J.C., Takano, H.K., Merotto, A. & Avila, L.A. 2022. Herbicide mixtures: Interactions and modeling. *Advances in Weed Science* 40(1): e02022005.

Bianchi, L., Anunciato, V.M., Gazola, T., Perissato, S.M., de Carvalho Dias, R., Tropaldi, L., Carbonari, C.A. & Velini, E.D. 2020. Effects of glyphosate and clethodim alone and in mixture in sourgrass (*Digitaria insularis*). *Crop Protection* 138: 105322

Chitband, A.A., Ghorbani, R., Hassan, M. & Mahbubeh Nabizade, M. 2019. Joint action of some broadleaf herbicides in sugar beet. *Pest Management Science* 67(3): 179-186.

Devine, M.D., Bestman, H.D. & Vanden Born, W.H. 1990. Physiological basis for different phloem mobilities of chlorsulfuron and clopyralid. *Weed Science* 38(1): 1-9.

Gerardo, R. & de Lima, I.P. 2022. Monitoring Duckweeds (*Lemna minor*) in small rivers using sentinel-2 satellite imagery: Application of vegetation and water indices to the Lis River (Portugal). *Water* 14(15): 2284.

Hatzios, K.K. & Penner, D. 1985. Interaction of herbicides with other agrochemicals in higher plants. *Reviews of Weed Science.* 1: 1-63.

Herrera-Herrera, A.V., Asensio-Ramos, M., Hernández-Borges, J. & Rodríguez-Delgado, M.Á. 2016. Pesticides and herbicides: Types, uses, and determination of herbicides. *Encyclopedia of Food and Health*. pp. 326-332.

Jugulam, M. & Shyam, C. 2019. Non-target-site resistance to herbicides: Recent developments. *Plants* 8(10): 417.

Kaushik, S., Inderjit, Streibig, J.C. & Cedergreen, N. 2007. Activities of mixtures of soil-applied herbicides with different molecular targets. *Pest Management Science* 62(11): 1092-1097.

Kim, S. & Vanden Born, W.H. 1997. Carbon allocation and translocation in chlorsulfuron-treated canola (*Brassica napus*). *Weed Science* 45: 466-469.

Kim, S. & Vanden Born, W.H. 1996. Chlorsulfuron decreases both assimilate export by source leaves and import by sink leaves in canola (*Brassica napus*) seedlings. *Pesticide Biochemistry and Physiology* 5: 141-148.

Kudsk, P. & Mathiassen, S.K. 2003. Joint action of amino acid biosynthesis-inhibiting herbicides. *Weed Research* 44: 313-322.

Lancaster, S., Jugulam, M. & Jones, J.F. 2021. *Herbicide Mode of Action*. Kansas State University. (C715). https://bookstore.ksre.ksu.edu/Item.aspx?catId=236&pubId=172

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

Martins, R.X., Vieira, L., Souza, J.A.C.R., Silva, M.G.F., Muniz, M.S., Souza, T., Queiroga, F.R., Machado, M.R.F., da Silva, P.M. & Farias, D. 2021. Exposure to 2,4-D herbicide induces hepatotoxicity in zebrafish larvae. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* 248: 109110.

Matzenbacher, F.O., Kalsing, A., Dalazan, G., Markus, C. & Merotto Jr., A. 2015. Antagonism is the predominant effect of herbicide mixture used for imidazoline-resistant barnyardgrass (*Echinochloa crus-galli*) control. *Planta Daninha* 33(3): 587-597.

Merritt, L.H., Ferguson, J.C., Brown-Johnson, A.E., Reynolds, D.B., Tseng, T-M. & Lowe, J.W. 2020. Reduced herbicide antagonism of grass weed control through Spray Application Technique. *Agronomy* 10(8): 1131.

Meyer, C.J., Norsworthy, J.K. & Kruger, G.R. 2020. Antagonism in mixtures of glufosinate + glyphosate and glufosinate + clethodim on grasses. *Weed Technology* 35(1): 12-21.

Ohio State University Extension. 2020. *Weed Control Guide for Ohio, Indiana, and Illinois*. Bulletin 789.

Ottis, B.V., Mattice, J.D. & Talbert, R.E. 2005. Determination of antagonism between cyhalofop-butyl and other rice (*Oryza sativa*) herbicides in barnyardgrass (*Echinochloa crus-galli*). *Journal of Agriculture and Food Chemistry* 53: 4064-4068.

Rao, V.S. 2002. *Principles of Weed Science*. 2nd ed. New Hampshire: Science Publishers.

Scherder, E.E., Talbert, R.E. & Lovelacc, M.I. 2005. Antagonism of cyhalofop grass activity by halosulfuron, triclopyr, and propanil. *Weed Technology* 19: 934-941.

Song, Y. 2013. Insight into the mode of action of 2,4-dichlorophenoxyacetic acid (2,4-D) as an herbicide. *Journal of Integrative Plant Biology* 56(2): 106-113.

Sørensen, H., Cedergreen, N., Skovgaard, I. & Streibig, J. 2007. An isobole – based statistical model and test for synergism/antagonism in binary mixture toxicity experiments. *Environmental and Ecological Statistics* 14: 383-397.

Tiwari, B., Kharwar, S. & Tiwari, D.N. 2019. Pesticides and rice agriculture. In *Cyanobacteria: From Basic Science to Application*, edited by Mishra, A.K., Tiwari, D.N. & Rai, A.N. Massachusetts: Academic Press. pp. 303-325.

Vølund, A. 1992. Dose response surface bioassay. In *XVIth International Biometric Conference,* Vol II. Hamilton, New Zealand. p. 249.

Willingham, S.D., Senseman, S.A., McCauley, G.N. & Chandler, J.M. 2008. Effect of temperature and propanil on penoxsulam efficacy, absorption, and translocation in alligatorweed (*Alternanthera philoxeroides*). *Weed Science* 56: 780-784.

Zhang, J., Hamill, A.S. & Weaver, S.E. 1995. Antagonism and synergism between herbicides: Trends from previous studies. *Weed Technology* 9: 86-90.

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