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Abstract. To test the effect of adjuvant on the efficacy of herbicides in a fast and non-destructive way is very helpful for selecting a right spray adjuvant for herbicide, which is an important strategy to enhance the efficacy of herbicides, reduce application dose, and enhance environmental safety. Experiments were conducted in the laboratory and greenhouse to study the effect of 2 adjuvants-Methylated seed oil (MSO) and organosilicone on the efficacy of topramezone on grass weed giant foxtail（*Setaria faberi* Herrm.） and broadleaved weed velvetleaf（*Abutilon theophrasti* Medik.) using weed leaves chlorophyll fluorescence measurement and whole plant biomass test. The results indicated that the top leaf maximum quantum efficiency (Fv/Fm) of two weeds treated by herbicide mixed with MSO adjuvant was significantly lower than that of treated by herbicide alone from the 2-3 days after treatment, while the difference between treatments of herbicide mixed with organosilicone adjuvant and herbicide applied alone was not significant. Results of the whole-plant pot tests showed biomass of the treatment of topramezone mixed with MSO was significantly lower than that of herbicide applied alone. This is similar to the result of chlorophyll fluorescence test. Chlorophyll fluorescence measurement has proven to be an attractive tool for studying the effect of the adjuvants on the efficacy of herbicide.

Key words: Weed; Chlorophyll fluorescence test; Adjuvant; Methylated seed oil (MSO); Fv/Fm

1 Introduction

Adjuvant is one substance of the herbicide formulation or added to the spray tank to improve herbicidal efficacy [1, 2]. Total formulation efficacy is usually a function of deposition, retention, uptake, translocation, and active ingredient toxicity. Adjuvants influence the physico-chemical and plant interactions involved for each factor above-mentioned. To select a right adjuvant for the herbicide is a difficult but very important work, because the efficacy of the herbicide is usually dependent on the herbicide species, weed species, the selected adjuvant and the environmental influence. By selecting the right adjuvant, herbicide application dose can be reduced, which is benefit to the environment food

safety.

As a kind of fatty acid, MSO enhanced the efficacy of herbicides on weeds by increasing the penetration of the active ingredient [3, 4, 5, 6]. It is a recommended adjuvant for herbicide topramezone in the whole world. Organosilicone surfactant was introduced to work as an adjuvant for the pesticide in 1980s [7], and since then, its mode of action have been researched extensively. MSO and organosilicone are nearly the most common used adjuvants for herbicide in the market of China. As a post-emergence hydroxyphenylpyruvate dioxygenase inhibitor (HPPD-inhibitor), topramezone could control many annual grass and broadleaved weeds [8] and has a good safety on maize (*Zea mays* L.) [9, 10]. MSO is the only adjuvant for this herbicide by now, so trying to find a new adjuvant for this herbicide is a very interesting and meaningful work.

The only way to judge the effect of an adjuvant on the efficacy of herbicide is testing the efficacy of herbicide after being mixed with the adjuvant. Conventional herbicide efficacy estimation is usually conducted by analyzing the variables, including plant fresh or dry weight, relative biomass, and visual ratings, additional variables including various growth parameters (e.g., rate of growth, height, leaf area and son on) [11] of weed response to the herbicide in the greenhouse and field experiment [12]. These estimation methods have some disadvantages, including lack of useful method to estimate plant response to herbicides quantitatively, time-consuming method for measuring and so on. Hence, scientists in the agricultural and horticultural sector are keen to invent some more automated, objective, and sensitive approaches to replace this largely manual process. So RGB, Multi spectral, Hyperspectral, thermal, Chlorophyll Fluorescence, 3D sensors and some other methods have been invited to this field. Such methods offer the opportunity to evaluate efficacy efficiently in a non-destructive way and at low cost [13, 14]. Chlorophyll fluorescence can be used as a sensitive indicator of the physiological status of plants, and it can monitor the spatial and temporal variations by providing images of the photosynthesis [15, 16, 17]. By utilizing this technology, Kaiser et al. [18] and Wang et al. [19] detected the herbicide resistance of *Alopecurus myosuroides* and Li et al. [20] identified the herbicide stress in soybean shortly after treatment.

The object for this study was that chlorophyll fluorescence test can be used (i) for measuring effects of two adjuvants on the efficacy of topramezone at early stage after treatment and (ii) that results of this method correspond well with the classical bioassay method in the greenhouse.

2 Materials and Methods

2.1 Chemicals and Plant Materials

Herbicide topramezone (336g L^{-1} SC) was given by BASF Co., Ltd. MSO (GY-HMax, methylated soybeans oil) adjuvant was given by the Central Research Institute of China Chemical Science and Technology. Organosilicone adjuvant (BREAK-THRU S240) was provided by Omya. Agro.AG, Switzerland.

Velvetleaf and giant foxtail were selected as broadleaved and grass weed in this study.

Pre-germination of weed seeds were conducted in 11×11×6cm plastic pots, which were filled with 2-3cm in depth vermiculite. The environmental temperature of the greenhouse was 25/20±1 ℃ day/night, additional light (122 μmol $m^{-2}s^{-1}$) was employed for 12h, and the relative humidity was 55±10%. 3 seedlings of velvetleaf were transplanted into a 11×11×12 cm plastic pot and 4 seedlings of giant foxtail into a 7×7×8 cm paper pot after germination. The nursery substrate in the pot was mixed with vermiculite: peat: clay at 1:1:1 by volume. All plants were irrigated daily with tap water except the first 24 hours after herbicide application.

2.2 Experiment Design and Conduct

Herbicide application solution was prepared according to table1. The homogeneous plants were selected as sample plants for the experiment when they grew up to 3-4 leaves. Herbicide solution was applied using a track sprayer (produced by Aro, Langenthal, Switzerland) and the carry water volume was 200 L ha^{-1}. The nozzle was 8002 EVS (Teejet® Spraying Systems Co., Wheaton, IL, USA) and the pressure was 3.2 kPa.

Table 1 Spray herbicide solution preparation for the experiment of 2 adjuvants on the efficacy of topramezone on weeds.

Treatment	Herbicide dose（g a.i.ha^{-1}）	Adjuvant dose (%)
Topramezone alone	6.3	0
Topramezone with MSO*	6.3	0.3 00 (v/v)
Topramezone with organosilicone	6.3	0.025 (v/v)
Control	0	0

*MSO means methylated seed oil.

Maximum quantum efficiency (Fv/Fm) of PSII of the top leaf (the fourth leaf) of both weeds was measured and recorded with an IMAGING-PAMM-Series MAXI Version Chlorophyll Fluorometer (produced by Heinz Walz GmbH, Germany) at 2, 3, 4 and 5 days after treatment (DAT). Equation (1) was employed to calculate Fv/Fm.

$$Fv/Fm = \frac{Fm - F0}{Fm} \quad (1)$$

Parameter Fm is the maximal fluorescence yield and F0 is the dark fluorescence yield. In order to guarantee the sample plants were only illuminated by the light source of the instrument and avoid other photosynthetic active radiation, all measurements were conducted in a dark background. Plants were dark adapted for half an hour before each measurement for determination of F0. After dark adaption, the whole plant was illuminated with a light saturation pulse of 2634 μMm^{-2} s^{-1} photosynthetic photon flux density. A wavelength of 450 nm was selected for determining Fv/Fm value. The whole plant was illuminated in the test room but only the fourth leaf (top leaf) was selected as the analysis target. Though the IMAGING-PAM fluorometer can also measures some other parameters related to chlorophyll fluorescence, the Fv/Fm of PSII was selected for analysis in this study because it was constant till the next F0, Fm detection.

Chlorophyll fluorescence images were shot by a CCD camera with spatial resolution of 640×480 pixels, and the field of view 10×13cm mounted above the plant pots while measuring the Fv/Fm value. Light-emitting diodes (LED) were placed around the lens of the camera. Only the target plants tested were shown and the background was segmented. To confine the detection window to wavelengths longer than 620 nm, a red long-pass filter was mounted in front of the CCD-chip. Twelve individual plants of giant foxtail and nine individual plants of velvetleaf were measured for each treatment totally.

At 3 weeks after herbicide application, above-ground biomass of plants were collected and dried at 80℃ in oven for 48h and then weighed. Completely randomized design was employed for the experiment. Three replicates were conducted for each treatment and the whole experiment was repeated twice.

2.3 Statistical Analysis

Data of Fv/Fm value and plants dry weight were subjected to the One-way ANOVA procedure of SPSS 22.0(V.22.0). Residual plots and the Shapiroe-Wilk normality test were conducted to ensure the residuals random, homogeneous, with a normal distribution. Data were combined to analyze for two repeated experiments and means were separated by Fisher's protected LSD test at α =0.05.

3 Results

3.1 Chlorophyll Fluorescence Measurement

The Fv/Fm value of the top leaf of giant foxtail and velvetleaf treated by topramezone with MSO was significantly lower （$P<0.05$）than that of treated by tap water and topramezone applied alone from 2 DAT to 5 DAT. While in the case of treatment of topramezone applied with organosilicone, the Fv/Fm value of the top leaf of treated plants were not significantly different from the treatment of topramezone applied alone at all time (Fig.1). The chlorophyll fluorescence images taken during the measurement from 2 to 5 DAT showed that there was no difference among different treatments at 2 DAT, while difference was showed apparently from 3 DAT. Top leaf of both weed species treated by herbicide with MSO were more injurious (the colour of the normal plant leaves was blue, while the colour of the leaves changing from green to yellow and even to black demonstrated that the plants were injured more severely) than the ones treated by herbicide alone and with organosilicone at 3 DAT (Fig.2), and the difference became more apparent at 4 and 5 DAT.

The above-mentioned results indicated that the MSO adjuvant enhanced the efficacy of topramezone significantly while organosilicone adjuvant had no effect on the efficacy of this herbicide on both weeds.

giant foxtail velvetleaf

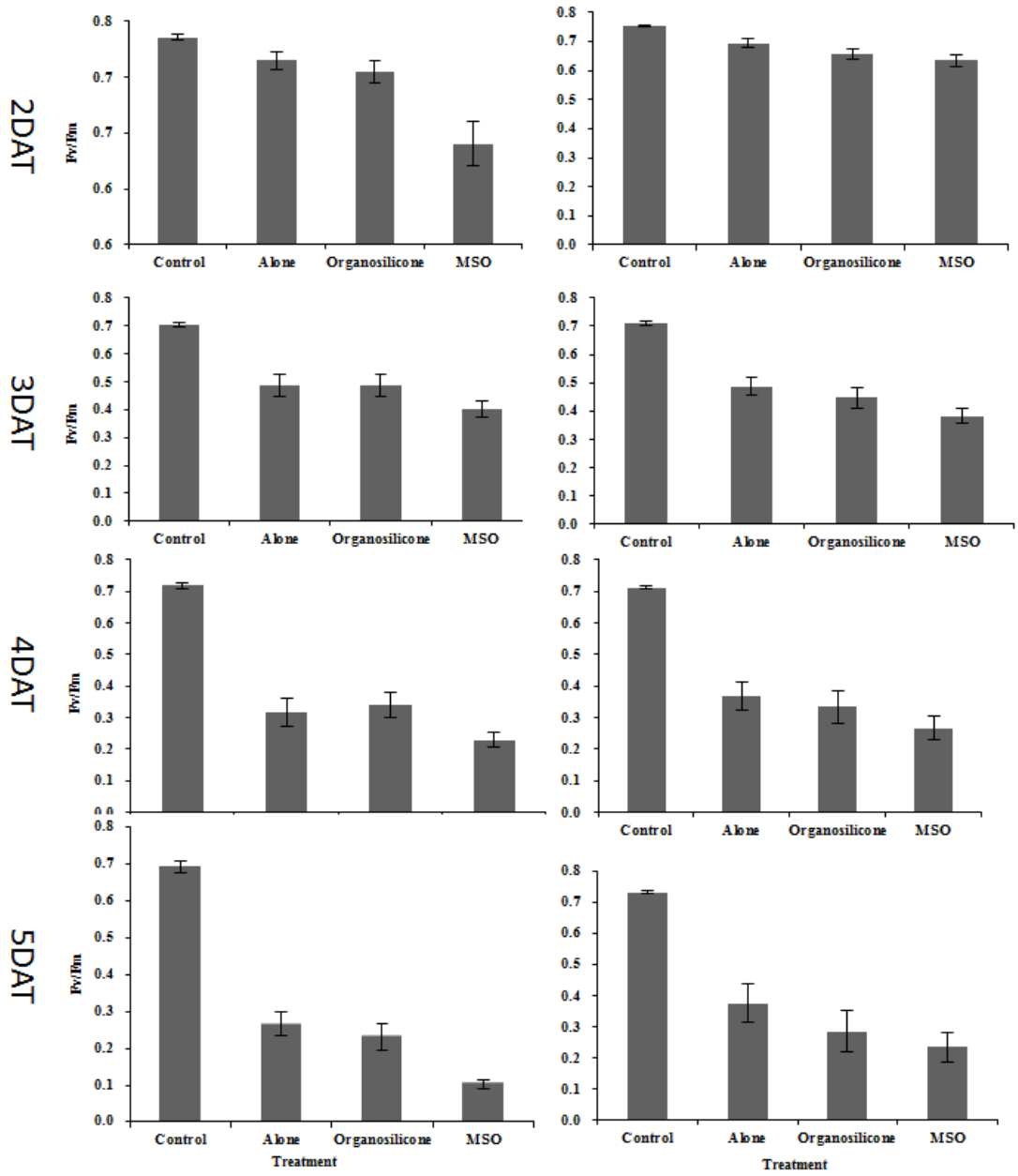

Fig.1. Analysis of Fv/Fm value of giant foxtail (left) and velvetleaf (right) treated by 4 different herbicide solutions during 2-5 days after treatment (DAT). Alone means topramezone was applied alone; Organosilicone means topramezone was applied with organosiilicone adjuvant; MSO means topramezone was applied with MSO adjuvant; control means treated with tap water.

Control Alone Organosilicone MSO

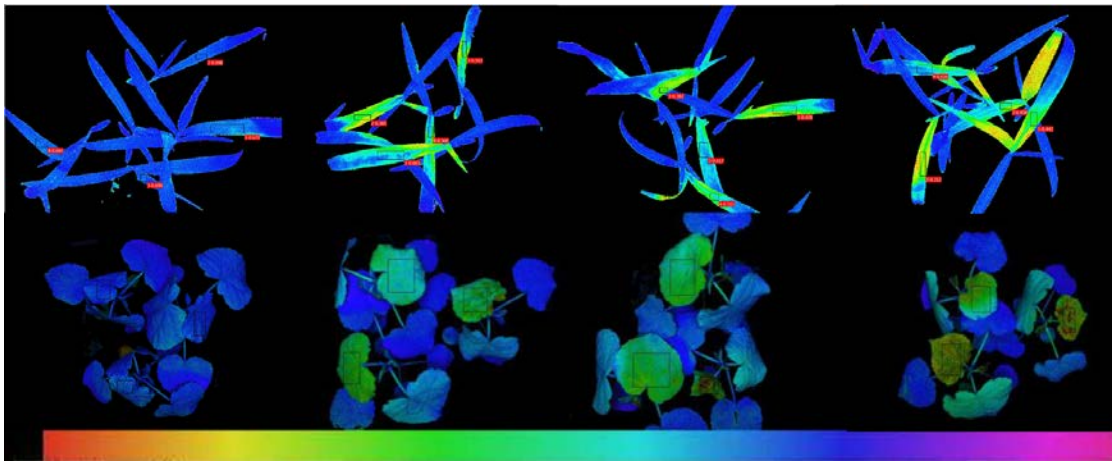

Fig.2. The chlorophyll fluorescence images of giant foxtail (above) and velvetleaf (below) treated by 4 different herbicide solutions at 3 days after treatment (DAT). Alone means topramezone was applied alone; Organosilicone means topramezone was applied with organosiilicone adjuvant; MSO means topramezone was applied with MSO adjuvant; control means applied with tap water.

3.2 The Whole Plant Bioassay

The dry biomass of both weeds treated by topramezone mixed with MSO adjuvant was significantly lower（$P<0.05$）than the treatments of topramezone applied alone and with organosilicone adjuvant, while the difference between treatment of herbicide applied alone and with organosilicone was not significant at 3 weeks after treatment (WAT, Fig.3). The pictures taken at harvest time (3 WAT) also showed apparently that the plants treated by topramezone with adjuvant MSO were injured more severely than the other adjuvant treatments for both weeds (Fig.4 and Fig.5). This result corresponds well with the result of chlorophyll fluorescence measurement.

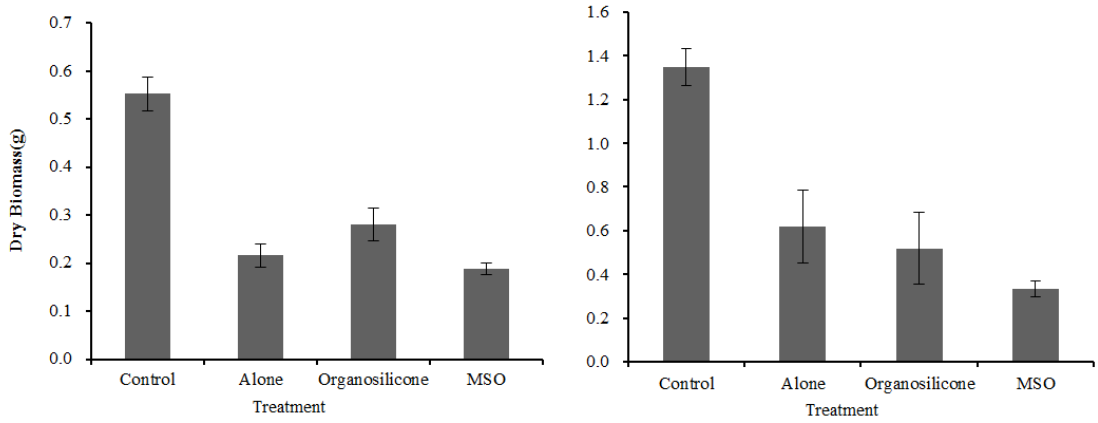

Fig.3 The plant dry biomass of giant foxtail (left) and velvetleaf (right) treated by 4 different herbicide solutions at 3 weeks after treatment. Alone means topramezone was applied alone; Organosilicone means topramezone was applied with organosiilicone adjuvant; MSO means topramezone was applied with MSO adjuvant; control means treated with tap water.

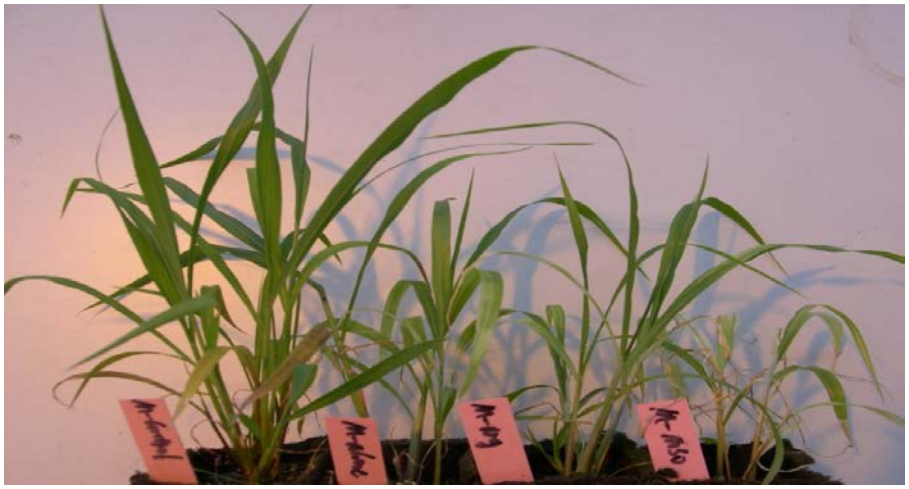

Fig.4. Pictures of giant foxtail taken at 3 weeks after application. M-control means treated with tap water; M-Alone means topramezone was applied alone; M-Org means topramezone was applied with organosiilicone adjuvant; M-MSO means topramezone was applied with MSO adjuvant.

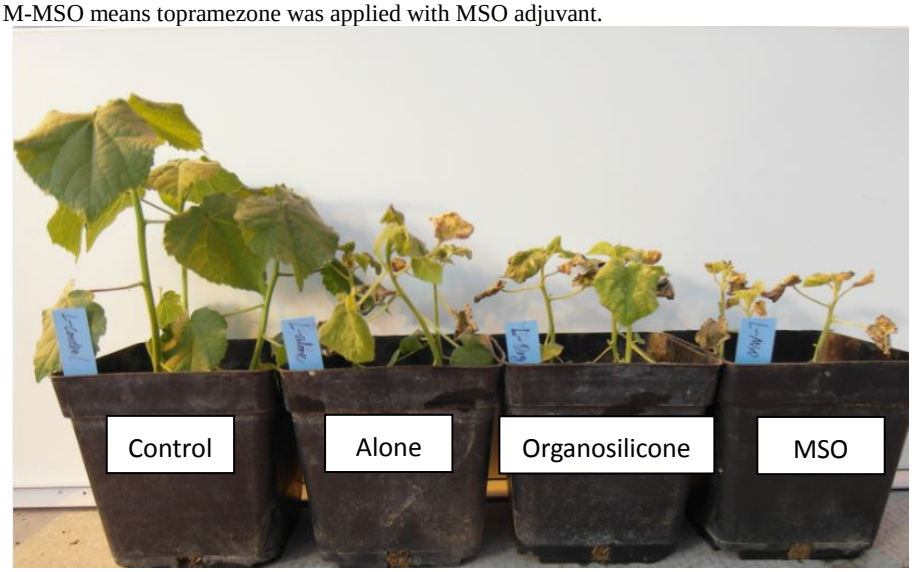

Fig 5 Pictures of velvetleaf taken at 3 weeks after application. Control means treated with tap water. Alone means topramezone was applied alone; Organosilicone means topramezone was applied with organosiilicone adjuvant; MSO means topramezone was applied with MSO adjuvant.

4 Discussion

4.1 Effect of Two Adjuvants on the Efficacy of Topramezone

In order to show the differences among different adjuvant treatments significantly, the topramezone dose we applied was only 1/4 (6.3 g a.i. ha^{-1}) of the recommended dose (22.5-27.0 g a. i. ha^{-1}in China). In spite of this, both the leaf chlorophyll fluorescence measurement and the whole plant bioassay results demonstrated that the MSO adjuvant enhanced the efficacy of topramezone significantly. Zollinger [21] has ever analyzed and demonstrated that MSO adjuvant may give herbicide a better weed control efficacy than other adjuvant types, for instances, 1) low humidity, hot and dry weather, and drought-stressed weeds, 2) herbicide used at reduced rates. So our result is

consistent with the report of Zollinger to most extent. Zhang et al. [22] found that this adjuvant enhanced the efficacy of topramezone on giant foxtail and velvetleaf by changing the solution surface tension, leaf contact angle, the spread areas on weed leaf surfaces, the wetting time, the active ingredient crystal amount, and finally the absorption and translocation. This may be the mechanism of this adjuvant enhancing the efficacy of this herbicide.

As for the result of organosilicone, it has no effect on the efficacy of topramezone. The organosilicone surfactants usually enhances the herbicide efficacy by reducing the surface tension of the spray solution, promoting infiltration of the active ingredient into stomata, and increasing the rate of droplet spreading over the leaf surface [23, 24]. Though there have been a large number of researches demonstrated the good effect of organosilicone on enhancing the efficacy of many different mode of action herbicides, there were also some reports indicated antagonistic action with glyphosate, and understanding of its mode of action involved is still very limited. Hence, the reason (perhaps from aspect of deposition, retention, spreading and evaporation, uptake and translocation and so on) needs further study in the future.

4.2 Chlorophyll Fluorescence Measurement in Evaluating the Effect of Adjuvant on Enhancing Herbicide Efficacy

Christensen et al. [25] had ever done their research on photosynthesis by measuring changes to the chlorophyll fluorescence induction curve (Kautsky curve). It is effective in providing a snap shot of the physiological status of a plant being suffered from various stress, and has been used to test the effect of photosystem II (PSII) inhibitors [26, 27, 28] and herbicides with other modes of mechanism [29]. This method is highly sensitive, fast and non-destructive, and it contains much more information about the photosynthesis which we could not find. In our study, we use this method to test the effect of two adjuvants on the efficacy of topramezone, an HPPD-inhibitor herbicide, and both the Fv/Fm value analysis and the images showed it could differentiate the various treatments well from 2-3 DAT. After conducting a conventional whole plant bioassay experiment in parallel, we found there was high correlation between the result of chlorophyll fluorescence measurement and the whole plant bioassay on both weeds. Hence, to use the chlorophyll fluorescence measurement should be a good method to evaluate the effect of adjuvant on the efficacy of HPPD-inhibitor herbicide. The ability to identify the response of weed to different herbicide treatments at early time after application would provide an opportunity for adjustment of weed management practices before they influence the crop yield. As a result of plants having different response to xenobiotics and the effect of adjuvant on herbicide is also various under different environmental conditions, attention should be paid while using this method.

5 Conclusions

To select a right spray adjuvant for herbicide is an important strategy to enhance the efficacy of herbicides, reduce application dose, and enhance environmental safety. It is interesting in the agricultural and horticultural sector to judge the effect of adjuvant on the efficacy of herbicide with more automated, objective, and sensitive approaches. Weed leaf chlorophyll fluorescence test and whole plant bioassay results both demonstrated that the MSO adjuvant enhanced the efficacy of topramezone significantly on grass weed giant foxtail and broadleaved weed velvetleaf. Both the

Fv/Fm value analysis and the images showed the chlorophyll fluorescence measurement method could differentiate the various treatments well from 2-3 DAT. There was a relatively good correspondence between chlorophyll fluorescence measurement and whole plant bioassay for both weeds. Hence, to use the chlorophyll fluorescence measurement evaluating the efficacy of different herbicide treatments should be a good method for selecting a good adjuvant for herbicide on weed under the normal environmental conditions, but attention should also be paid under different environmental conditions for some weed species. Organosilicone adjuvant had no effect on the efficacy of the herbicide on both weeds, but it is necessary to conduct some study on the reason (perhaps from aspect of deposition, retention, uptake and translocation and so on) and how to apply it more reasonably in the further study.

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