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Dose responses of selected weedy rice accessions, and commercial rice lines and varieties to bensulfuron-methyl

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ABSTRACT Dose responses of weed and crop species to herbicides form one of the basis of selectivity, delineating them into tolerant and susceptible types, species or varieties. A laboratory experiment was conducted to assess the differential response of weedy rice accessions, traditional rice varieties, and modern rice lines and varieties to bensulfuron-methyl. Dose response of weedy rice accessions and rice lines and varieties to the herbicide varied considerably with the rice line ACC 8474 being the most tolerant and weedy rice accession PA2 the most susceptible based on the respective seed germination scores of 99.7 and 38.9%. Based on the shoot length and at the dose up to 100 $\mu g\ g^{-1}$, rice lines ACC 4557 and ACC 8460 were most tolerant while the traditional rice variety PT and rice line ACC 4552 were most susceptible to bensulfuron- methyl. The respective I_{50} values of the tolerant rice lines were 51.9 and 28.8$\mu g\ g^{-1}$. The parallel I_{50} scores for the susceptible counterparts were 3.3 and 2.3 $\mu g\ g^{-1}$ respectively. Other tolerant rice lines include ACC 8460 and ACC 581 with the I_{50} respective values of 1.8 and 1.3. The I_{50} scores for the susceptible rice line ACC 1041 and weedy rice PAI were 9.2 x 10^{-3} and 1.2 x 10^{-2} $\mu g\ g^{-1}$, respectively.

ABSTRAK Tindak balas spesies-spesies rumpai and tanaman terhadap dos racun-racun herba merupakan salah satu asas pemilihan yang mengasingkan jenis, spesies dan varieti yang toleran atau peka. Untuk menilai perbezaan tindakbalas titisan-titisan padi angin, dan varieiti padi tradisional dan moden terhadap bensulfuron-methyl, satu percubaan telah dijalankan. Tindakbalas titisan-titisan padi angin, dan varieiti padi tradisional serta titisan dan varieti padi moden terhadap dos racun herba tersebut amat berbeza dimana titisan padi ACC 8474 adalah paling toleran sedangkan padi angin PA2 yang paling peka berdasarkan peratusan percambahan bihjibenih sebanyak 99.7 and 38.9%, masing-masingnya. Berdasarkan panjang pucuk dan pada dos sehingga 100 $\mu g\ g^{-1}$, titisan-titisan padi ACC 4557 dan ACC 8460 adalah paling toleran dan varieti padi tradisional rice PT dan titisan padi ACC 4552 paling peka terhadap bensulfuron- methyl. Nilai-nilai I_{50} titisan padi yang toleran adalah 51.9 dan 28.8 $\mu g\ g^{-1}$. Nilai-nilai I_{50} seiringan bagi titisan dan varieti yang peka adalah 3.3 dan 2.3 $\mu g\ g^{-1}$ masing-masingnya. Lain-lain titisan-titisan yang toleran termasuklah ACC 8460 dan ACC 581 dengan nilai I_{50} sebanyak 1.8 dan 1.3$\mu g\ g^{-1}$ masing-masingnya. Catitan-catitan I_{50} bagi titisan padi ACC 1041 dan padi angin PAI yang peka adalah masing-masingnya 9.2 x 10^{-3} dan 1.2 x 10^{-2} $\mu g\ g^{-1}$.

(*Oryza sativa* L., bensulfuron-methyl, herbicide)

INTRODUCTION

The Malaysian weedy rices (*Oryza sativa* L.) complex or *padi angin*, as they are known locally, is an aggregate of diverse, easy-shattering off-types, closely related to the cultivated varieties, evolving over the years with commercial varieties as possible parents [8]. These scrouges mimic cultivated commercial varieties, invading and infesting the rice ecosystems in Peninsular Malaysia since 1988.

Selective herbicidal control of weedy rice in the rice ecosystems is difficult because of their close biological and genetic affinities (Azmi, M. & Abdullah, M.Z., *pers. comms.* 2002). Consequently, the non-herbicide-based control regimes and methods, augmented with selective herbicide treatments and spot sprays provide the alternative means to combat these weedy rice scrouge populations especially at the seedling stage [2]. Bensulfuron-methyl is one of the herbicides used to control weeds in direct-seeded rice [3]. The herbicide inhibits acetolactate synthase (ALS), a key enzyme in biosynthetic pathway of branched-chain amino acids (isoleucine, leusine, and valine) in susceptible plants plant^{-1} [10].

The assessment of dose responses of weeds and crop plants to herbicide treatments can be made by indirect means through vegetative and reproductive growth measurements. These measurements include, *inter-alia,* shoot (fresh or dry) weights, grain or fruit yields, plant height and root lengths, shoot-to-root ratios, leaf area index (LAI), harvest index, and relative growth rates. Dose responses to herbicide treatments inhibiting photosynthesis and inhibition of target site enzymes can be assessed by means of CO_2 exchange [9]. Agronomist, agriculturists and farmers alike employ the visual impact assessment of injury inflicted on target species, and crops where applicable, upon herbicide application. One of the ways to assess the effect of herbicides on the growth of crops and the target weed species is through the I_{50} value. The I_{50} value is the concentration of herbicide inflicting 50% damage or growth reduction *vis-à-vis* the control. Weed species registering higher I_{50} value are considered more tolerant than those registering lower values. Others employed the GR_{50} and GR_{70} value to indicate the dosage of herbicide inflicting the respective 50 and 70% reductions in growth of tested weed or crop species [6,7].

The principal objective of this experiment was to assess the dose response of selected Malaysian weedy rice accessions, traditional rice varieties, and modern rice breeding lines and varieties towards bensulfuron-methyl.

MATERIALS AND METHODS

Seeds of twenty-nine different rice accessions were obtained from MARDI Research Station, Bertam, Penang. Seeds of two traditional rice varieties, namely Padi Tinggi (PT) and Padi Rendah (PR) were collected from rice fields in Papar, Sabah while two weedy rice accessions, PA1 and PA2 were sampled from the Sungei Burong granary, Selangor. These seeds were kept in a refrigerator at a temperature of *ca.* 5 ± 2 °C before use.

Experiments were conducted in a growth chamber (Model E8, Controlled Environment Ltd. Winnipeg, Manitoba, Canada) with the temperature regimes of 25 ±2 °C, humidity 99%, and 12 hours of light/day. Twenty seeds of each weedy rice accession, the traditional rice varieties, and modern rice lines and cultivars were sown in a petri-dish, previously-lined with 99 mm diameter Whatman No. 1 filter papers. Bensulfuron methyl (commercial grade) was dissolved in distilled water to obtain the needed concentrations of 0, 0.01 0.1, 1, 10, and 100 μg g^{-1}. Each petri-dish received 5 ml of the aliquots. Each treatment (= concentration) was replicated three times. The number of germinated seeds, shoot and root lengths of emerged weedy rice and rice seedlings were measured 8 days after treatment. About 5ml of distilled water were added into every petri-dish 2, 4, and 8 days after treatment.

The germination and seedling growth data were independently fitted into an exponential decay model with a lower asymptote ($y = a + be^{mlgx}$) or a linear model ($y = mlgx + c$), where y = shoot or root lengths, a, b are the regression coefficients, mglx, the rates or the concentration of bensulfuron. Then choice of the regression model depends on the model which fits the data better [5]. Regression analysis was performed by Microsoft Excel Program using the percentage of seedling growth (shoot and root lengths) against log of concentration. The analysis of variance was performed using the Microsoft Excel Program and the Null Hypotheses (NH) were tested using F test ($\alpha = 0.05$) [13] to determine significance with respect to differential dose response of weedy rice accessions, rice varieties and lines to the bensulfuron-methyl. Where applicable the I_{50} values of shoot and root lengths were further tested for significant difference by Tukey's HSD test [13].

RESULTS AND DISCUSSION

Seed germination prevailed in all weedy rice accessions and cultivated rice varieties and lines and concentrations of bensulfuron-methyl. Germination rates ranged from 91.3% to 93.1%, with the dose of 10 and 0.01 μg g^{-1} registering the highest and lowest germination rates, respectively. However, no significant difference was observed in seed germination of weedy rice accessions or cultivated rice varieties and lines subjected to the concentrations 0.01 0.1, 1, 10, and 100 μg g^{-1} of bensulfuron-methyl. Based on the NH that all varieties (concentrations) means are effectively equal requires that the group's mean square be compared with the residual mean square in an *F*-test [13] and these values were not significantly different from other. This fortifies the earlier contention that concentrations of bensulfuron-methyl employed in the experiment did not result in significant differential response among the accessions of weedy or varieties and lines of cultivated rice, based on the ANOVA. The hierarchical sequence of germination percentages based on the herbicide dose were as follows: 10μg g^{-1} (93.16%) > 0.1 μg g^{-1} (92.47%) > 100μg g^{-1} (92.41%) > 1.0 μg g^{-1} (91.84%) > 0.01μg g^{-1} (91.72%) > 0 μg g^{-1} (91.32%) **(Fig. 1).**

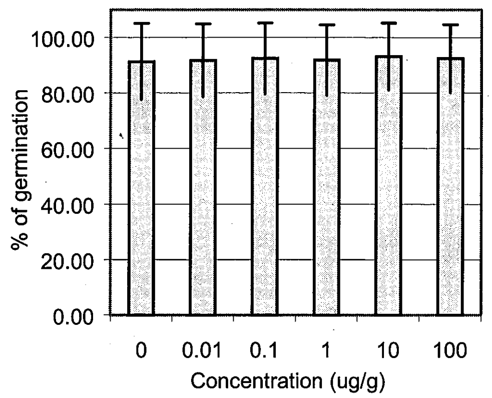

Figure 1. Mean percentages of germination of seeds of weedy rice accessions, cultivated rice varieties and breeding lines treated with different concentrations of bensulfuron-methyl. Bars represent ±1 S.D. value for each concentration of bensulfuron-methyl.

Significant differences were registered in seed germination among the weedy rice accessions, and rice varieties or lines following treatments with bensulfuron-methyl. The highest rate of germination was recorded for the rice line ACC 8474 (99.72%) and the lowest was by PA2 (38.89%) **(Fig. 2)**. Arguably, differences in the germination rates were due to the intrinsic character of the seeds themselves, and nothing to do with the herbicide exposure.

The study did clearly indicate that the germination rates were only being influenced by the different weedy rice accessions or varieties or lines or cultivated rice, but not the different concentrations used. Seeds still germinated, even at high concentrations (100 μg g^{-1}) although some died soon after they germinated.

Arguably, dose-dependent responses should prevail in the germination rates of weedy rice accessions, and varieties and lines of cultivated rice if bensulfuron-methyl influences seed germination. Germination rates should be higher at the lower concentrations and lower at the higher concentrations. Percentage of germination could then be plotted against concentration and the corresponding I_{50} values would be obtained. Since this was not the case, bensulfuron methyl (up to 100 μg g^{-1}) did not have any influence in germination rate. Therefore, the germination rate is not a sensitive parameter to delineate weedy rice accessions, varieties or lines of cultivated rice subjected to bensulfuron-methyl treatment either as tolerant or susceptible entities towards the herbicide.

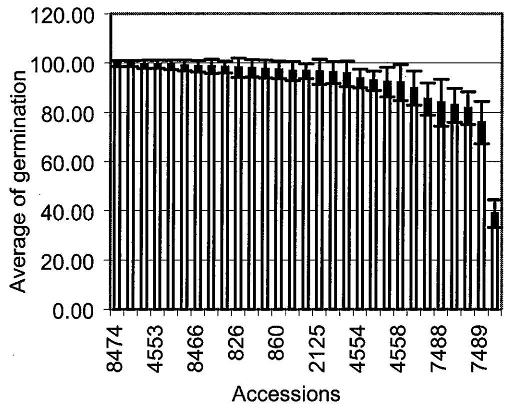

Figure 2. Mean percentages of germination of seeds of weedy rice accessions, cultivated rice varieties and breeding lines irrespective of the concentrations of bensulfuron-methyl. Bars represent ±1 S.D. value for each accession of weedy rice, cultivated rice varieties and breeding lines.

One method to assess the effect of herbicides on the growth of crops and the target weed species is through the I_{50} value. The I_{50} value is the concentration of herbicide inflicting 50% damage or growth reduction *vis-à-vis* the control. Weed and crop species or cultivars registering higher I_{50} value are considered more tolerant than those registering lower values.

Weedy rice accessions and cultivated rice cultivars or lines exposed to bensulfuron-methyl registered significant difference in shoot growth shoot growth prevailed among weedy rice accessions, and the cultivated rice cultivars and breeding lines as exemplified by the calculated the I_{50} values for shoot lengths. The resultant I_{50} values for shoot lengths generated seven homogeneous groups prevailed **(Table 1).**

Accessions sharing at least one common letter were considered in the same homogenous group, which means that no significant difference prevailed between them based on I_{50} values. In one group, the I_{50} values for shoot lengths ranged from as low as 2.2648 µg g^{-1} for the rice breeding line ACC 4552 to as high as 15.4221 µg g^{-1} or rice line ACC 4558, but being in the same homogeneous group they were not significantly different from each other. However, the latter rice line was relatively more tolerant to bensulfuron-methyl than the former rice line. The second homogeneous group registered I_{50} values ranging from 4.8658 µg g^{-1} (rice line ACC 4559) to 18.0164 µg g^{-1} (rice line ACC 826). By the same argument rice line ACC 826 was more tolerant to the herbicide than rice line ACC 4559. In the same vein, the I_{50} values for shoot lengths for the other five homogeneous groups, were relatively higher than the previous two homogeneous groups, rendering them as relatively more tolerant to bensulfuron-methyl. The most tolerant rice line was ACC 4557 with the I_{50} value of 51.9137 µg g^{-1}.

The hierarchical sequence of tolerance to bensulfuron-methyl based on I_{50} values for ten most tolerant entities was ACC 4557 > ACC 8460 > ACC 2618 > ACC 8458 > PA2 > ACC 826 > ACC 4558 > ACC 581 > ACC 839 > ACC 2123. It appeared that the cultivated rice var. MR 84, the traditional var. PT and PR, the weedy rice accession PA1, and rice breeding lines ACC 1041, ACC 1478, ACC 2125, ACC 4552, ACC 4556 and ACC 4559 were less tolerant to bensulfuron-methyl based on shoot length and the calculated I_{50} values **(Fig. 3)**

Table 1. Homogeneous subsets for I_{50} values of root and shoot lengths of weedy rice accessions, cultivated rice cultivars and breeding lines subjected to bensulfuron-methyl treatment. Means for groups in homogeneous subsets are displayed *.

Cultivated rice varieties, breeding lines & weedy rice accessions	I_{50} values (µg g^{-1})	
	Root lengths	Shoot length
ACC 1041	0.00916 a	2.2648 a
PA1	0.01180 a	3.3324 a
ACC 4551	0.02600 a	3.4849 a
PT	0.02700 a	3.7965 a
ACC 4556	0.02760 a	3.8688 a
ACC 2618	0.02870 a	4.0126 a
ACC 860	0.02870 a	4.8658 ab
ACC 4557	0.03550 a	5.4486 ab
ACC 1478	0.03920 a	5.6235 ab
ACC 4553	0.04420 a	6.0855 ab
ACC 2126	0.04580 a	6.3707 ab
MR84	0.04780 a	6.5438 ab
PA2	0.04920 a	6.8933 ab
ACC 2125	0.05870 a	7.3579 ab
ACC 7488	0.06270 a	7.5441 ab
ACC 4552	0.06420 a	8.0744 ab
PR	0.06470 a	8.4385 ab
ACC 4554	0.06890 a	8.6982 ab
ACC 7489	0.06910 a	9.9265 ab
ACC 2123	0.08040 a	10.1085 ab
ACC 4558	0.08190 a	10.4254 ab
ACC 826	0.08300 a	14.5281 ab
ACC 4559	0.08830 a	15.4221 ab
ACC 839	0.15700 a	18.0164 bcdef
ACC 8466	0.23130 a	18.3637 cdef
ACC 8474	0.25970 a	20.9354 def
ACC 8458	0.36210 a	20.0147 ef
ACC 581	1.25250 b	28.8912 f
ACC 8460	1.84860 c	51.9137 g

Root lengths: Subset for α = 0.05 (a = 0. 105; b = 1.00; c = 1.00); Shoot lengths: Subset for α = 0.05 (a = 0. 055 b = 0.053; c = 0.057; d = 0.51; e = 0.063; f = 0.082; g = 1.00); * Values followed by common lower case letters are not significantly different at $p < 0.05$ (Tukey's HSD tests).

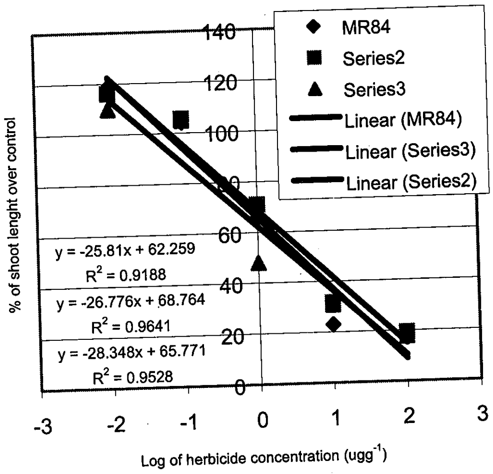

Fig. 3. The relationships between mean shoot lengths of emerged seedlings of weedy rice accessions, cultivated rice varieties and breeding lines as the percentages of control and concentrations of bensulfuron-methyl.

There was a strong negative linear relationship between the shoot length of weedy and cultivated rices (based on I_{50} values), and the concentrations of bensulfuron-methyl, with R^2 values ranging from 91.88% to 95.28%. This fortifies the earlier arguments that dose-mediated differences in shoot lengths occurred among weedy rice accessions, and the cultivated rice cultivars and breeding lines following exposures to bensulfuron-methyl **(Fig. 4)**.

The root growth of weedy rice accessions, cultivated rice cultivars and breeding lines was affected when exposed to bensulfuron-methyl. The effects were both dose- and cultivars/ breeding lines/ accessions-mediated. This argument was fortified when the root length data was fitted into an exponential decay model with a lower asymptote ($y = a + be^{mlgx}$) depicting strong negatively exponential relationships prevailed between root lengths (as percentage of the control) of weedy rice accessions, cultivated rice varieties and breeding lines and concentrations of bensulfuron-methyl to which these plants were exposed (with R^2 values ranging from 94.02% to 97.73%) **(Fig. 5)**.

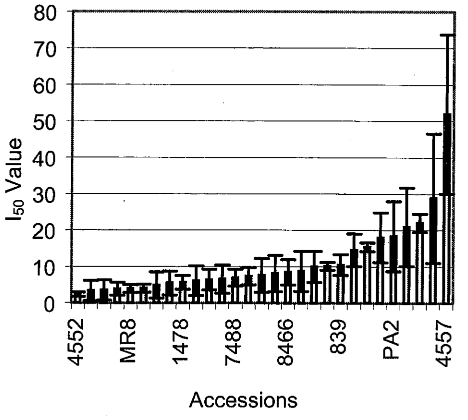

Fig. 4. The I_{50} values of mean shoot lengths of emerged seedlings of weedy rice accessions, cultivated rice varieties and breeding lines exposed to different concentrations of bensulfuron-methyl. Bars represent ±1 S.D. value for each accession of weedy rice, cultivated rice varieties and breeding lines.

Based on the analysis of I_{50} values of root lengths, there were only three homogeneous groups with rice breeding lines ACC 581 and ACC 8640 being singled out as the relatively more tolerant entities *vis-à-vis* the other 27 entities (Table 1). Again as in the case of the analysis based on the I_{50} values of shoot lengths, the cultivated rice cultivars MR84 and PT and PR together with scores of rice breeding lines and the weedy rice accessions PA1 and PA2 were less tolerant to bensulfuron-methyl treatment, registering lower I_{50} values than ACC 8640 **(Fig. 6)**.

Overall, albeit inconsistencies, the I_{50} values of shoot lengths of weedy rice accessions, and the cultivated rice cultivars and breeding lines were higher than those registered for root lengths. It follows that the shoots of weedy rice accessions, and the cultivated rice cultivars and breeding lines was more tolerant to bensulfuron-methyl exposure than the roots. Again there were those cultivars/ breeding lines/ accessions-mediated differences in their reactions to exposures to bensulfuron-methyl. Differential sensitivity of roots *vis-à-vis* shoots to herbicide exposures have been reported [1, 4, 11]. Baki [4] cited such greater sensitivity of roots of wheat compared to shoots to ethofumesate due to the fact that the radicles were emerged earlier and came into close proximity with and exposed to the herbicide. The fact that the uptake of bensulfuron-methyl by the rice plants can occur through absorption by the shoots and roots make the latter organ more likely to experience the phytotoxic effect of the herbicide, earlier than the shoots.

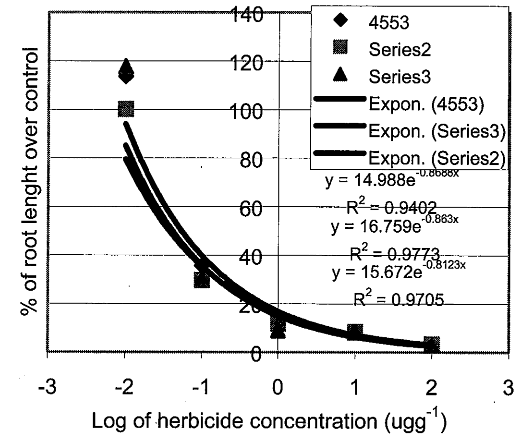

Fig. 5. The relationships between mean root lengths of emerged seedlings of weedy rice accessions, cultivated rice varieties and breeding lines as the percentages of control and concentrations of bensulfuron-methyl.

No firm conclusions can be ascertained from this study to delineate weedy rice accessions, and the cultivated rice cultivars and breeding lines as either tolerant or susceptible to bensulfuron-methyl. For a particular weed species or crop cultivars or species to be classified as either tolerant or susceptible to a particular herbicide, we should consider the sensitivity of both roots and shoots within the context of whole plant physiology. Nevertheless, ACC 8460 was relatively more tolerant to bensulfuron-methyl than the cultivated rice var. MR 84, PT or PR, and other rice breeding lines and weedy rice accessions PA1 and PA2, based on the I_{50} values of the shoot and root lengths. It would interesting to undertake studies at the molecular level to determine the inherent factors responsible for the apparent tolerance of ACC 8460 to bensulfuron-methyl.

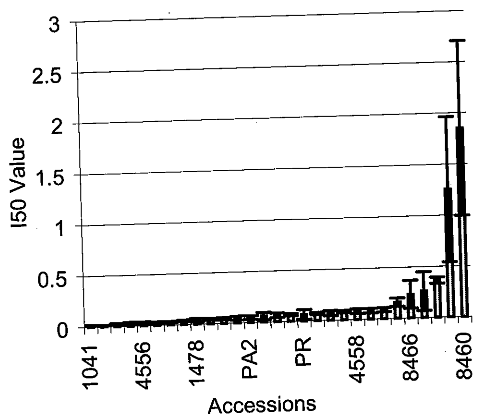

Fig. 6. The I_{50} values of mean root lengths of emerged seedlings of weedy rice accessions, cultivated rice varieties and breeding lines exposed to different concentrations of bensulfuron-methyl. Bars represent ±1 S.D. value for each accession of weedy rice, cultivated rice varieties and breeding lines.

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