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ARTICLE

Evaluation of Pre-Emergence and Post-Emergence Herbicides for Weed Management in *Miscanthus sacchariflorus* and *Miscanthus sinensis*

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ABSTRACT

Miscanthus, is a promising bioenergy crop, considered superior to other bioenergy crops because of its higher water and nutrient use efficiency, cold tolerance, and higher production of biomass. Broadleaf weeds and grass weeds, cause major problems in the *Miscanthus* field. A field experiment was conducted in 2018 and 2019, to assess the effects of pre-emergence (alachlor and *napropamide*) and post-emergence herbicides (nicosulfuron, dicamba, bentazon, and glufosinate ammonium) on broadleaf and grass weeds in *M. sinensis* and *M. sacchariflorus* fields. The weed control efficiency and phytotoxicity of pre- and post-emergence herbicides were evaluated at 30 days after treatment (DAT) and compared to those of the control plots. The results showed wide variations in the susceptibility of the weed species to the treated herbicides. Treatment with nicosulfuron 40 g.a.i.ha^{-1} provided the most effective overall weed control (with 10% visual injury), without affecting the height and biomass of neither *Miscanthus* species in the field. Post-emergence herbicides such as glufosinate ammonium 400 g.a.i.ha^{-1} and dicamba 482 g.a.i.ha^{-1} were effective and inhibited the growth and density of the majority of weeds to a 100%; however, they showed significant phytotoxicity (toxicity scale of 1–10) to both species of *Miscanthus*. The application of glufosinate ammonium caused severe injuries to the foliar region (90% visual injury) of both *Miscanthus sps*. Comparatively, *M. sinensis* showed a slightly higher tolerance to the herbicides nicosulfuron, bentazon and napropamide with 10% visual injury at the recommended dose than *M. sacchariflorus*. The present study clearly showed that infestation of broadleaf and grass weeds in *Miscanthus* fields can cause significant damage to the growth and biomass of *Miscanthus* and applying pre-emergence and post-emergence herbicides effectively controls the high infestation of these weeds.

KEYWORDS

miscanthus; herbicides; weeds; biomass; chlorophyll; visual injury

1 Introduction

Miscanthus sacchariflorus (Maxim.) Hack., a rhizomatous C4 grass species, also called as silver banner grass has received considerable attention as a potential bioenergy crop because of its high biomass production [1]. *M. sacchariflorus* has a broad geographic range (up to 50° N in Eastern Russia) [2–4]. It has also been investigated as a feedstock, for ensilage, large-scale paper production [5–7] and higher bioremediation capacity of heavy metals [7]. The vigorous rhizomes of *M. sacchariflorus* are useful in preventing soil erosion [8]. Some ornamental accessions of *M. sacchariflorus* are sold in nurseries [9] and the inflorescence is used as a winter bouquet [10]. Its annual biomass production is higher than any other capable perennial bioenergy crop [7]. It is fairly tolerant to a wide range of environmental conditions and propagates in varied water and nitrogen contents with minimal input [5,11–13]. *Miscanthus sinensis* Andersson (Poaceae), a C4 perennial bioenergy crop and also called Chinese silver grass is native to Asia, primarily in China, Korea and Japan, and grows in a wide range of environments and altitudes (from sea level to 2500 m) [14,15]. They originate in a region ranging as far north as Sakhalin and as far south as the Indochinese peninsula [4]. It is suitable for thatching, animal feeds and the production of compost, sold as an ornamental plant in nurseries, and as raw material for industrial use for the production of paper pulp [15].

Both species differ morphologically, phylogenetically, and ecologically [14,15]. Compared to *M. sacchariflorus*, the accessions of *M. sinensis* are not an aggressive competitor at the early growing stages due to a weak rhizome spread [12,16]. They occasionally grow in the same area forming a sympatric population from ~29° N to 43° N [3], and are considered a promising renewable biomass resource [14,17]. *M. sinensis* usually has a caespitose form, prefers aerobic soils and grows mostly in hills, and seminatural grasslands on sloppy lands [18] and is typically diploid with a monoploid genome size of about 2.5–2.8 pg. *M. sinensis* is an early colonizer [15,19] with great cold tolerance and yield in cold regions [15,20,21], whereas *M. sacchariflorus*, which has a rhizomatous habit, prefers to grow under wetter soil conditions, in riparian environments, on river banks, mostly on mesic, viscid, sandy soil formed by floodings, and dominates fertile lowland habitats on alluvial plains [14,22,23]. *M. sacchariflorus* can be diploid or tetraploid with a monoploid genome size of about 2.1–2.3 pg [22,24,25]. The population of *M. sacchariflorus* from China and Korea are generally diploid [26,27], whereas its accessions from Japan are predominantly tetraploid [28]. *M. sacchariflorus* flowers less readily than *M. sinensis* [11,29,30] and produce a lower number of stems per plant (average 26 stems during 3rd year of cultivation) than *M. sinensis* (average 49 stems) [31]. Climate change is posing a serious threat to the global environment. Fossil fuels emit a huge amount of polluting gases contributing to an increased greenhouse effect, triggering environmental pollution and climate change [32]. Due to the fast depletion of fossil reserves and the rapid growth of the world population, the demand for bioenergy is expected to increase by 10 fold by 2050 in both energy and transport sectors [33,34]. Therefore, the use of renewable energy (bioenergy) is inevitable and must be carried out in a sustainable way to reduce the environmental impacts [35]. *Miscanthus* is characterized by a rapid growth, and thus a high productivity, that can be grown with a low input of nutrients and water consumption, and may confer a high capacity to sequester carbon in the soil that enables to mitigate the global warming by reducing greenhouse gas emissions [35].

Weeds are a major threat to the growth of bioenergy crops as they constantly compete with them for nutrients, space, sunlight, and moisture. Some important broadleaf weeds and grass weeds that commonly grow in *Miscanthus* fields contribute to the loss of biomass yield of the crop [36]. The growth rate of Miscanthus, at the beginning of the establishment year, is slow and sensitive to weed interference [36], which reduces its ability to compete with weeds for growth. Previous studies on *Miscanthus* revealed difficulties in its establishment due to prolonged and various degrees of dormancy, cold stress, and water deficiency during growth [36]. These plants take considerable time to fully develop the canopy and fill the wide gap between seedlings [36]. The bare land between the seedlings resulting from low densities allows weeds to grow and compete with the young shoots of *Miscanthus*. In the absence of weed control, approximately a 97% reduction in *M. sacchariflorus* biomass yield was reported under field conditions by grass weeds such as *Echinochloa crus-galli, Digitaria sanguinalis, Setaria viridis*, and broadleaf weeds including *Stellaria aquatic, Amaranthus lividus, Galinsoga ciliate, Calystegia sepium, Chenopodium album, Acalypha australis, Viola mandshurica, Rorippa palustris, Ipomoea hederacea* and *Trifolium repens* [36]. The higher rate of competition of *Miscanthus* plants with weeds was observed during the first year of growth of *Miscanthus* in the field, and it was reduced in the subsequent years [37–41]. As the rate of growth of these weeds is higher than that of *Miscanthus*, it has a detrimental effect on the growth of the crop due to the deprivation of nutrients, space, light, and moisture. In the absence of a proper weed control system, young shoots and canopy die, show poor growth, or produce less biomass. Therefore, controlling or suppressing weed growth at or before the critical point of weed growth not only minimizes yield loss but also protects plants from diseases. Moreover, several previous studies indicated an improvement in the biomass of bioenergy crops by using pre-emergence or post-emergence herbicides [42,43].

Previous work on weed control on these perennial crops has mainly focused on *Miscanthus* × *giganteus*, which is widely cultivated in the UK, European countries and USA for biomass production [1,44]. Several pre-emergence and post-emergence herbicides have been tested for use in the early growth of *Miscanthus* × *giganteus* [1,40,41,44]. The herbicides such as pendimethalin, S-metolachlor, and isoxaflutole, applied at planting, and POST herbicides, such as 2,4-D (1060 g ai ha^{-1}), bromoxynil (840 g ai ha^{-1}), and dicamba (560 g ai ha^{-1}) were found to be effective on controlling the weeds and safety for *Miscanthus* × *giganteus* [44]. Other herbicides such as atrazine were effective in controlling a wide range of broadleaves weeds, whereas, acetochlor was less effective in controlling the emerged weeds (68%) in a *Miscanthus* × *giganteus* field [45]. Recently, interest in *M. sinensis* and *M. sacchariflorus* has been increased for use as biomass energy crops in Europe, North America, and South Korea for productivity trials [44,46,47]. However, very few field studies have been conducted on weed control systems for *M. sacchariflorus* using pre-and post-emergence herbicides [36]. Also, no study has comparatively evaluated herbicide-based weed management systems for *M. sinensis* and *M. sacchariflorus* under field conditions. This research hypothesized that (1) all the tested herbicides will reduce the emergence of weeds, sustain toxic effects and lowers the survivability of the distinct weed community of a *M. sinensis* and *M. sacchariflorus* field, (2) mulches pretreated with pre-emergence herbicides would impact early *Miscanthus* growth and development but have no adverse effect on yield, and (3) mulches pretreated with the pre-emergence and post-emergence herbicides would provide effective weed control by reducing the density and biomass (FW and DW) of weeds. Therefore, the main objective of the present study was to determine the effectiveness of specific pre-and post-emergence herbicides to control weeds. In addition, we investigated the safety of pre-and post-emergence applications of herbicides on *M. sinensis* and *M. sacchariflorus* under field conditions, and also evaluated the phytotoxicity of herbicides on *M. sinensis* and *M. sacchariflorus*.

2 Materials and Methods

The bioefficacy of pre-and post-emergence herbicides was evaluated in the *M. sinensis* and *M. sacchariflorus* fields at the Kangwon National University, South Korea, (37°56009.96″ N; 127°46055.21″ E; Fig. 1) at an average altitude of 100 m for two years (during the years 2018, 2019). Two different plots were used to study the effect of herbicides on the two species of *Miscanthus* and weeds. Meteorological data were obtained from the database of the Korea Meteorological Administration. The average temperature of the cultivated field ranged from −9.9°C (January) to 29.8°C (August). Soil samples in each plot were collected in April 2018 after plot construction by randomly selecting three sampling points. Briefly, 10 g of soil samples (10–20 cm) were gathered using a sterilized spatula. The collected soil samples were placed in individual whirl-pack bags and immediately transported to laboratory on ice at 4°C until use. Before the analysis, soil samples were air-dried, milled and sieved using a 2 mm sieve. The pH value and the characteristics of the soil samples were determined in the Department of Biological Environment, Kangwon National University, Chuncheon 24341, South Korea by following the methods described by Kim et al. [48].

Figure 1: Location of experimental plots for the weed management in *M. sinensis* and *M. sacchariflorus* field

The soil of the experimental field had a sandy loam texture. The *M. sinensis* and *M. sacchariflorus* experimental field was irrigated during the initial year of establishment on a regular basis. The research field was located in a temperate monsoon climate, with a wet and humid summer. In the first set of experiments, the harvested rhizomes of *M. sinensis* and *M. sacchariflorus* were planted 8 cm deep in the field (100 m × 100 m in size) in the year 2015. The same *M. sinensis* and *M. sacchariflorus* accessions were maintained at the experimental field until 2017 and transferred to the plots for herbicides treatment. There were six herbicide treatments and a control (without herbicide treatments) in each plot. Each experimental plot consisted of six rows of 10 m in length, spaced 1 m apart. Each row contained eight plants (in a 10 m length row), with an inter-plant distance of 1.25 m. The treatments consisted of two pre-emergence herbicides and four post-emergence herbicides (applied in recommended standard doses). Pre-emergence herbicides (alachlor and napropamide) were sprayed a week after planting *M. sinensis* and *M. sacchariflorus* in the field. Post-emergence herbicides (nicosulfuron, dicamba, bentazon, and glufosinate ammonium) were sprayed 30 days after planting the *Miscanthus* spp. (Table 1). None treated *Miscanthus* spp. in the areas were considered as the control for evaluating injury. The field experiments were a randomized block design, each with three replications. All the herbicides were applied within two days of planting *Miscanthus* plants. Both pre- and post-emergence herbicides were applied using a pressurized backpack sprayer equipped with a flat fan nozzle fitted to a knapsack stainless steel sprayer (MT-009, Taizhou, Zhejiang, China) with the capacity of 18 L at 1.0 MPa. The amount of herbicide sprayed on the Miscanthus spp. was based on the recommended rate. The fresh weight (FW) and dry weight (DW) of the weeds were measured at 30 DAT, after the post-emergence herbicide treatment. Weed density and measurement of FW and DW of weed samples were taken from three randomly selected spots with the help of a quadrat (0.5 m^2). After 30 days, all weeds were collected and classified into broadleaf and grass weeds. Different weed species (both broadleaf species and grasses) were counted, and the FW of each weed plant was measured. To measure the DW, the collected weeds were dried at 90°C in an oven for 24 h. Weed densities in both the herbicide-treated and control plots were recorded 30 days after planting of *Miscanthus* spp. in the field.

2.1 Density, FW, and DW of Weeds

The number of weed species that emerged in the *M. sinensis* and *M. sacchariflorus* fields was counted at 30 DAT using a 0.5 m^2 quadrat. The counted weeds were harvested and placed in paper envelopes, and the FW was recorded immediately using an electronic balance. The weeds were dried at 90°C in an oven for 24 h, and the DW of the weeds was recorded.

Table 1: Pre-emergence and post-emergence herbicides tested for phytotoxicity and weed control in the *Miscanthus* spp. field

Trade name	Formula (% of a.i.)2	Name of herbicide	Mode of action	Standard dose (g.a.i/ha)1	Manufacturer
Onehope	SC (4%)	Nicosulfuron (C15H18N6O6S)	Acetolactate synthase (ALS)	40	Hankook Sam Gong Co., Ltd., South Korea
Banvel	SL (48.2%)	Dicamba (C8H6Cl2O3)	Auxin	482	Sungbo Chemical Co., Ltd., South Korea
Basagram	SL (40%)	Bentazon (C10H12N2O3S)	Photosystem II (PSII)	1200	Sungbo Chemical Co., Ltd., Kyungi Do, South Korea
Synster	SN	Glufosinate ammonium (C5H15N2O4P)	Glutamine synthetase	400	KyungNong Co., Ltd., South Korea
KyungNong	EC (43.7%)	Alachlor (C14H20ClNO2)	Cell division	951	KyungNong Co., Ltd., South Korea
Debranolgold	SC (21.8%)	Napropamide (C17H21NO2)	Cell division	1120	Kyung Nong Co., Ltd., Seoul, South Korea

Note: [1]g.a.i/ha: Grams of active ingredient per hectare. [2]SL; Suspension concentration, SC; Soluble concentration, SN; Solution, EC; emulsifiable concentration.

2.2 Scoring for Herbicide Tolerance of M. sinensis and M. sacchariflorus

In the second set of experiments, fifty *M. sinensis* and fifty *M. sacchariflorus* were planted in a plot of size 100 m × 100 m and used for screening and assessing the phytotoxicity of herbicides. After the screening, herbicide-tolerant, partially tolerant, sensitive, and highly sensitive *Miscanthus* were separated and characterized to assess the effect of herbicides on morphological traits and biomass yield. The degree of damage by the treated herbicides was evaluated on a scale of 1 (No visible leaf injury, healthy tissues) to 10 (Damage to 100% of plants) (Table 2). Phytotoxicity of the sprayed herbicides on the *M. sinensis* and *M. sacchariflorus* was evaluated by observing symptoms such as chlorosis, epinasty, hyponasty, necrosis, stunting, wilting, death or no phytotoxicity at 30 days after treatment (DAT).

Table 2: Qualitative assessment of herbicide injury in *M. sinensis* and *M. sacchariflorus*

Scale	Injury (%)	Effects on weeds
1	0	No visible leaf injury, healthy tissues
2	1–10	Mild foliar damage, some stunting
3	11–30	Stunting growth and leaf discoloration (mild yellowing)
4	31–49	Distinct yellowing or browning of leaves but not persistent
5	50	Permanent damage but a higher possibility of recovery
6	51–70	Higher rate of injury and recovery doubtful
7	71–80	Near severe injury and no possibility of recovery
8	81–90	Severe injury and only a few plants survived
9	91–99	Very severe damage to plants and loss of plants
10	100	Damage to 100% of plants and total destruction of plants

2.3 Effect of Pre- and Post-Emergence Herbicides on Morphological Traits and Biomass of M. sinensis and M. sacchariflorus

The effect of pre- and post-emergence herbicide treatments on the morphological traits of *M. sinensis* and *M. sacchariflorus* was measured and compared with the *Miscanthus* spp. grown as a control. Six random plants from each *Miscanthus* spp. were taken to measure plant height, DW, and FW. The plant height of all the treated and control plants was taken from the base of the plant to the tip of the main shoot at the time of maturity. To measure the DW, collected herbicide-treated *Miscanthus* shoots were dried at 90°C in an oven for 24 h.

2.4 Relative Chlorophyll Content

The relative chlorophyll content of the control and treated *M. sinensis* and *M. sacchariflorus* was measured using a chlorophyll meter SPAD-520 (Minolta Co., Ltd., Osaka, Japan). Data were collected from ten healthy leaves. Measurements were made on a sunny day (at 1 PM). Leaves were selected from five different plants from each *Miscanthus* plot.

2.5 Statistical Analysis

The number of weeds, fresh weight and dry weight of weeds, height, visual injuries, fresh weight, and dry weight of *Miscanthus* species were collected during the vegetative season. Weeds were collected from the area of 1 m^2 after the herbicide treatment, 30 days after the last herbicide treatment. They were then separated according to species and counted and weighed immediately. The collection of the above-ground *Miscanthus* was carried out in the month of November 2018 and 2019. Normal distribution and homogeneity of variances of the collected data were assessed. The average of two years (2018 and 2019) data were subjected to Two-way ANOVA analysis, and significant differences between the means were assessed using Duncan's multiple range test at a significance level of $p < 0.05$ using SPSS version 20 (SPSS, 2011). All the linear correlation analysis was subsequently established to obtain a correlation coefficient between various parameters and the p-value for the significance of the correlation, using the EXCEL extension XLSTAT software (Version, 2021.2.2) at $p < 0.05$. Relationships within and between the parameters were determined by Pearson correlation analysis.

3 Results

3.1 Effect of Herbicide Treatments on Density, FW, and DW of Weeds in the M. sinensis Growing Field

The efficacy of pre-emergence and post-emergence herbicides on the various weeds species was assessed visually at 30 DAT. The major weeds recorded in the *M. sinensis* and *M. sacchariflorus* fields were *Digitaria ciliaris* (Retz.) Koeler, *Erigeron canadensis* L., *Chenopodium album* L., *Alopecurus aequalis* Sobol., *Calystegia sepium* L., *Ixeris dentate* Thunb. Ex Thunb.*, Setaria viridis* L., *Oenothera biennis* L., *Capsella bursa-pastoris* (L.) Medik., *Trifolium repens* L., *Agropyron tsukushiense* var. transiens (Hack.), *Rumex crispus* L., and *Artemisia princeps* Pamp. Different pre- and post-emergence herbicide treatments had significant influences on the FW and DW of weeds compared to the control plots. All the treated herbicides inhibited the growth, reduced the FW and DW, and the density of weed species. The density and FW of both broadleaf and grass weeds decreased more in the second year of weed management (2019).

Pre-emergence herbicides, including napropamide (21.8% of a.i.) and alachlor (43.7% of a.i.), displayed a higher rate of injuries to the foliar part of the broadleaf and grass weed species at the recommended dose, resulting in a reduced weed density, FW, and DW after 30 DAT with minimal negative effects on the growth of *M. sinensis* plants in the field after 30 DAT in both 2018 and 2019 (Table 3). Treatment of napropamide reduced the FW and DW of the emerged broadleaf weeds *Chenopodium album* var. *centrorubrum* (FW: 1.2 g, DW: 0.4 g), *Oenothera* biennis (FW: 2.0 g, DW: 1.00 g), and *Plantago asiatica* (FW: 16.5 g, DW: 2.0 g), and the grass weed *Digitaria ciliaris* (FW: 8.0 g, DW: 2.53 g). Similarly, the broadleaf weeds *O. biennis*, and *Erigeron canadensis* L. emerged in the alachlor (43.7% of a.i.) treated *M. sinensis* field (Table S1). Application of post-emergence herbicides such as Dicamba and glufosinate ammonium showed 100% control of grass weeds such as *Setaria viridis, Digitaria ciliarie, Agropyron tsukushiense* during both weed management years (2018 and 2019). Treatment with these herbicides completely inhibited the growth of the broadleaf weeds including *Oenothera biennis*, *Chenopodium album A. princeps, A. tsukushiense*, and *B. frondosa* (Tables S2–S4). However, the emergence of some grass weeds in the *Miscanthus* field indicated that a single application of these herbicides is not enough to restrict the growth of weeds due to insufficient injuries to the foliar part of them, and weeds may re-emerge later in the season and cause *M. sinensis* biomass reduction.

Table 3: Effect of pre-emergence and post-emergence herbicides on the density, fresh weight, and dry weight of weed species in *M. sinensis* growing field in the years 2018 and 2019

Time of application	Herbicides	Density (plants/m^2)	FW (g)	DW (g)	Density (plants/m^2)	FW (g)	DW (g)
		2018			2019		
	Control	169.0^e	1470.0^g	491.2^f	111.0^d	1292.0^f	353.9^f
Pre-emergence	Napropamide	10.0^b	27.7^c	5.9^b	8.0^b	25.7^c	7.4^c
	Alachlor	13.0^c	112.0^e	51.0^e	3.0^a	22.2^c	8.8^c
Post-emergence	Bentazon	9.0^b	49.0^d	15.5^d	4.0^{ab}	40.0^d	16.5^e
	Nicosulfuron	25.0^d	127.0^f	16.5^d	11.0^c	73.4^e	12.1^d
	Dicamba	6.0^a	20.5^b	10.4^c	2.0^a	5.0^a	1.9^a
	Glufosinate ammonium	5.0^a	14.5^a	3.5^a	3.0^a	8.5^b	3.0^b

Data having the same letter in a column did not differ significantly according to Duncan's multiple comparison test ($p < 0.05$).

3.2 *Effect of Herbicides Treatments on Density, FW, and DW of Weeds in M. sacchariflorus Growing Field*

Application of pre-emergence herbicides and post-emergence herbicides showed a wide range of effects on the growth, density, FW, and DW of weed species (Table 4). In the present study, pre-emergence herbicide treatment suppressed weeds but was not effective in controlling all weed species (Table 4) that emerged under field conditions. Both the pre-emergence herbicides that were tested showed great efficacy in controlling broadleaf and grass weeds and significantly reduced the density of weed species that emerged in the *M. sacchariflorus* field. Application of pre-emergence herbicides significantly reduced the FW, DW, and density of major broadleaf and grass weeds to various degrees. Treatment with pre-emergence herbicides reduced weed density, FW and DW of *Trifolium repens* L., *Agropyron tsukushiense* Hack., *Rumex crispus* L., *Artemisia princeps* Pamp., *Taraxacum platycarpus* H., and *I. dentata* to 100% (Table S5). Treatment of napropamide incompletely suppressed the growth of *Oenothera biennis* (FW: 6.5 g, DW: 2.2 g), *Digitaria ciliaris* (FW: 3.2, FW: 0.9 g), and *C. album* (FW: 4.0 g, DW: 1.3 g) in 2018 and this trend continued in 2019. Comparatively, alachlor was more effective in controlling both broadleaf and grass weeds in the *M. sacchariflorus* field when compared to napropamide at the recommended dose of treatment. Overall the efficacy of pre-emergence herbicides was higher in 2019 than in 2018 in *M. sacchariflorus* growing field (Tables S5–S9).

Table 4: Effect of pre-emergence and post-emergence herbicides on the density, fresh weight and dry weight of weed species in *M. sacchariflorus* growing field in the years 2018 and 2019

Time of application	Herbicides	Density (plants/m^2)	FW (g)	DW (g)	Density (plants/m^2)	FW (g)	DW (g)
		2018			2019		
	Control	151.0^d	2116.8^g	620.7^d	110.0^e	1119.4^f	338.2^e
Pre-emergence	Napropamide	16.0^c	27.2^d	7.4^b	9.0^c	17.2^c	4.2^b
	Alachlor	14.0^c	30.0^e	11.0^c	6.0^b	17.0^c	5.5^b
Post-emergence	Bentazon	3.0^{ab}	10.5^b	3.0^a	9.0^c	71.0^d	11.5^c
	Nicosulfuron	15.0^c	94.5^f	29.5^d	13.0^d	110.5^e	23.5^d
	Dicamba	6.0^b	19.5^c	10.0^c	2.0^a	5.0^b	1.0^a
	Glufosinate ammonium	2.0^a	7.5^a	2.0^a	1.0^a	2.5^a	0.9^a

Note: Data having the same letter in a column did not differ significantly according to Duncan's multiple comparison test ($P < 0.05$).

Application of post-emergence herbicides showed great efficacy in controlling broadleaf and grass weeds in the *M. sacchariflorus* field (Table 4). The majority of the broadleaf weeds that emerged in the *M. sacchariflorus* field were inhibited to 100% by the treatment of post-emergence herbicides. The highest FW and DW of weeds were recorded in the nicosulfuron, which was statistically superior to all the other treatments. Comparatively, treatment with dicamba and glufosinate ammonium reduced the majority of the weed species at the recommended dose. The efficacy of these herbicides for the inhibition of grass weeds and broadleaf weeds was higher in 2019 than in 2018 in *M. sacchariflorus* growing field. Most of the weed species showed leaf discoloration, followed by foliar injuries within a week of herbicide treatment. These weeds showed various degrees of tolerance and their biomass was affected to different extents. On average for the year 2019, the treatment of post emergence herbicides including bentazon, nicosulfuron, dicamba and glufosinate ammonium reduced the FW of weeds by 93.66%, 90.13%, 99.5%, 99.7%, respectively. Significant changes among the treatments were observed for the density ($F = 54.11$, $p < 0.01$), FW ($F = 19.21$, $p < 0.01$), and DW ($F = 11.35$, $p < 0.002$) of weeds for *M. sinensis*. Similar trends were observed for *M. sacchariflorus* (Table S5). Likewise, significant differences were observed in the interaction between years and treatments in density ($F = 4.77$, $p < 0.001$), FW ($F = 6.52$, $p < 0.003$), and DW ($F = 3.22$, $p < 0.025$) of weeds for the *M. sinensis*. Similar trends were observed for *M. sacchariflorus* (Table S5). This emphasizes that for proper control of weeds, proper application of pre-and post-emergence herbicides can effectively control them. Additional research is required to evaluate combinations with other herbicides, as well as sequential application of herbicides to identify those that cause higher levels of injury to the weeds and provide a sufficient level of weed control in the *M. sinensis* field, indicating that these herbicides might also be useful in the management of grass weeds in the *Miscanthus* field.

3.3 Effect of Herbicides Treatments on Miscanthus spp. Height

Treatment with pre- and post-emergence herbicides significantly affected the height of *Miscanthus* plants under field conditions (Fig. 2). In the present study, it was found that treatment with the herbicide glufosinate ammonium resulted in the lowest height of *M. sinensis* (70.00 ± 8.14 cm), which differed (not significantly) from the other treatments. This was followed by treatment with dicamba (105.00 ± 10.80 cm). Treatment with nicosulfuron resulted in taller plants (176.67 ± 12.47 cm); however, it was not statistically different from the height of *M. sinensis* in the control plots (170.00 cm). Similar results were also observed for *M. sacchariflorus*. A significant reduction in the *M. sacchariflorus* plant height was observed when treated with the pre- and post-emergence herbicides under field conditions (Fig. 2). Treatment of *M. sacchariflorus* plots with dicamba showed the lowest height (80.00 ± 8.05 cm), followed by glufosinate treatment (90.00 ± 8.16 cm). Treatment with nicosulfuron resulted in taller plants (183.33 ± 9.43 cm); however, it was not statistically different from the height of *M. sacchariflorus* in the control plots (180.00 ± 8.17 cm).

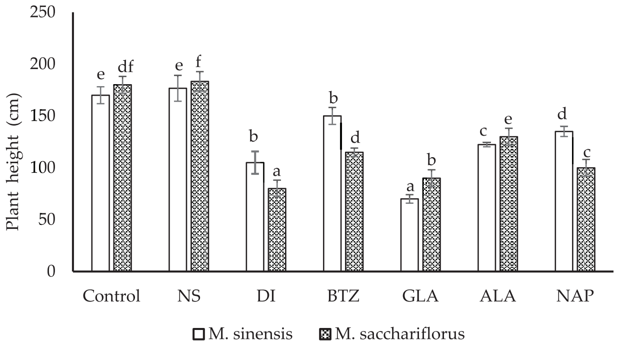

Figure 2: Effect of pre-emergence and post-emergence herbicides on the plant height of *M. sinensis* and *M. sacchariflorus*. Data are means ± standard deviation (n = 3). Means with different small letters indicate significant differences at $P < 0.05$. NS, Nicosulfuron; DI, Dicamba; BTZ, Bentazon; GLA, Glufosinate ammonium; ALA, Alachlor; NAP, Napropamide

3.4 Assessment of Visual Injury and Biomass Production of M. sinensis and M. sacchariflorus Resulted by Herbicides Treatment

Herbicidal toxicity was assessed after treating the two *Miscanthus* species with pre- and post-emergence herbicides. Significant variation in the degree of tolerance to the treated herbicides was observed in the *Miscanthus* species (Fig. 3, Supplementary Figs. 1 and 2). Comparatively, *M. sinensis* showed slightly higher tolerance to the herbicides nicosulfuron (10%), bentazon (10%), and napropamide (10%) at the recommended dose than *M. sacchariflorus* (Fig. 3). As a result, the FW and DW of *M. sinensis* were higher in the pre- and post-emergence herbicide application than in unweeded control plots in 2019 (Table 5). The DW of *M. sinensis* treated with nicosulfuron, bentazon and napropamide were lower than the unweeded control plots in 2018 (Table 5). Similarly, except in the case of napropamide, the FW and DW of *M. sacchariflorus* were significantly reduced by the Pre- emergence and Post emergence herbicides treatment in 2018 (Table 5). Interestingly, with some exception, the treatment of herbicides increased the biomass of *M. sacchariflorus* in the second year (2019) of weed management (Table 5), demonstrating that the herbicides are effective in improving the biomass yield of *M. sacchariflorus*. Among these herbicides, napropamide treatment showed a greater increase in the FW and DW (4900.0 and 2700.3 g, respectively), compared to the FW and DW of control *M. sinensis* (1800.0 and 158.0 g, respectively). The results showed that the application of dicamba and glufosinate ammonium caused severe injuries to the foliar region, chlorosis, and stunt growth on both species of *Miscanthus* plants and resulted in a significant loss in biomass at its recommended dose during its initial year of weed management. It is interesting to note that the degree of visual injuries caused by the application of glufosinate ammonium was similar (90%) for both *M. sinensis* and *M. sacchariflorus* plants (Fig. 3), which may be due to its higher phytotoxic effects on the growing plant in 2018. Both species were susceptible to dicamba (visual injury of 80% and 90%, respectively), resulting in a significant reduction in FW (467.7 and 328.7 g, respectively) compared to the control plants in the first year (2018) of weed management (Table 5). Such damage in the biomass appeared temporal as there was significant increase in the FW and DW of both *M. sinensis* and *M. sacchariflorus* in the second year (2019), which may be due to the effective controls of weeds and the lower competition with weeds for light, moisture, space and nutrients at the critical stages of growth (Table S10). This in turn favoured *Miscanthus* to utilize available resources for growth and gaining biomass, demonstrating that herbicides treatments are effective in reducing the biomass loss of *M. sinensis* and *M. sacchariflorus* plants. Significant changes were recorded to FW (F = 2.66, $p < 0.05$), DW (F = 4.82, $p < 0.007$) among the years for *M. sinensis*. Similar trend were recorded in FW (F = 31.78, $p < 0.05$), and DW (F = 11.10, $p < 0.02$) among the years for *M. sacchariflorus*. Likewise, significant differences were observed in the interaction between years and treatments for *M. sinensis* FW (F = 1.74, $p < 0.05$) and DW (F = 4.89, $p < 0.006$). Similar trend were recorded in FW of *M. sacchariflorus* (F = 6.19, $p < 0.02$) and DW (F = 3.46, $p < 0.03$) (Table S10). Furthermore, the present results support the hypothesis that sequences of pre-emergence and post-emergence herbicides will be necessary to achieve maximum control of weeds and increase the biomass of *M. sinensis* and *M. sacchariflorus*.

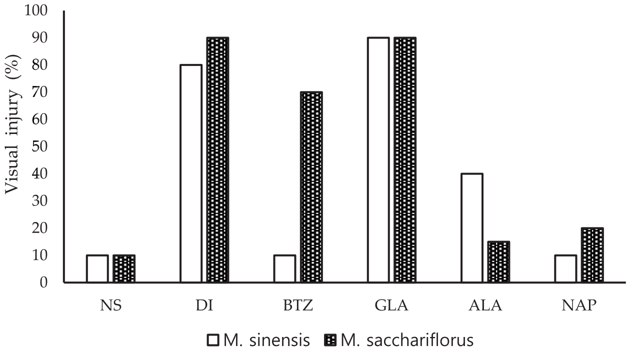

Figure 3: Visual damage of pre-emergence and post-emergence herbicides on *M. sinensis* and *M. sacchariflorus*. NS, Nicosulfuron; DI, Dicamba; BTZ, Bentazon; GLA, Glufosinate ammonium; ALA, Alachlor; NAP, Napropamide

Table 5: Effect of pre-emergence and post-emergence herbicides on the biomass of *M. sinensis* and *M. sacchariflorus* 30 days after the application on field conditions in the first year (2018) and second year (2019)

Time of application	Herbicides	*M. sinensis*				*M. sacchariflorus*			
		FW (g)	DW (g)	FW (g)	DW (g)	FW (g)	DW (g)	FW (g)	DW (g)
		2018		2019		2018		2019	
	Control	900.0b	475.0ef	1800.0a	158.0a	520.0f	193.3d	620.0b	391.3d
Pre-emergence	NAP	2003.7e	511.3f	4900.0f	2700.3g	612.0g	225.8e	1500.0f	525.0f
	AlA	405.7a	209.7b	2900.0d	1821.0e	220.3b	72.7bc	827.3c	420.8e
Post-emergence	BTZ	1247.0c	386.3d	3900.0e	2020.6f	268.3c	100.7c	668.3b	300.7c
	NS	432.7a	258.8c	2800.0c	199.0b	83.7a	10.3a	140.2a	70.3a
	DI	467.7a	162.7a	2500.0b	1575.0d	328.7d	101.5c	828.7d	301.5c
	GLA	1542.7d	415.0e	3900.0e	258.0c	373.5e	65.7b	973.0e	169.7b

Note: Data having the same letter in a column did not differ significantly according to Duncan's multiple comparison test ($P < 0.05$).

3.5 Total Chlorophyll Content (SPAD Value)

The total chlorophyll (TC) content in *M. sinensis* and *M. sacchariflorus* leaves under the different herbicide treatments is shown in Fig. 4. In the present study, there was a significant difference in the chlorophyll content between herbicide-treated and control plants. At 30 days after application of pre- and post-emergence herbicides to the *M. sinensis*, higher TC content was observed for the herbicide alachlor (38.35 ± 2.37 SPAD unit), followed by the treatment with Napropamide (35.03 ± 3.01 SPAD unit) and nicosulfuron (34.80 ± 2.51 SPAD unit). A significant reduction in TC was observed in the plants treated with dicamba (20.30 ± 4.21 SPAD unit) and glufosinate ammonium (20.90 ± 4.23 SPAD unit), with no significant difference between them. Similar to *M. sinensis*, the chlorophyll content of *M. sacchariflorus* was affected by treatment with different herbicides. The TC content of *M. sacchariflorus*, when exposed to napropamide, was similar to that of *M. sacchariflorus* grown in the control plots. Chlorophyll content was higher in *M. sacchariflorus* treated with napropamide and nicosulfuron (43.15 ± 2.86 and 41.53 ± 3.69 SPAD units, respectively). The chlorophyll content of *M. sacchariflorus* was significantly affected by the treatment with glufosinate ammonium, dicamba, and alachlor. However, this value was significantly lower from the chlorophyll content of *M. sacchariflorus* in the control plots. In contrast, the highest reduction in chlorophyll content was observed in *M. sacchariflorus* treated with glufosinate ammonium followed by dicamba (22.45 ± 3.84 and 28.55 ± 3.95 SPAD units, respectively).

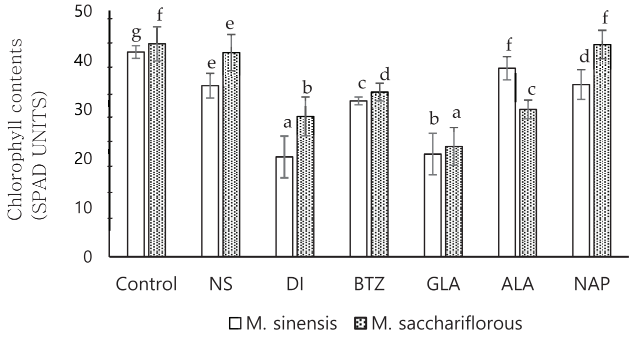

Figure 4: Effect of pre-emergence and post-emergence herbicides on the total chlorophyll content of *M. sinensis* and *M. sacchariflorus*. Data are means ± standard deviation (n = 3). Mean with different lowercase letters indicate a significant difference at $P < 0.05$. NS, Nicosulfuron; DI, Dicamba; BTZ, Bentazon; GLA, Glufosinate ammonium; ALA, Alachlor; NAP, Napropamide

3.6 Pearson's Correlations among Selected Variables

The Pearson's correlation analysis shows a wide range of correlations between the different parameters (Table S11). The weed density had a significant negative correlation with the fresh weight ($r = -0.595$, $p < 0.05$) and dry matters ($r = -0.425$, $p < 0.05$) of *M. sinensis* (Figure), implying that the fresh weight of the *M. sinensis* increased with a proportional decrease in the weed density and its biomass in the field. A similar relationship was also observed between the fresh weight of *M. sinensis* with the fresh weight ($r = -0.612$, $p < 0.05$) and dry weight ($r = -0.604$, $p < 0.05$) of weeds. Another attribute such as plant height was positively correlated with the total chlorophyll content ($r = 0.773$, $p < 0.05$) and fresh weight of *M. sinensis* ($r = 0.327$, $p < 0.05$). These results indicate that the decrease in the weed density using per-emergence and post-emergence herbicides significantly impact the plant growth and biomass of *M. sinensis*. The Pearson's correlation analysis shows that the density of weeds was significantly and negatively correlated to the fresh weight of *M. sacchariflorus* ($r = -0.227$, $p < 0.05$), and dry weight of it ($r = -0.220$, $p < 0.05$). A similar trend was observed between fresh weight of *M. sacchariflorus* with the fresh weight and dry weight of weeds, indicating a significant impact on the plant growth and biomass of

M. sacchariflorus (Table S12). In contrast, plant height and chlorophyll content of the *M. sacchariflorus* showed a positive correlation with its biomass, indicating a direct impact of weed competition on the reduction in *M. sacchariflorus* growth rate and total biomass.

4 Discussion

Proper control of weeds in bioenergy crop fields is necessary as the infestation of weeds causes competition for available nutrients, water, light, and space, which may cause the inhibition of the growth of bioenergy crops, reducing their biomass [49,50]. These challenges can be addressed by the appropriate application of registered herbicides for weed control [51]. In this study, *Miscanthus* grown in the control plots without weeding resulted in poor growth and less biomass production compared to weed management by herbicides treatments. This is because the application of herbicides reduced the density of broadleaf and grass weeds in the field, which reduced competition and caused the surplus flow of nutrients to the growing *Miscanthus* plants. In the present study, comparatively, the biomass of both the *M. sinensis* and *M. sacchariflorus* was reduced by the treated herbicides during the first year of weed management (2018), and then in the second year (2019). These results corroborate the report of Everman et al. [52], who observed an increase in the biomass of *M. giganteus* after the treatment of herbicides in field experiments. In the present study, treatment with pre-emergence herbicides showed a significant variation in the impact on weeds and achieved good control over the broadleaf and grass weeds.

In this study, treatment with the pre-emergence herbicides showed that recommended dose of alachlor when compared to napropamide was more effective in controlling both broadleaf and grass weed species that grew in the *M. sinensis* and *M. sacchariflorus* fields. A significant decrease in weed density, FW, and DW was observed in the plot treated with alachlor. Higher efficiency of weed growth control was also reported for other crops treated with alachlor [53]. It has been reported that alachlor causes a reduction in plant growth by inhibiting cell division and cell enlargement [49]. Furthermore, Hemanth Kumar et al. [49] observed nuclear lesions and chromosomal abnormalities in alachlor-treated weeds. Moreover, they observed that the treatment of alachlor caused the inhibition of the activity of elongase and geranylgeranyl pyrophosphate (GGPP) enzymes and inhibited cell division, leading to the ultimate death of emerging weeds. In this study, treatment with post-emergence herbicides effectively reduced the density, FW, and DW of dominant broadleaf and grass weed species grown in the *M. sinensis* and *M. sacchariflorus* fields. The efficacy of these herbicides for the inhibition of the broadleaf and grass weeds was higher in 2019 than in 2018 in *Miscanthus* growing field.

The treatment of Pre-emergence herbicides, partially controls the density, FW, and DW of *D. ciliaris, C. album* L., and *O. biennis* L. whereas the treatment of post-emergence herbicides such as Bentazon, dicamba, Glufosinate ammonium completely suppressed these weeds species in *Miscanthus* field. According to Somerville et al. [54], a shorter length of soil residual activity of pre-emergence herbicides does not provide sufficient weed control when used alone. Moreover, others argued that due to the continuous use of different chemical herbicides in the cultivation land, many weeds have evolved resistance against different herbicides and its mode of action [55]. Other than chemical herbicides, genetic factors, biological characteristics of weed species, agronomic practices and characteristics of herbicides also play important role the evalution of and spread of herbicides resistance in weeds species [55]. In the present study, presence of *D. ciliaris, C. album* L., and *O. biennis* L. in the pre-emergence herbicides treated *Miscanthus* field could be due to insufficient weed control, rather than herbicides resistance of weeds. Others believed that weeds escapes are due to their ability to germinate for a long time during the growing season [56], indicating that the timing of herbicides applications is the key to its efficacy. Based on the present study, the combination of both pre-and post-emergence herbicides is necessary to obtain a good efficacy in total control of weeds in *Miscanthus* fields.

Among the four post-emergence herbicides, treatment with nicosulfuron inhibited the growth of most of the dominant weeds without affecting the biomass of the growing *Miscanthus* plants, indicating that the application of nicosulfuron, in particular, is necessary to control the late emergence or escape weeds. Moreover, in a similar study, nicosulfuron has been reported to control weed species without adversely affecting the growth of bioenergy crops [57–59]. In the present study, treatment with nicosulfuron partially inhibited the growth and density of grass weeds *D. ciliaris* (Retz.) and *S. viridis* L., which is in line with an earlier report by Zhang et al. [60], who observed nicosulfuron tolerance in *D. sanguinalis*. It has been reported that nicosulfuron herbicides are responsible for inhibiting acetolactate synthase (ALS), which is located in the photosynthetic tissues (chloroplast and plastids) of plants and is more susceptible in young foliar tissues [61]. In contrast, the application of dicamba and glufosinate ammonium resulted in severe visual injuries and caused various degrees of necrosis, stunting of growth, leaf distortion, twisting of leaves, and reduction in the FW and DW of the weeds in both *M. sinensis and M. sacchariflorus* fields. In the present study, broadleaf weeds *P. asiatica* L. and *E. canadensis* appeared in the glufosinate ammonium-treated *Miscanthus* plot. In contrast, the treatment with glufosinate ammonium completely inhibited the growth and density of grass weeds. The lower susceptibility of grass weeds to glufosinate ammonium was attributed to the presence of multiple meristematic zones in a single plant, which makes grasses generally possess a higher tendency to survive than broadleaf plants when exposed to glufosinate ammonium [62].

Recently, the application of herbicides has increased in weed management programs due to the scarcity of labour and the higher efficiency of herbicides in controlling their growth. Several studies have reported an increase in the FW of newly established bioenergy crop fields. However, herbicides may degrade the growth of bioenergy crops and cause severe losses in biomass yield. Therefore, it is important to assess the phytotoxicity of herbicides on bioenergy crops. Application of pre- and post-emergence herbicides at the recommended dose caused various degrees of visual injury in the two species of *Miscanthus*. Treatment with the recommended dose of bentazon resulted in lower phytotoxicity to the growing *M. sinensis*. However, treatment with bentazon at the same dose caused higher visual injury to the *M. sacchariflorus* plants. Application of glufosinate ammonium on plants either damaged plant tissues or killed them completely due to disruption in the biochemical and physiological processes of the cells. Both *M. sinensis* and *M. sacchariflorus* were susceptible to dicamba and glufosinate ammonium, resulting in a significant reduction in FW and DW compared to the control plants. In the present study, the phytotoxicity of dicamba and glufosinate ammonium was characterized by severe leaf chlorosis in the midrib region. Eventually, the plant showed rapid recovery after 30 DAT. Chlorophyll content of *M. sinensis* and *M. sacchariflorus* was significantly reduced by treatment with glufosinate ammonium, bentazon, dicamba, and alachlor compared to the chlorophyll content of plants in the control plot.

In this study, the application of dicamba and glufosinate ammonium in the *Miscanthus* field showed higher efficacy in reducing the weed density, FW, and DW of both broadleaf and grass weeds. However, application of these herbicides resulted in severe intoxication in both species of *Miscanthus*, leading to stunting of the plant and necrosis in the foliar part of the plants, which contributed to the reduced FW and DW. Glufosinate ammonium targets glutamine synthetase (the second most abundant protein in plant leaves [63–66] and is responsible for the production of the amino acid glutamine and ammonia detoxification [52]). Inhibition of glutamine synthetase causes decreased glutamine production and rapid accumulation of ammonia in plant tissues [63,67,68] and toxic accumulation of glyoxylate, which inhibits RuBP-carboxylate and carbon dioxide fixation [69]. It has been further observed that an interruption of photorespiration results from a deficiency of intermediates of the Calvin cycle [70,71]. Accumulation of higher levels of ammonia causes acute toxicity to plants, causing severe chlorosis of leaves, suppression of growth, and eventually plant death [72]. According to Takano and Dayan [73], glufosinate ammonium disrupts both photorespiration and the light reactions of photosynthesis, which causes photoreduction of molecular oxygen, which generates reactive oxygen species. Moreover, it can also inhibit carbon assimilation and plant growth [63,74]. In the present study, the application of dicamba and glufosinate ammonium not only suppressed the dominant broadleaf and grass weeds but also reduced the growth of *Miscanthus* plants. Therefore, before applying these herbicides, all of these factors should be considered. In the present study, *M. sinensis* showed higher tolerance to nicosulfuron and napropamide. In particular, treatment with nicosulfuron resulted in higher FW and DW of *Miscanthus*. Moreover, *Miscanthus* treatment with nicosulfuron caused higher chlorophyll content in both species of *Miscanthus*, which could contribute to the increased biomass in the plant. According to Barroso et al. [75], some plant species can absorb nicosulfuron in non-toxic compounds and can reduce phytotoxicity. Moreover, in the present study, *M. sinensis* was less susceptible to bentazon. However, treatment with bentazon resulted in reduced FW and DW, which is possibly due to the insufficient injuries to the broadleaf weeds such as *Erigeron canadensis, Artemisia princeps*, and *Plantago asiatica* L., which causes the re-growth of weeds in the *M. sinensis* field and competition for space, light, water, and nutrition. In the present study, treatment with bentazon resulted in lower chlorophyll content, higher visual injuries, and reduced FW and DW of *Miscanthus* species. Bentazon, a photosystem II (PSII) electron flow inhibitor, causes changes in the chloroplast ultrastructure, photosynthetic pigment ratios, and levels of chlorophyll proteins in the photosynthetic apparatus of plants [76]. Therefore, in the present study, it is possible that the reduction in photosynthetic pigments could reduce the FW and DW of *Miscanthus* species. In similar research, Hassannejad et al. [77] observed a lower photosynthetic performance index (PIABS) in *Xanthium strumarium* L. when treated with bentazon as compared to nicosulfuron.

5 Conclusions

In the present study, none of the herbicides alone treatments were found effective in controlling all the types of weeds. It was observed that the application of pre-emergence herbicides such as alachlor and napropamide at 951 and 1120 g.a.i.ha^{-1}, respectively, caused less visual injuries and biomass loss in *M. sinensis* and *M. sacchariflorus*, while the effect was inadequate to control invasive weeds such as *biennis* L., *D. ciliaris* (Retz.) and *O. biennis* L. The highest weed control was achieved by the treatment of glufosinate ammonium and dicamba at 400 and 482 400 g.a.i/ha^{-1}, respectively, which also showed severe phytotoxicity with a considerable amount of loss in the *Miscanthus* biomass, but these were often temporary. Treatment with nicosulfuron (40 g.a.i/ha^{-1}) showed good control of the broadleaf and grass weeds at the recommended dose with lower phytotoxicity and caused least visual injuries to the *M. sinensis* and *M. sacchariflorus*. The present study can be relevant for weed management in *Miscanthus* fields with multiple pre- and post-emergence herbicide options.

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Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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Supplementary Materials

Figure S1: *M. sinensis* field in Chuncheon, South Korea treated with (i) Nicosulfuron, (ii) Dicamba, (iii) Bentazon, (iv) Glufosinate ammonium, (v) Alachor, (vi) Napropamide, (vii) Control

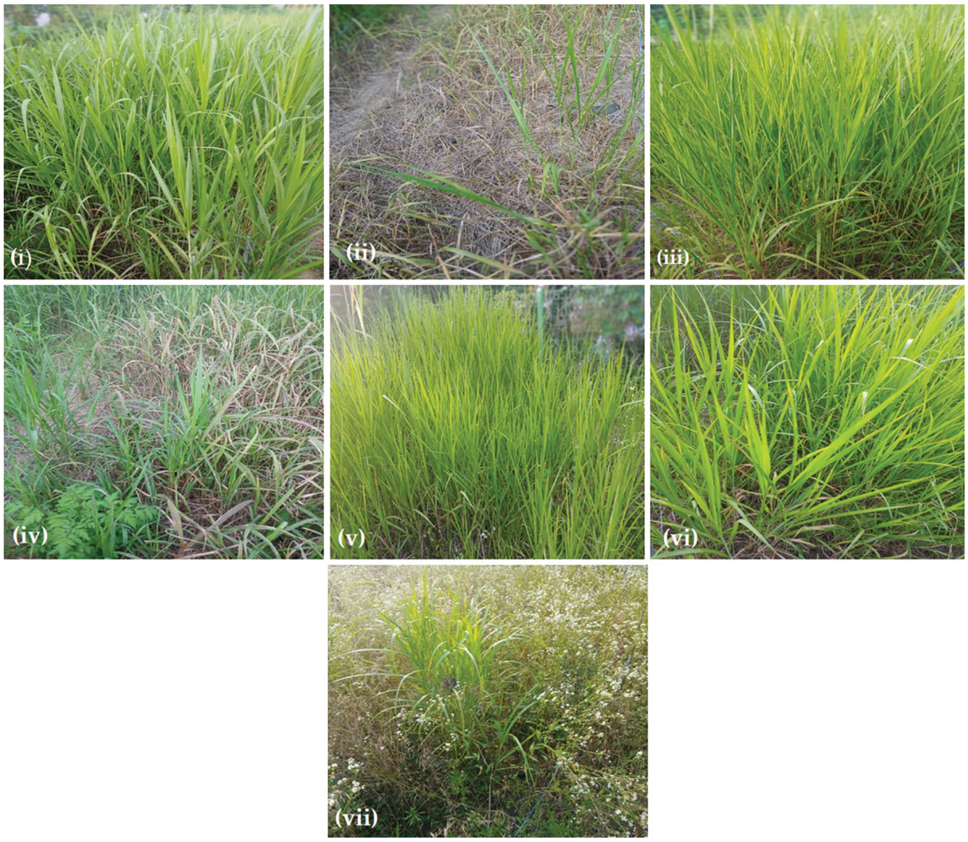

Figure S2: *M. sacchariflorus* field in Chuncheon, South Korea treated with (i) Nicosulfuron, (ii) Dicamba, (iii) Bentazon, (iv) Glufosinate ammonium, (v) Alachor, (vi) Napropamide, (vii) Control

Table S1: Effect of pre-emergence herbicides on the density, fresh weight and dry weight of different weed species in *M. sinensis* field in 2018

Weeds	Control			Napropamide			Alachlor		
	Density (plants/m^2)	FW (g)	DW (g)	Density (plants/m^2)	FW (g)	DW (g)	Density (plants/m^2)	FW (g)	DW (g)
Setaria viridis L.	28.0	88.5	28.5	-	-	-	-	-	-
Oenothera biennis L.	20.0c	119.7c	45.2c	1.0a	2.0a	1.0a	7.0b	39.0b	21.5b
Ixeris dentate	5.0	44.0	9.7	-	-	-	-	-	-
Erigeron Canadensis L.	14.0b	151.5b	53.9b	-	-	-	6.0a	73.0a	29.5a
Chenopodium album L.	18.0b	169.6b	25.8b	1.0a	1.2a	0.4a	-	-	-
Digitaria ciliaris (Retz.)	16.0b	51.7b	21.1b	2.0a	8.0a	2.5a	-	-	-
Taraxacum platycarpus H.	4.0	20.5	5.0	-	-	-	-	-	-
Artemisia princeps Pamp.	5.0	73.0	21.0	-	-	-	-	-	-
Rumex crispus L.	6.0	431.0	111.0	-	-	-	-	-	-
Agropyron tsukushiense	33.0	190.0	130.5	-	-	-	-	-	-
Plantago asiatica L.	13.0b	30.5b	12.5b	6.0a	16.5a	2.0a	-	-	-
Trifolium repens L.	7.0	100.0	27.0	-	-	-	-	-	-

Note: Data having the same letter in a row did not differ significantly according to Duncan's multiple comparison test ($p < 0.05$).

Table S2: Effect of post-emergence herbicides on the density, fresh weight, and dry weight of different weed species in *M. sinensis* field in 2018

Weeds	Control			Bentazon			Nicosulfuron			Dicamba			Glufosinate ammonium		
	Density (plants/m^2)	FW (g)	DW (g)	Density (plants/m^2)	FW (g)	DW (g)	Density (plants/m^2)	FW (g)	DW (g)	Density (plants/m^2)	FW (g)	DW (g)	Density (plants/m^2)	FW (g)	DW (g)
Commelina communis L.	13.0b	100.5b	10.5b	3.0a	13.5a	3.5a	-	-	-	-	-	-	-	-	-
Bidens frondosa L.	11.0	51.1	8.0	-	-	-	-	-	-	-	-	-	-	-	-
Erigeron canadensis L.	12.0b	151.5b	23.9b	-	-	-	-	-	-	2.0a	5.0a	1.9a	2.0a	5.5a	1.0a
Oenothera biennis L.	14.0b	130.7b	19.2b	-	-	-	7.0a	50.5a	2.0a	-	-	-	-	-	-
Chenopodium album L.	16.0	169.6	25.8	-	-	-	-	-	-	-	-	-	-	-	-
Ipomoea purpurea (L.)	4.0	101.9	17.8	-	-	-	-	-	-	-	-	-	-	-	-
Artemisia princeps Pamp.	12.0b	74.8b	21.5b	3.0a	16.5a	5.0a	-	-	-	-	-	-	-	-	-
Setaria viridis L.	14.0b	55.9b	23.0b	-	-	-	8.0a	35.5a	8.5a	-	-	-	-	-	-
Plantago asiatica L.	12.0c	37.5d	13.5c	23.0b	19.0c	7.0b	-	-	-	3.0a	11.5b	7.0b	3.0a	9.0a	2.5a
Ixeris dentata (Thunb.)	2.0b	9.5b	2.5b	-	-	-	-	-	-	1.0a	4.0a	1.5a	-	-	-
Digitaria ciliaris (Retz.)	11.0b	50.7b	22.1b	-	-	-	6.0a	41.0a	6.0a	-	-	-	-	-	-
Agropyron tsukushiense	12.0	187.0	20.8	-	-	-	-	-	-	-	-	-	-	-	-

Note: Data having the same letter in a row did not differ significantly according to Duncan's multiple comparison test ($p < 0.05$).

Table S3: Effect of pre-emergence herbicides on the density, fresh weight and dry weight of different weed species in *M. sinensis* field in 2019

Weeds	Control			Napropamide			Alachlor		
	Density (plants/m^2)	FW (g)	DW (g)	Density (plants/m^2)	FW (g)	DW (g)	Density (plants/m^2)	FW (g)	DW (g)
Setaria viridis L.	13.0	29.2	12.6	-	-	-	-	-	-
Oenothera biennis L.	12.0b	54.7c	18.3c	2.0a	2.0a	1.0a	2.0a	19.0b	7.5b
Ixeris dentate Thunb.	5.0	45.0	10.8	-	-	-	-	-	-
Erigeron Canadensis L.	7.0b	30.0b	8.0b	2.0a	14.5a	3.5a	-	-	-
Chenopodium album L.	9.0b	70.3d	18.2d	1.0a	1.2b	0.4a	1.0a	3.2c	1.3b
Digitaria ciliaris	16.0b	52.7b	26.1b	3.0a	8.0a	2.5a	-	-	-
Taraxacum platycarpus H.	5.0	29.5	6.0	-	-	-	-	-	-
Artemisia princeps Pamp.	6.0	117.9	20.5	-	-	-	-	-	-
Rumex crispus L.	8.0	631.0	156.0	-	-	-	-	-	-
Agropyron tsukushiense	15.0	44.8	10.5	-	-	-	-	-	-
Trifolium repens L.	15.0	187.0	67.0	-	-	-	-	-	-

Note: Data having the same letter in a row did not differ significantly according to Duncan's multiple comparison test ($p < 0.05$).

Table S4: Effect of post-emergence herbicides on the density, fresh weight and dry weight of different weed species in *M. sinensis* field in 2019

Weeds	Control			Bentazon			Nicosulfuron			Dicamba			Glufosinate ammonium		
	Density (plants/m^2)	FW (g)	DW (g)	Density (plants/m^2)	FW (g)	DW (g)	Density (plants/m^2)	FW (g)	DW (g)	Density (plants/m^2)	FW (g)	DW (g)	Density (plants/m^2)	FW (g)	DW (g)
Commelina communis L.	10.0	98.0	8.5	-	-	-	-	-	-	-	-	-	-	-	-
Bidens frondosa L.	9.0	50.0	7.0	-	-	-	-	-	-	-	-	-	-	-	-
Erigeron Canadensis L.	10.0b	150.0c	20.0c	2.0a	20.0b	12.0b	-	-	-	2.0a	5.0a	1.9a	2.0a	5.5a	2.0a
Oenothera biennis L.	11.0	119.0	15.1	-	-	-	-	-	-	-	-	-	-	-	-
Chenopodium album L.	12.0	160.0	20.8	-	-	-	-	-	-	-	-	-	-	-	-
Ipomoea purpurea (L.)	2.0	100.0	17.0	-	-	-	-	-	-	-	-	-	-	-	-
Artemisia princeps	10.0c	70.0c	20.0c	2.0a	9.5a	2.5a	4.0b	20.0b	4.0b	-	-	-	-	-	-
Setaria viridis L.	11.0	50.0	20.0	-	-	-	-	-	-	-	-	-	-	-	-
Plantago asiatica L.	8.0b	39.5b	13.0b	2.0a	10.5a	2.0a	-	-	-	-	-	-	-	-	-
Ixeris dentata (Thunb.)	2.0	9.0	2.0	-	-	-	-	-	-	-	-	-	-	-	-
Digitaria ciliaris (Retz.)	9.0b	42.7b	20.1b	-	-	-	3.0a	12.5a	2.5a	-	-	-	-	-	-
Agropyron tsukushiense	12.0b	187.0b	20.8b	-	-	-	4.0a	40.9a	5.6a	-	-	-	-	-	-

Note: Data having the same letter in a row did not differ significantly according to Duncan's multiple comparison test ($p < 0.05$).

Table S5: Results of two-way analysis of variance (ANOVA) for effects of pre-emergence and post emergence herbicide (H) on density, fresh weight (FW) and dry weight (DW) of weeds in 2018 and 2019

	M. sinensis				*M. sacchariflorus*			
	df	Density of weeds	FW of weeds	DW of weeds	df	Density of weeds	FW of weeds	DW of weeds
H	1	54.11 (0.01)*	19.21 (0.01)*	11.35 (0.002)*	1	9.49 (0.004)*	4.36 (0.045)*	1.39 (0.01)*
Y	5	25.42 (ns)	36.04 (ns)	10.03 (ns)	5	20.61 (ns)	68.28 (ns)	70.57 (ns)
H × Y	5	4.77 (0.001)*	6.52 (0.003)*	3.22 (0.025)*	5	4.29 (0.0036)*	6.65 (0.0005)*	8.51 (0.0001)*

Notes: Means were compared by two-way ANOVA, and the *F* calculated for the two factors; herbicides treatment (H) and year (Y). Only significant factors and interactions are shown.
* denotes significant differences at $p < 0.05$. ns = not significant.

Table S6: Effect of pre-emergence herbicides on the density, fresh weight and dry weight of different weed species in *M. sacchariflorus* field in 2018

Weeds	Control			Napropamide			Alachlor		
	Density (plants/m^2)	FW (g)	DW (g)	Density (plants/m^2)	FW (g)	DW (g)	Density (plants/m^2)	FW (g)	DW (g)
Setaria viridis L.	33.0b	230.0b	50.0b	5.0a	4.5a	0.3a	-	-	-
Oenothera biennis L.	24.0c	120.0c	35.0c	4.0a	6.5a	2.2a	6.0b	15.0b	5.0b
Ixeris dentate Thunb. Ex	4.0	37.0	7.0	-	-	-	-	-	-
Erigeron canadensis L.	7.0b	310.5b	104.5b	3.0a	9.0a	2.7a	-	-	-
Chenopodium album L.	9.0b	80.3b	28.2b	2.0a	4.0a	1.3a	2.0a	3.0a	1.0a
Digitaria ciliaris (Retz.)	16.0d	44.0d	18.0d	2.0a	3.2a	0.9a	6.0b	12.0b	5.0c
Taraxacum platycarpus H.	5.0b	28.0	5.0	-	-	-	-	-	-
Artemisia princeps Pamp.	15.0b	290.0	73.5	-	-	-	-	-	-
Rumex crispus L.	8.0b	600.0	100.0	-	-	-	-	-	-
Agropyron tsukushiense	15.0b	197.0	139.5	-	-	-	-	-	-
Trifolium repens L.	15.0b	180.0	60.0	-	-	-	-	-	-

Note: Data having the same letter in a row did not differ significantly according to Duncan's multiple comparison test ($p < 0.05$).

Table S7: Effect of post-emergence herbicides on the density, fresh weight and dry weight of different weed species in *M. sacchariflorus* field in 2018

Weeds	Control			Bentazon			Nicosulfuron			Dicamba			Glufosinate ammonium		
	Density (plants/m^2)	FW (g)	DW (g)	Density (plants/m^2)	FW (g)	DW (g)	Density (plants/m^2)	FW (g)	DW (g)	Density (plants/m^2)	FW (g)	DW (g)	Density (plants/m^2)	FW (g)	DW (g)
Commelina communis L.	10.0b	98.5b	8.5b	3.0a	10.5a	3.0a	-	-	-	-	-	-	-	-	-
Bidens frondosa L.	9.0b	50.1	9.0	-	-	-	-	-	-	-	-	-	-	-	-
Erigeron Canadensis L.	11.0b	131.5c	20.0b	-	-	-	-	-	-	2.0a	5.1a	2.0a	2.0a	7.5b	2.0a
Oenothera biennis L.	12.0b	110.8b	25.2b	-	-	-	5.0a	40.0a	15.0a	-	-	-	-	-	-
Chenopodium album L.	12.0b	160.6	20.0	-	-	-	-	-	-	-	-	-	-	-	-
Ipomoea purpurea L.	3.0b	98.0	16.8	-	-	-	-	-	-	-	-	-	-	-	-
Artemisia princeps	10.0d	69.0	17.0	-	-	-	-	-	-	-	-	-	-	-	-
Setaria viridis L.	11.0b	50.0b	20.0b	-	-	-	4.0a	40.5a	7.5a	-	-	-	-	-	-
Plantago asiatica L.	11.0b	30.5b	10.0b	-	-	-	-	-	-	3.0a	11.5a	7.0a	-	-	-
Ixeris dentata (Thunb.)	2.0b	8.2b	2.5b	-	-	-	-	-	-	1.0a	3.0a	1.0a	-	-	-
Digitaria ciliaris (Retz.)	9.0b	42.7b	20.1b	-	-	-	6.0a	14.0a	7.0a	-	-	-	-	-	-
Agropyron tsukushiense	10.0b	140.0	10.6	-	-	-	6.0	-	-	-	-	-	-	-	-

Note: Data having the same letter in a row did not differ significantly according to Duncan's multiple comparison test ($p < 0.05$).

Table S8: Effect of pre-emergence herbicides on the density, fresh weight and dry weight of different weed species in *M. sacchariflorus* field in 2019

Weeds	Control			Napropamide			Alachlor		
	Density (plants/m^2)	FW (g)	DW (g)	Density (plants/m^2)	FW (g)	DW (g)	Density (plants/m^2)	FW (g)	DW (g)
Setaria viridis L.	9.0b	41.5	8.9	-	-	-	-	-	-
Oenothera biennis L.	14.0b	30.0b	10.3b	3.0a	4.5a	1.3a	2.0a	5.0a	2.5a
Capsella bursa pastoris L.	7.0b	29.5b	13.6b	1.0a	3.5a	1.1a	-	-	-
Digitaria ciliaris (Retz.)	16.0b	57.0c	25.1b	2.0a	3.2a	0.9a	3.0a	8.0b	1.5a
Erigeron Canadensis L.	13.0b	68.5b	23.3b	2.0a	5.0a	0.7a	-	-	-
Chenopodium album L.	3.0b	17.3c	4.5c	1.0a	1.0a	0.3a	1.0a	4.0b	1.5b
Alopecurus aequalis Sobol.	5.0b	88.5	32.3	-	-	-	-	-	-
Calystegia sepium L.	2.0b	20.0	4.2	-	-	-	-	-	-
Ixeris dentate (Thunb.)	4.0b	9.0	2.5	-	-	-	-	-	-
Trifolium repens L.	15.0b	44.8	10.5	-	-	-	-	-	-
Agropyron tsukushiense	15.0b	187.0	67.0	-	-	-	-	-	-
Artemisia princeps Pamp.	7.0b	526.6	136.0	-	-	-	-	-	-

Note: Data having the same letter in a row did not differ significantly according to Duncan's multiple comparison test ($p < 0.05$).

Table S9: Effect of post-emergence herbicides on the density, fresh weight and dry weight of different weed species in *M. sacchariflorus* field in 2019

Weeds	Control			Bentazon			Nicosulfuron			Dicamba			Glufosinate ammonium		
	Density (plants/m^2)	FW (g)	DW (g)	Density (plants/m^2)	FW (g)	DW (g)	Density (plants/m^2)	FW (g)	DW (g)	Density (plants/m^2)	FW (g)	DW (g)	Density (plants/m^2)	FW (g)	DW (g)
Commelina communis L.	12.0b	120.5b	13.5b	5.0a	47.5a	5.5a	-	-	-	-	-	-	-	-	-
Setaria viridis L.	10.0b	54.5b	13.5b	-	-	-	3.0a	30.5a	7.5a	-	-	-	-	-	-
Bidens frondosa L.	10.0b	50.0	7.0	-	-	-	-	-	-	-	-	-	-	-	-
Erigeron Canadensis L.	11.0b	150.0e	20.0e	2.0a	10.0c	3.5c	2.0a	19.0d	6.0d	2.0a	5.5b	1.9b	1.0a	2.5a	0.9a
Oenothera biennis L.	13.0b	114.7	12.2	-	-	-	-	-	-	-	-	-	-	-	-
Chenopodium album L.	15.0b	160.0	23.8	-	-	-	-	-	-	-	-	-	-	-	-
Ipomoea purpurea L..	11.0b	100.9	15.8	-	-	-	-	-	-	-	-	-	-	-	-
Polygonum hydropiper L.	12.0b	45.5	11.7	-	-	-	-	-	-	-	-	-	-	-	-
Digitaria ciliaris Retz.	11.0b	54.1b	23.1b	-	-	-	4.0a	21.0a	6.0a	-	-	-	-	-	-
Artemisia princeps Pamp.	7.0b	526.8b	136.7b	2.0a	13.5a	2.5a	-	-	-	-	-	-	-	-	-
Rumex crispus L.	4.0b	22.4	2.9	-	-	-	-	-	-	-	-	-	-	-	-
Trifolium repens L.	10.0b	105.7	12.0	-	-	-	-	-	-	-	-	-	-	-	-
Agropyron tsukushiense	15.0b	67.8b	15.0b	-	-	-	4.0a	40.0a	4.0a	-	-	-	-	-	-

Note: Data having the same letter in a row did not differ significantly according to Duncan's multiple comparison test ($p < 0.05$).

Table S10: Results of two-way analysis of variance (ANOVA) for effects of pre-emergence and post emergence herbicide (H) on fresh weight (FW) and dry weight (DW) of *M. sinensis* and *M. sacchariflorus* in 2018 and 2019

	M. sinensis			*M. sacchariflorus*		
	df	FW	DW	df	FW	DW
H	1	57.63 (ns)	47.55 (ns)	1	118.73 (ns)	62.58 (ns)
Y	4	2.66 (0.05)*	4.82 (0.007)*	4	31.78 (0.05) *	11.10 (0.02) *
H × Y	4	1.74 (0.05)*	4.89 (0.006)*	4	6.19 (0.02)*	3.46 (0.03)*

Notes: Means were compared by two-way ANOVA, and the *F* calculated for the two factors; herbicides treatment (H) and year (Y). Only significant factors and interactions are shown.
* denotes significant differences at $p < 0.05$. ns = not significant.

Table S11: . Correlation coefficients matrix among different characteristics of weeds and *M. sinensis*

Variables	Density of weeds	^{1}FW of weeds	DW of weeds	FW of *M. sinensis*	DW of *M. sinensis*	Plant height	Chlorophyll content
Density of weeds	**1**						
FW of weeds	0.999*	**1**					
DW of weeds	0.997*	0.999*	**1**				
FW of *M. sinensis*	–0.595*	–0.612*	–0.604*	**1**			
DW of *M. sinensis*	–0.425*	–0.450*	–0.451*	0.902*	**1**		
Plant height	0.418*	0.416*	0.435*	0.327*	0.263	**1**	
Chlorophyll content	0.493*	0.497*	0.507*	–0.063	–0.036	0.773*	**1**

Notes: ^{1}FW: Fresh weight of weeds; ^{2}DW: Dry weight of weeds.
*Correlation is significant at the 0.05 level (2-tailed).

Table S12: Correlation Coefficients matrix among different characteristics of weeds and *M. sacchariflourus.*

Variables	Density of weeds	^{1}FW of weeds	DW of weeds	FW of *M. sacchariflourus*	DW of *M. sacchariflourus*	Plant height	Chlorophyll content
Density of weeds	**1**						
FW of weeds	0.998*	**1**					
DW of weeds	0.998*	0.999*	**1**				
FW of *M. sacchariflourus*	–0.227*	–0.260*	–0.235*	**1**			
DW of *M. sacchariflourus*	–0.220*	0.177	0.196	0.723*	**1**		
Plant height	0.578*	0.550*	0.558*	0.488*	0.622*	**1**	
Chlorophyll content	0.418*	0.409*	0.427*	0.627*	0.434*	0.660*	**1**

Notes: ^{1}FW: Fresh weight of weeds; DW: Dry weight of weeds.
*Correlation is significant at the 0.05 level (2-tailed).