

## Research Article

# Weed control of glufosinate, oxyfluorfen, and paraquat as affected by the application time of day

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### Conflict of Interest:

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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## HIGHLIGHTS

- The application time of day effect for contact herbicides is dose-dependent.
- The contact herbicides are more active at night-time applications than at day-time ones.
- At night-time applications, the application after sunset is preferable to the application before sunrise.

## ABSTRACT

**Background:** The application of appropriately timed herbicide is an affordable approach to optimize the activity of herbicides.

**Objective:** The study aims to determine the best night-time to apply glufosinate, oxyfluorfen, and paraquat with emphasis on the role of the period of dark exposure after herbicide application.

**Methods:** The study was conducted at two fields in Iran, the fresh weight: dry weight (FW/DW) ratio of five weed species treated with glufosinate, oxyfluorfen, and paraquat at 0.5x and 1x of labeled doses after sunset or before sunrise was assessed.

**Results:** The application time-of-day effect for tested herbicides was affected by application dose of herbicide and was more pronounced when the herbicides were applied at the 0.5x as compared to at the 1x. In 14 out of 18 cases, foliage tissues were more desiccated with the sunset applications of herbicides at the 0.5x than with the sunrise applications. When applications were made after sunset, an increased foliage tissues desiccation of *Chenopodium album* L. treated with paraquat (29.6%) in field 1 and *C. album* treated with glufosinate (27.2%) or paraquat (23.7%), and *Malva neglecta* Wallr. treated with glufosinate (21.6%) or oxyfluorfen (31.6%) in field 2 was detected by increasing the dose from 0.5x to 1x. Except 4 out of 18 cases, the herbicides applied either at the 0.5x after sunset or at the 1x before sunrise resulted in similar FW/DW ratio in all weed species.

**Conclusions:** If contact herbicides are used at night-time after sunset, it is possible to take advantage of their reduced doses.

## 1 INTRODUCTION

Chemical weed control is increasing worldwide due to be an affordable option (Kudsk, 2008). There is a recommendation to apply no-tillage systems and/or crop rotation with cover crop (Sims et al., 2018), a

suggestion to remove a failed crop before planting a subsequent crop (Norsworthy et al., 2011), or a tendency to convert annual crop into higher-value perennial crop, e.g. pistachio and saffron in Iran, due to relatively high soil salinity (Shrestha et al., 2018). These can most likely increase the application of

contact herbicides, with little or no activity in soil. Such herbicides belong to the group of Photosystem I inhibitors (D/22), protoporphyrinogen oxidase inhibitors (E/14), and glutamine synthetase inhibitors (H/10) on the basis of WSSA (2012). Although they have different modes of action, they have almost the same mechanism of action in plants. So that, a range of oxidizing species, including  $\cdot\text{OH}$  by electron deflectors at Photosystem I, e.g. paraquat,  $^1\text{O}_2$  by protoporphyrinogen oxidase inhibitors, e.g. oxyfluorfen, and  $^3\text{CHL}$  by glutamine synthetase inhibitors, e.g. glufosinate, in plants treated with these groups of herbicides can be formed. They can initiate a series of reactions, resulting in cell membrane disruption, leading to rapid leaf desiccation and, subsequently, plant death (Cobb and Reade, 2010).

Although contact herbicides are important to agriculture, there is an increasing focus on the non-target effects of them on the fauna, flora, and particularly humans (Ahn et al., 2001; Carpenter and Boutin, 2010; Zhao et al., 2013; Prosser et al., 2016). Therefore, they are considered a global human rights concern (UN, 2017), especially glufosinate, which has been identified as a highly hazardous herbicide since 2009 (PANI, 2018). In this regard, it is important to optimize the activity of contact herbicides for applying them at doses below the labeled dose (1x), leading to the reduction of non-target effects (Kudsk, 2008). This is the first step towards an integrated weed management system (Zhang et al., 2013).

The application of appropriately timed herbicide can be used as an affordable approach to optimize the activity of herbicides. Previous studies have shown that the activity of contact herbicides such as acifluorfen (Lee and Oliver, 1982), bentazon (Stopps et al., 2013), bromoxynil (Stewart et al., 2009), carfentrazone-ethyl, diquat (Wersal et al., 2010), fluthiacet-methyl, flumiclorac (Fausey and Renner, 2001), fomesafen (Miller et al., 2003; Stopps et al., 2013; Cieslik et al., 2014), glufosinate (Martinson et al., 2002; Sellers et al., 2003; Miller et al., 2003; Stewart et al., 2009; Montgomery et al., 2017), ioxynil (Skuterud et al., 1998), paraquat (Norsworthy et al., 2011), and saflufenacil (Montgomery et al., 2017) is significantly affected by the application time-of-day (TOD). As reported in the literature, weeds are more sensitive when they have been treated with the above-mentioned herbicides at night-time than at day-time. Except for fluthiacet-methyl, flumiclorac (Fausey and Renner, 2001) and ioxynil (Skuterud et al., 1998), better weed control has been achieved when herbicides were applied at night-time after

sunset than at night-time before sunrise. Therefore, this background was the first reason why our study was conducted only at the time of sunrise and sunset.

Many factors can affect the application TOD effect for herbicides. It has been established that the absorption of contact herbicides, e.g. fomesafen (Cieslik et al., 2014), fluthiacet, flumiclorac (Fausey and Renner, 2001), glufosinate (Ramsey et al., 2002), and paraquat (Preston et al., 2005), into the plant tissues can increase by increasing air temperature due to the fluidity of cuticle and plasma membrane can increase, resulting in a subsequent improvement in herbicide activity. Also, the absorption of contact herbicides, e.g. acifluorfen (Wills and McWhorter, 1981), fomesafen (Cieslik et al., 2014; Wichert et al., 1992), bentazon (Koukkari and Johnson, 1979), glufosinate (Ramsey et al., 2002) and lactofen (Wichert et al., 1992), into the plant tissues increases as relative humidity increases due to the prolonged drying time of spray droplets, resulting in a subsequent improvement in herbicide activity. In field conditions, diurnal fluctuation in air temperature and relative humidity is negatively correlated (Cieslik et al., 2014). Therefore, it is challenging to conclude which one is responsible for the application TOD effect for herbicides. Dew affecting the application TOD effect often occur at night-time in cool air temperature. It has been hypothesized that a run-off problem may occur when there is much dew on the leaves at the time of herbicide application, resulting in a reduction in herbicide activity. While, the drying time of spray droplets may be longer when there is little dew on the leaves at the time of herbicide application, resulting in improved herbicide activity (Stopps et al., 2013). Wind speed effect is often lower at night-time than at day-time in summer (Waltz et al., 2004). It is common knowledge that the higher the wind speed, the greater the spray exo-drift (Alves et al., 2017). As a result, the spray exo-drift can be significantly reduced with night-time applications (Stopps et al., 2013). For this reason, although its applicability is difficult, there is a recommendation to apply all types of herbicides at night-time, leading to an improvement in herbicide efficacy and/or a reduction in non-target effects (Mohr et al., 2007). On the other hand, leaf orientation at night-time (leaf angle  $> -90^\circ$ ) distinctly differs from those at day-time (leaf angle  $< 0^\circ$ ) due to the so-called foliar nyctinasty (Norsworthy et al., 1999; Sellers et al., 2003; Mohr et al., 2007; Dalazen and Merotto Jr, 2016), affecting spray retention on leaf surfaces and, subsequently, herbicide efficacy. Therefore, this background was the second reason why our experiment was conducted only at the time

of sunrise and sunset. In this way, it will be possible to ignore two important factors affecting the application TOD effect, i.e. wind speed and leaf orientation; instead, we will focus on another factor affecting the application TOD effect, i.e. the period of dark exposure after herbicide application (Wersal et al., 2010). It has been well-established that the application TOD effect for herbicides is also affected by weed species (Fausey and Renner, 2001; Stopps et al., 2013; Montgomery et al., 2017) and mode of action of herbicides (Skuterud et al., 1998; Stopps et al., 2013; Montgomery et al., 2017).

So far, no study has been conducted to evaluate the application TOD effect for oxyfluorfen. Unlike previous studies in this field, the fresh weight: dry weight (FW/DW) ratio of plant was considered in this study to evaluate the application TOD effect on the activity of glufosinate, oxyfluorfen, and paraquat against various weed species. To fill these gaps in knowledge, the current study was performed to determine the best night-time to apply the three above-mentioned contact herbicides, with emphasis on the role of the period of dark exposure after herbicide application.

## 2 MATERIALS AND METHODS

Two field experiments were conducted, one in Namivand-e-Olya village located at 34°24'10"N, 46°44'18"E, 1343 m a.s.l., Kermanshah Province, Iran (hereafter referred to as field 1) and the other in Dastjerd village located at 35°00'33"N, 48°31'34"E, 1774 m a.s.l., Hamadan Province, Iran (hereafter referred to as field 2), both where no crop was planted. In both fields, winter wheat (*Triticum aestivum*) had been planted in the last year. The soil in field 1 was a silty clay soil with 11% sand, 46% silt, 42% clay, 1% organic matter, and pH of 7.4; field 2 was a loam soil with 46% sand, 39% silt, 14% clay, 1% organic matter, and pH of 7.6. The fields were ploughed and disc-harrowed twice in the first week of June 2018 after applying a composted cattle manure at 10 t ha<sup>-1</sup> and triple superphosphate 46% at 50 kg ha<sup>-1</sup>. Then, an overhead sprinkler system was applied to irrigate the field 1 and 2 every six days for a period of five and six weeks, respectively, allowing

to establish natural weed populations from the soil seedbank. The field 1 was predominantly infested with *Chenopodium album*, *Convolvulus arvensis* and *Solanum nigrum*; while the field 2 was predominantly infested with *Amaranthus retroflexus*, *C. album*, and *Malva neglecta*.

In each field, the experiment was arranged in a randomized complete block design with a 3 × 2 × 2 factorial arrangement of treatments using three factors and four replications. The plot size was 2 × 4 m. The factors were comprised of herbicides with different modes of action (glufosinate 1x = 1000 g a.i. ha<sup>-1</sup> (Basta® 20% SL, Bayer), oxyfluorfen 1x = 480 g a.i. ha<sup>-1</sup> (Goal™ 24% EC, Dow), and paraquat 1x = 600 g a.i. ha<sup>-1</sup> (Gramoxone® 20% SL, Syngenta)), application doses of herbicide (0.5x and 1x), and application times of day (before sunrise and after sunset). The application times of day started 15 min after sunset (20:45) on 19 and 26 July and ended 15 min before sunrise (5:45) on 20 and 27 July in the field 1 and 2, respectively. A daily cycle of 15/9 h day/night happened. In this way, the plants treated after sunset were approximately exposed period of 9 h of darkness before they were exposed to light at sunrise on the next day. In each block, a non-treated plot was included to compare the results visually but was not included in data analysis.

The weeds were 6 to 20 cm in height at the time of herbicide application. Herbicide treatments were applied with 260 L ha<sup>-1</sup> at 300 kPa using a compressed-air-sprayer equipped with a low drift 11002-VP nozzle (Abba Spray-Jet, Italy) providing a fine spray quality ranged from 144 to 235 μm. This nozzle type was selected to ignore the factor of wind speed affecting the application TOD effect for herbicides. The atmospheric conditions at the time of herbicide application are shown in Table 1.

The aboveground plant material within four 50 × 50 cm quadrats placed in each plot was sampled at 24, 48, and 96 h after the sunrise application of paraquat, oxyfluorfen, and glufosinate, respectively. To do this, the weed species were carefully identified, clipped about 1 cm above the soil surface, and immediately weighed to obtain the fresh weight.

**Table 1** - Atmospheric conditions at the time of herbicide application

Field	Time of day	Air temperature (°C)	Relative humidity (%)	Wind speed (m s <sup>-1</sup> )	Dew presence
Field 1	Sunset	30	17	0.5	No
	Sunrise	25	19	0.3	No
Field 2	Sunset	28	17	0.8	No
	Sunrise	23	20	0.6	No

Data were recorded in the fields.

Then, they were oven-dried at 70 °C for 48 h and reweighed to obtain the dry weight. As described by Rytwo and Tropp (2001), the desiccation/dehydration of foliage tissues resulted from the action of herbicide can be evaluated by the FW/DW ratio having a starting range from one to up. If this ratio is equal to one, a complete desiccation of plant tissues occurred from the action of the herbicide. The larger the FW/DW ratio, the less evident is the desiccation of plant tissues.

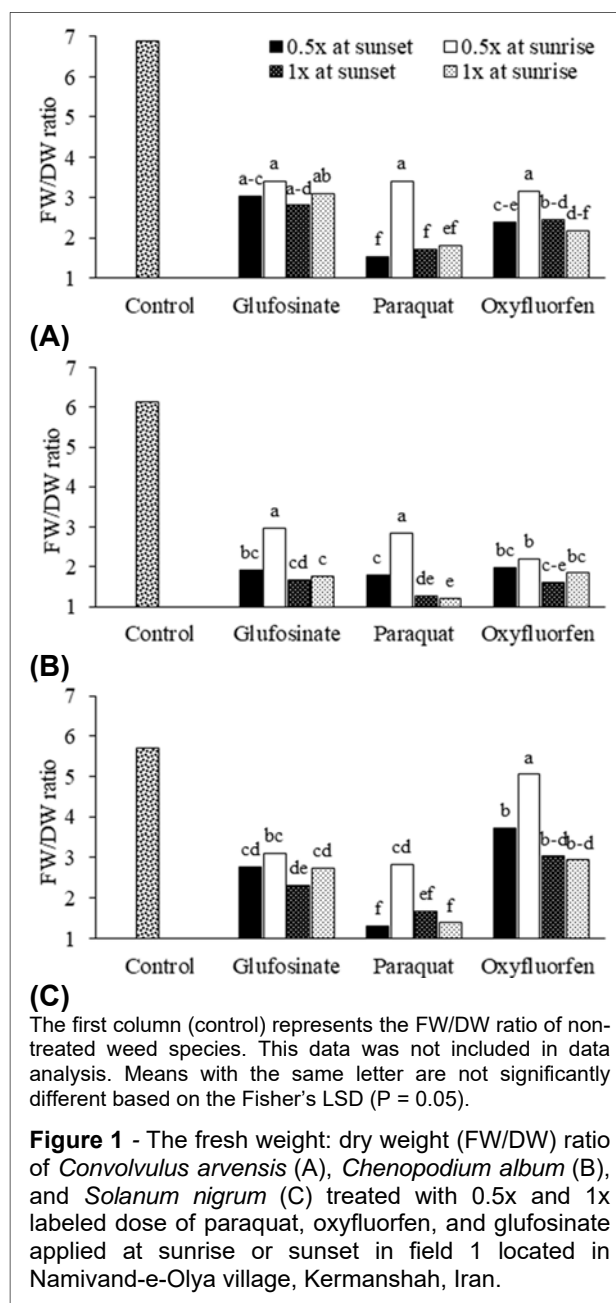
After checking the data for normality, the non-transformed data were subjected to analysis of variance (ANOVA) using the PROC GLM in SAS software. The means were separated using the Fisher's Least Significant Difference (LSD) at the 0.05 level.

### 3 RESULTS AND DISCUSSION

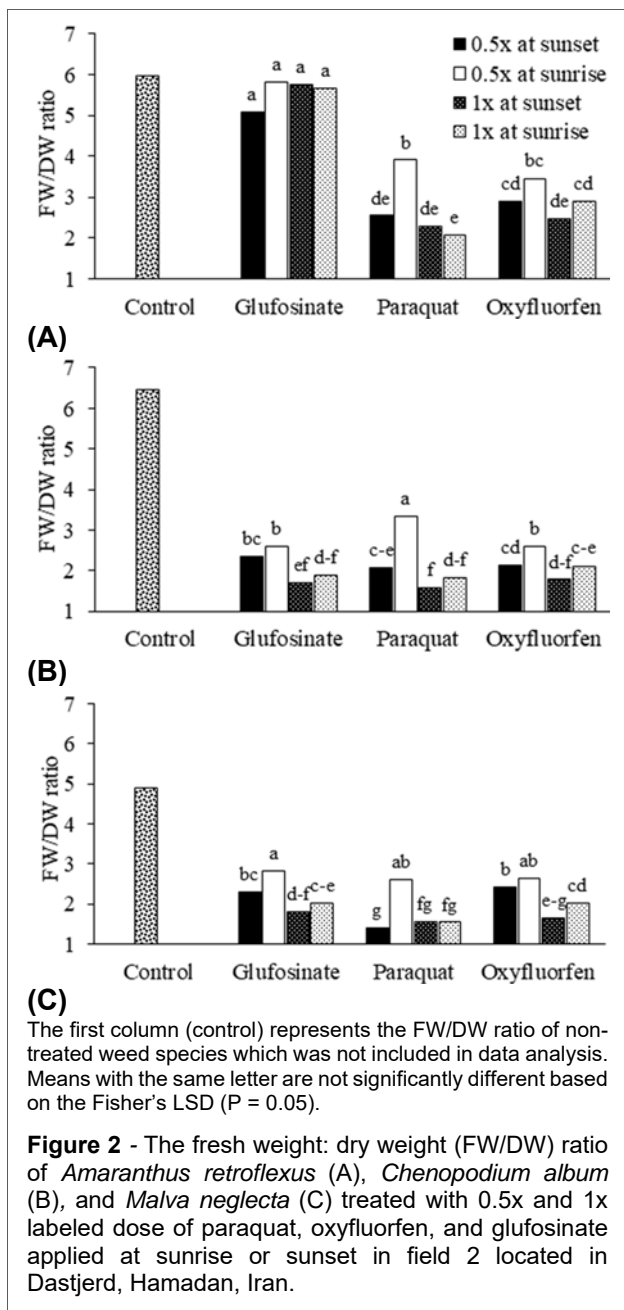
In both fields, ANOVA showed that the FW/DW ratio in all weed species was significantly affected by all simple effects (mode of action of herbicide, application dose of herbicide, and application time of day). The interaction of herbicides and applied doses was significant for the FW/DW ratio of *S. nigrum* in field 1 and *C. album* in both fields. The interaction of herbicides and application time of day was significant for the FW/DW ratio of *C. album* in field 2. Except in the case of *A. retroflexus* in field 2, the interaction of applied doses and application time of day was significant for the FW/DW ratio in all weed species in both fields. In both fields, the three-way interaction was significant in all weed species.

Except for *M. neglecta* treated with oxyfluorfen at 480 g ha<sup>-1</sup> (1x) in field 2 (Figure 2C), our observations in both fields showed that when the herbicides were applied at the 1x after sunset or before sunrise, no significant difference between the FW/DW ratio of weed species was observed, indicating no application TOD effect for herbicides at the 1x (Figures 1A, B, C and 2A, B). In field 1, a foliage desiccation of 77.6 and 50.5% on *C. arvensis* treated with paraquat, 66.2 and 53.0% on *C. arvensis* treated with oxyfluorfen (Figure 1A), 70.8 and 53.6% on *C. album* treated with paraquat, 64.4 and 59.5% on *C. album* treated with glufosinate (Figure 1B), 35.0 and 11.6% on *S. nigrum* treated with oxyfluorfen, 52.3 and 44.7% on *S. nigrum* treated with glufosinate, and 77.1 and 50.6% on *S. nigrum* treated with paraquat (Figure 1C) was detected in comparison with control when they were applied at the 0.5x after sunset and before sunrise, respectively. In field 2, a foliage desiccation of 57.3 and 34.7% on *A. retroflexus* treated with paraquat

(Figure 2A), 67.9 and 48.2% on *C. album* treated with paraquat, 66.8 and 57.1% on *C. album* treated with oxyfluorfen, 64.5 and 57.5% on *C. album* treated with glufosinate (Figure 2B), 52.9 and 41.4% on *M. neglecta* treated with glufosinate, 71.6 and 46.9% on *M. neglecta* treated with paraquat, and 53.4 and 44.1% on *M. neglecta* treated with oxyfluorfen (Figure 2C) was detected in comparison with control when they were applied at the 0.5x after sunset and before sunrise, respectively. In the above mentioned cases in both fields, the plant tissues desiccation from the sunset applications of herbicides at the 0.5x were significantly greater than that from the sunrise applications of them at the 0.5x, indicating an application TOD effect at the 0.5x. Current results suggested the application TOD effect for tested herbicides was affected by application dose of







herbicide. The application TOD effect for herbicides was more evident when they were applied at the 0.5x as compared to 1x. This finding might be related to differences in the surface tension of 0.5x and 1x spray solutions. The surface tension of spray solutions was not measured in this study; however, based on Gauvrit and Lamrani (2008), the surface tension of spray solution decreased by increasing the concentration of commercial formulation of clodinafop-propargyl and fenoxaprop-P-ethyl in a fixed spray volume. It is common knowledge that the lower the surface tension of spray solution, the higher the spray coverage on the leaf surface (Castro et al., 2018). This led us to suppose that the application TOD effect for herbicides at the 1x can be overcome with more coverage of leaf surface. In this regard, a study dealing with surfactant is needed to support

or reject our hypothesis. A similar result was observed by other researchers who reported that the application TOD effect for diquat, carfentrazone-ethyl (Wersal et al., 2010), and glufosinate (Sellers et al., 2003) was discernible when they were applied at doses below the labeled dose. On the contrary, Miller et al. (2003) reported that the application TOD effect for glufosinate and fomesafen did not disappear by increasing the application dose of herbicide.

Dew, wind speed (Table 1) and leaf orientation can not explain variations in herbicide activity to weeds after sunset and before sunrise. Moreover, air temperature and relative humidity at the time of herbicide application created a complex environment to interpret which one is responsible for the application TOD effect for herbicides. However, increased plant tissues desiccation from the sunset applications is most likely a result of increased herbicide absorption and translocation. Light is required to activate contact herbicides (Cobb and Reade, 2010). In this study, the plants treated after sunset were allowed for a 9 h dark exposure period. A dark period following herbicide application can temporarily prevent the activation of them. As a result, it has not the potential to disrupt cell membrane, allowing more herbicide absorption at the point of contact and translocation out of the point of contact. This has been proved in case of paraquat by Preston et al. (2005) and oxyfluorfen by Guh et al. (1995) who reported that a dark period after herbicide application can improve herbicide absorption and translocation. For this reason, the plant tissues desiccation not be limited to the point of contact, allowing more plant tissues desiccation.

In field 1, when applications were made after sunset, only the FW/DW ratio of *C. album* treated with paraquat was significantly reduced (29.6%) by increasing the dose from 0.5x to 1x (Figure 1B). In field 2, when applications were made after sunset, increasing the dose of herbicides from 0.5x to 1x decreased significantly the FW/DW ratio of *C. album* treated with glufosinate (27.2%), *C. album* treated with paraquat (23.7%) (Figure 2B), *M. neglecta* treated with glufosinate (21.6%), and *M. neglecta* treated with oxyfluorfen (31.6%) (Figure 2C). These results indicated that it is more feasible to reduce the labelled doses by 50% for these three herbicides in field 1 than in field 2. This finding might be related to differences in growth stage. In this study, the weeds were allowed to grow for a period of five and six weeks in field 1 and 2, respectively. This is in accordance with Steckel et al. (1997) who reported that *C. album* has been controlled more effectively with

glufosinate at reduced doses when it was 10 cm than 15 cm in height.

In field 2, glufosinate applied at both doses on *A. retroflexus* caused a poor desiccation, varying from 2.5 to 15.0% in comparison with control (Figure 2A). So far, there was no history of glufosinate-resistant *A. retroflexus* in Iran. The most likely explanation for the results is that *A. retroflexus* is a C<sub>4</sub> plant species having negligible rates of photorespiration. In such plant species, a starvation for the amino acid glutamine to biosynthesize proteins is the primary cause for phytotoxicity of glufosinate. While, in C<sub>3</sub> plant species, the formation of <sup>3</sup>CHL disrupting cell membrane is the primary cause for phytotoxicity of glufosinate (Cobb and Reade, 2010; Monaco et al., 2002). It seems that *A. retroflexus* in this study did not have enough time to show treatment effects because it takes a relatively longer time for glufosinate to kill C<sub>4</sub> plant species than C<sub>3</sub> plant species (Wendler et al., 1990).

In conclusion, the most notable observation, except for *C. album* treated with paraquat (Figure 1B) in field 1 and *C. album* treated with glufosinate (Figure 2B), and *M. neglecta* treated with oxyfluorfen (Figure 2C) in field 2, was that all herbicides applied either at the 0.5x after sunset or at the 1x before sunrise resulted in similar FW/DW ratio in all weed species. Current results suggested the application of appropriately timed glufosinate, paraquat, and oxyfluorfen can be used to improve the activity of them against the target species, resulting in a reduction in non-target effects of them.

#### 4 CONCLUSIONS

The application time of day effect for glufosinate, oxyfluorfen, and paraquat was dose-dependent. The application TOD effect for the herbicides was more evident when they were applied at the 0.5x as compared to 1x. They were more active at night-time applications than at day-time ones. At night-time applications, the application after sunset is preferable to the application before sunrise. If contact herbicides are used at night-time after sunset, it is possible to take advantage of their reduced doses.

#### 5 CONTRIBUTIONS

AA, GA, and BEM: contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

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