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Behavior of Sethoxydim Alone or in Combination with Turnip Oils on Chlorophyll Fluorescence Parameter

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Abstract

Sethoxydim is an acetyl-coenzyme A carboxylase (ACCase) inhibitor that changed the shape of the chlorophyll fluorescence curve (kautsky curve) in wild oat (*Avena ludoviciana* Durieu.) in greenhouse experiment. This experiment was conducted as completely randomized factorial design with three replications at the College of Agriculture, Ferdowsi University of Mashhad, Iran, during 2012. Results of this study revealed that sethoxydim only and plus emulsifiable turnip oil changed the shape of the chlorophyll fluorescence curve (kautsky curve) 7 days after spraying. Sethoxydim plus emulsifiable turnip oil changed the shape of the kautsky curve more than for sethoxydim only. We found that in our study the fv/fm (maximum quantum efficiency) was closely linked to the fresh and dry weight dose-response. Sethoxydim plus emulsifiable turnip oil proved more rapidly effect on fv/fm in comparison with sethoxydim only. The fresh and dry weight dose-response relationship with fv/fm showed a similar behavior. This study revealed a good relation between fresh and dry weight according with values of 28 DAS and fv/fm 7 DAS. In general, the findings of this study revealed that Fv/Fm is a good parameter for evaluating effect of sethoxydim little time after spraying. Also, this research showed that 4 folds more time for classical screening methods comparing to chlorophyll fluorescence method. Thereupon, classical screening methods may be replaced by chlorophyll fluorescence method in future.

Keywords: kautsky curve, sethoxydim, turnip oil, wild oat

Abbreviation: DAS=days after spraying

Introduction

Among the most harmful weeds in the world (18 species), 10 species that belongs to Poaceae family. Wild oat is the most important among these 10 species weeds in more than 20 crops in 55 countries (Salehian and Eshaghi, 2012). Also wild oat is the most important grass weed in Iranian cropping systems (Bijanzadeh *et al*., 2010). Among the methods of weed management, the application of herbicides is the most common method in Iran (Baghestani *et al*., 2008).

Sethoxydim is a selective post emergence and foliar-absorbed herbicide that belongs to aryloxy phenoxy propionate (AOPP) group that was registered for numerous broad-leaved crops including cotton, soybean, canola, alfalfa, sunflower, sugar beet, tobacco, ornamental trees, shrubs, flowers and ground cover (Senseman, 2007). It inhibits the enzyme acetyl coenzyme-A carboxylase and disrupts fatty acid biosynthesis in grasses such as wild oat (*Avena fatua* L. and *Avena ludoviciana* Durieu.), bahiagrass (*Paspalum notatum*), crabgrass (*Digitaria sanguinalis*), downybrome (*Bromus tectorum*), quackgrass (*Elytrigia repens*), annual ryegrass (*Lolium multiflorum*) and witchgrass (*Panicum* spp.) (Basf, 2000; Senseman, 2007) but dicotyledonous species are not sensitive to sethoxydim and it doesn't have effect on broadleaf herbs and crops. Non-susceptible broadleaf species have a different acetyl CoA carboxylase binding site rendering them immune to the effects of sethoxydim. Increasing the effectiveness of the post-emergence herbicides such as sethoxydim, imazamethabenz-methyl and sulfosulfuron by approved vegetable oils were reported (Izadi-Darbandi *et al*., 2013). So, use of vegetable oils is known as a tool for reducing herbicide usage which allows to decrease the environmental risk (Izadi-Darbandi *et al*., 2013).

Chlorophyll fluorescence method is non-destructive with high sensitive, fast and easy to measure, and it contains important information about the photosynthetic apparatus (Barbagallo *et al*., 2003), so researcher researchers would be able to quickly prescribe herbicide performance before the visual symptoms appear in plants (Riethmuller-Haage *et al*., 2006). Chlorophyll fluorescence can be altered by many processes that are not directly involved in photosynthesis (Crudace, 2000) so evaluation of changes to the

chlorophyll fluorescence curve (kautsky curve) can be used for study of the effect of the herbicides with each mode of action (Barbagallo *et al*., 2003). For example Barbagallo *et al*. (2003) showed change in chlorophyll fluorescence parameters in Mousear Cress (*Arabidopsis thaliana*) seedlings by asulam, bifenox, 2,4-D, glyphosate, diclofop-methyl, and imazapyr. Also, Avarsaji *et al*. (2012) reported the same with Dicamba+2, 4-D herbicides on wild mustard (*Sinapis arvensis*), which don't have a direct impact on photosynthesis. The advantage of this approach is represented by the possibility of using a portable instrument with rapid collection of data and advanced software enabling statistical analysis (Klem *et al*., 2002).

Kautsky curve has three phases based on the O, J, I, and P steps (Fig. 1). These phases indicate photochemical events related to PSII (Govindjee, 1995) and interpreted as follows: (I) (O–J) phase corresponds to a complete reduction of the primary electron acceptor QA of PSII, this phase takes place within 50 μs to 2 ms, (II) (J–I) phase corresponds to electron transfer from QA to QB (the release of fluorescence quenching during the [J–I] phase is controlled by the PSII donor side, This phase takes place within 2 to 30 ms and (III) (I–P) phase corresponds to the release of fluorescence quenching by the oxidized plastoquinone pool that takes place within 30-500 ms (Fig.1) (Avarseji *et al*., 2012; Elahifard *et al*., 2013; Abbaspoor *et al*., 2006; Abbaspoor and Streibig, 2005).

Illumination of dark-adapted leaves produces a rise in fluorescence from the ground state (Fo) at the O step to its maximum value (Fm) at the P step within a second. Under this condition, QA is completely reduced and the value of maximum quantum efficiency (Fv/Fm) can be determined, this value in all unstressed leaves plants, independent of species, is approximately equal to 0.83 (Abbaspoor and Streibig, 2005; Appenroth et *al*., 2000; Strasser and Stirbet, 2001). The shape of the kautsky curve is affected by various factors, such as temperature, water stress, pathogens and herbicides (Abbaspoor and Streibig, 2005).

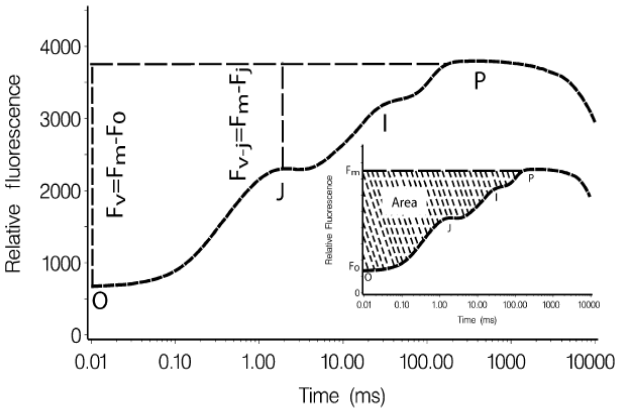

Fig. 1. Chlorophyll fluorescence curve (kautsky curve) recorded with Handy PEA instrument in a 30 min dark-adapted leaf (adopted by Abbaspoor and Streibig, 2005)

J step [Fvj= (Fm -Fj)/Fm] (Fig. 1) have been selected to be a common response parameter for an herbicide with various modes of actions (Christensen *et al*., 2003).

The goals of this study were to assess the effect of sethoxydim and sethoxydim plus turnip oil on the shape of the kautsky curve in wild oat and related parameters.

Materials and methods

Plant growth

About 200 gr wild oat caryopsis fruit were collected from plants in the field adjacent to the Research Greenhouse at the Ferdowsi University of Mashhad, Iran and preserved in a refrigerator (at 4±1 °C). To break seed dormancy before the start of experimentation, caryopsis fruits were dehulled and seeds were placed in 11 cm diameter Petri dishes over the surface of a single layer of Whatman no. 1 filter paper. Ten ml of KNO_3 solution (2g L^{-1}) were added to each Petri dish and they were placed in a refrigerator at 4-5 °C in the dark for two days and then transferred to an incubator with 20/10 °C temperature in 45/65% relative humidity for a 16/8 h day/night for germination (Hammami *et al*., 2011). Five seeds were sown in potting trays (3×3×5 cm) filled with moistened peat. One week after sowing, when the seedlings had one leaf, each of them were transplanted in 2 L plastic pots that were filled with a mixture of sand, clay loam soil, and peat (1:1:1; v/v/v). The pots were irrigated every three days with tap water. The seedlings were thinned to five per pot at the two leaf stage and 40 mL of a water-soluble N:P:K (20:20:20) fertilizer, at a concentration of 3 g of fertilizer per liter of tap water, were supplied to each pot. The greenhouse temperature varied from 24±3 °C during the day and 16±2 °C at night.

Dose-response study

Sethoxydim (Nabo-S, 12.5% EC, Basf, Germany) treatment consisted of six doses against wild oat (0, 45, 94, 187, 281, and 375 g ai ha^{-1}). The experiment was arranged in a randomized complete factorial design with three replications and carried out in a greenhouse at the Faculty of Agriculture, Ferdowsi University of Mashhad, Iran in 2012. Turnip (*Eruca sativa* L.) oil seed was extracted via mechanical extraction method (Kemper, 2005). A non-ionic emulsifier, Citogate (100% alkyl aryl polyglycol ether prepared from Zarnegaran Pars Company, Karaj, Iran) was added to the turnip oil at 10% (v/v), and 0.5% (v/v) of this compound (90% vegetable oil + 10% emulsifier) was added to the sethoxydim solution. The experimental treatments were sethoxydim doses in six levels at 0 (control), 45, 94, 187, 281, and 375 g ai ha^{-1} with and without emulsifiable turnip oil. The spray treatment was done at the four leaf stage by using an overhead trolley sprayer (Matabi 121030 Super Agro 20 L sprayer; Agratech Services-Crop Spraying Equipment, Rossendale, UK), equipped with an 8002 flat fan nozzle tip delivering 240 L ha^{-1} at 2 bar spray pressure. Four weeks after spraying, the control and treated plants above-ground biomass from each pot (all of the plants in each pot) were harvested and weighted (fresh weight) then oven dried at 75 °C for 48 h and reweighed (dry weigh).

Statistical analysis

Weight data of all the herbicide treatments were subjected to non-linear regression analyses by using a logistic dose-response model (Kudsk and Mathiassen, 2004):

$$U = C + \frac{D - C}{1 + \exp[b(\log(z) - \log(ED_{50}))]}$$

where, U is the plant response to the herbicide treatment, z is the dose, D and C are the upper and lower limits of the curve respectively. ED_{50} denotes the required dose of herbicide to give 50% wild oat control and b is proportional to the slope of the curve around the ED_{50}.

Fluorescence measurement

Chlorophyll fluorescence was measured using a portable chlorophyll fluorometer (Handy-PEA, Hansatech Instruments, King's Lynn, Norfolk, UK) after at 1, 2, 3, 5 and 7 DAS which emits a light of 650 nm wave length with an intensity of 3000 μmol photons m^{-2} s^{-1} for 10 seconds on dark-adapted leaves (30 minutes dark adapted by covering the leaves with a clip). Kautsky curves and their parameters, obtained by the pea plus program. The parameter analyzed in this experiment was Fv/Fm= (Fm-F0)/Fm where Fm: maximum fluorescence and F0: ground state fluorescence (Fig. 1).

Results and discussion

Dose-response study

The results from this research revealed that when sethoxydim was combined with emulsifiable turnip oil, wild oat control was considerably increased. The ED_{10}, ED_{50} and ED_{90} values of sethoxydim were remarkably decreased (Tab. 1). The performance of sethoxydim (Nabo-S, 12.5% EC, Basf, Germany), in the presence of emulsifiable turnip oil, were equal to 2.285 and 2.876 fold compare with sethoxydim alone, for fresh weight and dry weight respectively (Tab. 1). The results indicated that emulsifiable turnip oil has potency in enhancing the foliage activity and reducing biomass wild oat of sethoxydim. The improvement of the tested sethoxydim plus emulsifiable turnip oil may be related to a theory which shows the solubilizing, softening or disrupting nature of cuticular waxes by the methylated seed oils (Hazen, 2000).

Chlorophyll Fluorescence study

The katusky curves

Sethoxydim affected the shape of the kautsky curves significantly at 7 DAS at all doses (Fig. 2), although the onset of these changes was observed at 2 DAS (Fig. 2). By increasing the dose of the Sethoxydim OJIP steps of the kautsky curve were eliminated; at the recommended dose (375 gr. ai. ha^{-1}) the kautsky curves turned into approximately straight lines (Fig. 2).

The effect of sethoxydim plus emulsifiable turnip oil on the shape of the kautsky curve (Fig. 3) clearly showed that the curves were severely affected at much lower doses than by comparison with sethoxydim alone (Fig. 2) (kautsky curves changed at sethoxydim plus emulsifiable turnip oil more rapidly than at sethoxydim alone).

The findings of this study revealed the non phytotoxic effect of emulsifiable turnip oil on wild oat because when emulsifiable turnip oil was applied only, kautsky curves didn't change in compare control (Fig. 2, 3). Likewise, Izadi-Darbandi *et al.* (2013) reported that emulsifiable turnip oil non phytotoxic effect on wild oat (*Avena ludoviciana* L.) With the difference that chlorophyll fluorescence method was non-destructive, high sensitive, fast and easy to measure compare the measuring fresh and dry weight (Barbagallo *et al*., 2003). Actually in according to findings of this study use of chlorophyll fluorescence method supplies the measuring of sethoxydim and sethoxydim plus emulsifiable turnip oil effect after 7 DAS, whereas more than 4 folds time needs for measuring of sethoxydim and sethoxydim plus emulsifiable turnip oil with use of measuring fresh and dry weight (Izadi-Darbandi *et* al., 2013). In general, sethoxydim and sethoxydim plus emulsifiable turnip oil changed kautsky curves but changes for sethoxydim plus emulsifiable turnip oil happen more rapidly by comparison with sethoxydim only.

The fluorescence parameters

The inhibition of acetyl CoA carboxylase prevents fatty acid production, which leads to I) failure of cell membrane integrity especially in regions of active growth II) breakdown of membrane and accumulation of polyunsaturated fatty acids III) produces reactive oxygen species (ROS) with lipoxygenase activity on polyunsaturated fatty acids (Theodoulou *et al*., 2003; Luo *et al*., 2004; Senseman, 2007).

Tab. 1. The ED10, ED50 and ED90 (g a.i. ha^{-1}) of sethoxydim alone and in mixture with turnip oil against wild oat (*Avena ludoviciana* L.)

Herbicide + vegetable oil	ED_{10} (g a.i. ha^{-1}) ± SE	ED_{50} (g a.i. ha^{-1}) ± SE	ED_{90} (g a.i. ha^{-1}) ± SE
Dry Weight			
Sethoxydim alone	32.18 ±2.88	80.00 ±2.85	222.16 ±2.16
Sethoxydim + turnip	4.86 ±1.12	27.81 ±0.13	139.82 ±1.06
Fresh Weight			
Sethoxydim alone	47.60 ±1.14	100.93 ±1.43	215.83 ±1.56
Sethoxydim +turnip	12.01 ±0.38	44.17 ±0.08	88.93 ±0.31

The Turnip oil added at 0.5% (v/v) that 5% of the vegetable oils were non-ionic alkyl aryl polyglycol ether emulsifier.

Due to the production of ROS, numerous damaging reactions are initiated, collectively called oxidative stress, that causes a destruction of the electron transport chain from PSII to PSI (Sofo *et al.* 2004). This destruction has an influence on chlorophyll fluorescence, probably allowing the detection of herbicide efficacy by measuring maximum quantum efficiency of PSII. So, chlorophyll fluorescence can be used as tool for detection of herbicide performance with other mode of action such as acetyl coenzyme-A carboxylase inhibitor (Abbaspoor and streibig, 2005), phenoxy (Avarseji *et al.*, 2012) and glyphosate (Christensen *et al.*, 2003).

Changes in the shape of the kautsky curves are described in Figs. 2, 3. Important fluorescence parameters Fv/Fm was plotted at the range of doses of sethoxydim at 1, 2, 3, 5 and 7 DAS and relationship between Fv/Fm with fresh and dry weight is shown in Figs. 4, 5, 6. The Fv/Fm was greatly decreased as the dose was increased in sethoxydim plus emulsifiable turnip oil in compare with sethoxydim only (Figs. 2, 3). The value of the Fv/Fm parameter is about 0.83 in healthy leaves, regardless of plant species (Abbaspoor and Streibig, 2007; Stribet and Govindjee, 2011) but it is reduced under stress conditions such as high temperature, salinity, drought and herbicides.

In this experiment, Fv/Fm-values of the control plants

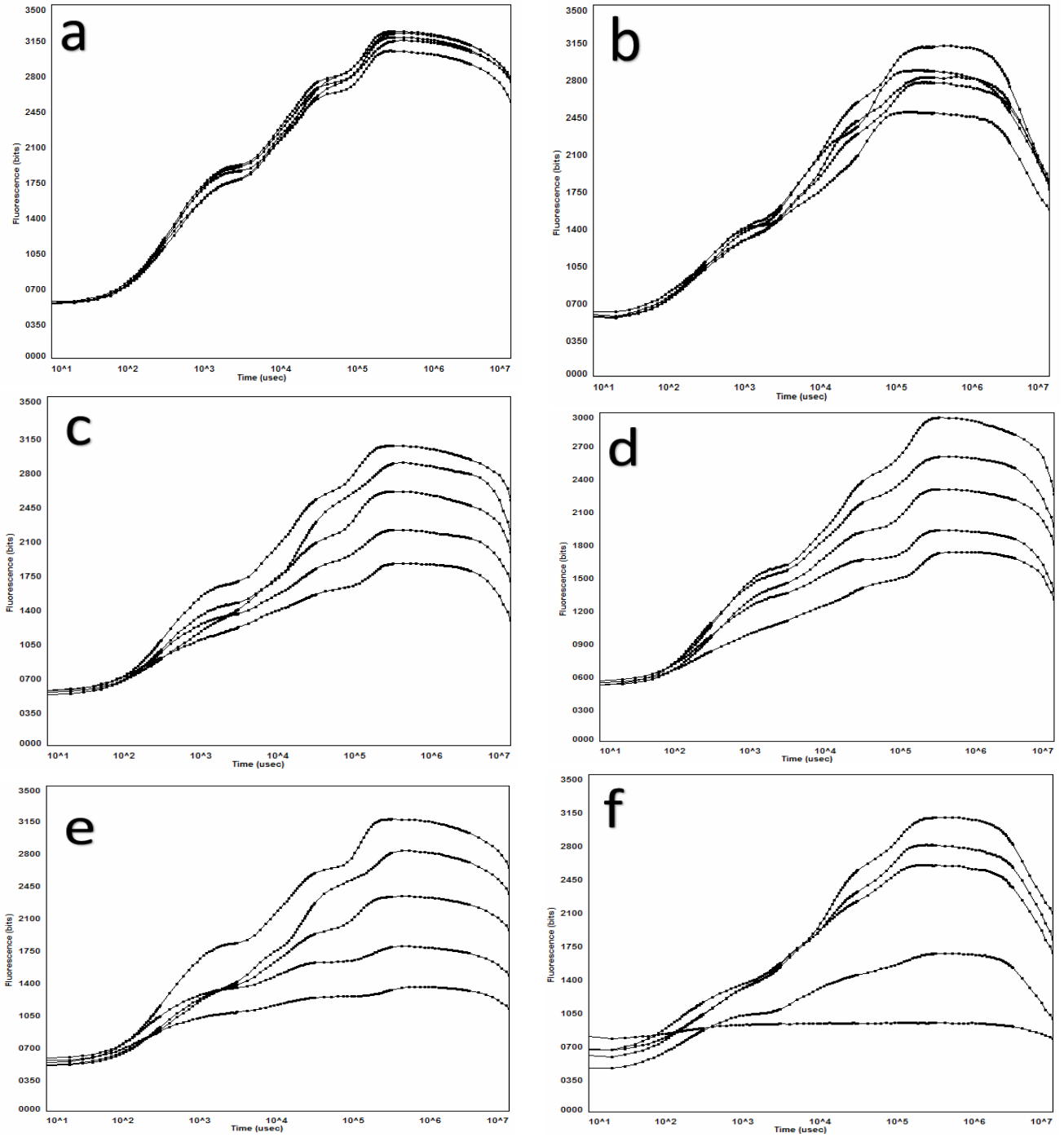

Fig. 2. Effect of sethoxydim only on the shape of the katusky curve in wild oat at 1, 2, 3, 5 and 7 days after treatment: a; 0, b; 45, c; 94, d; 187, e; 281 and f; 375 gr a.i. /ha

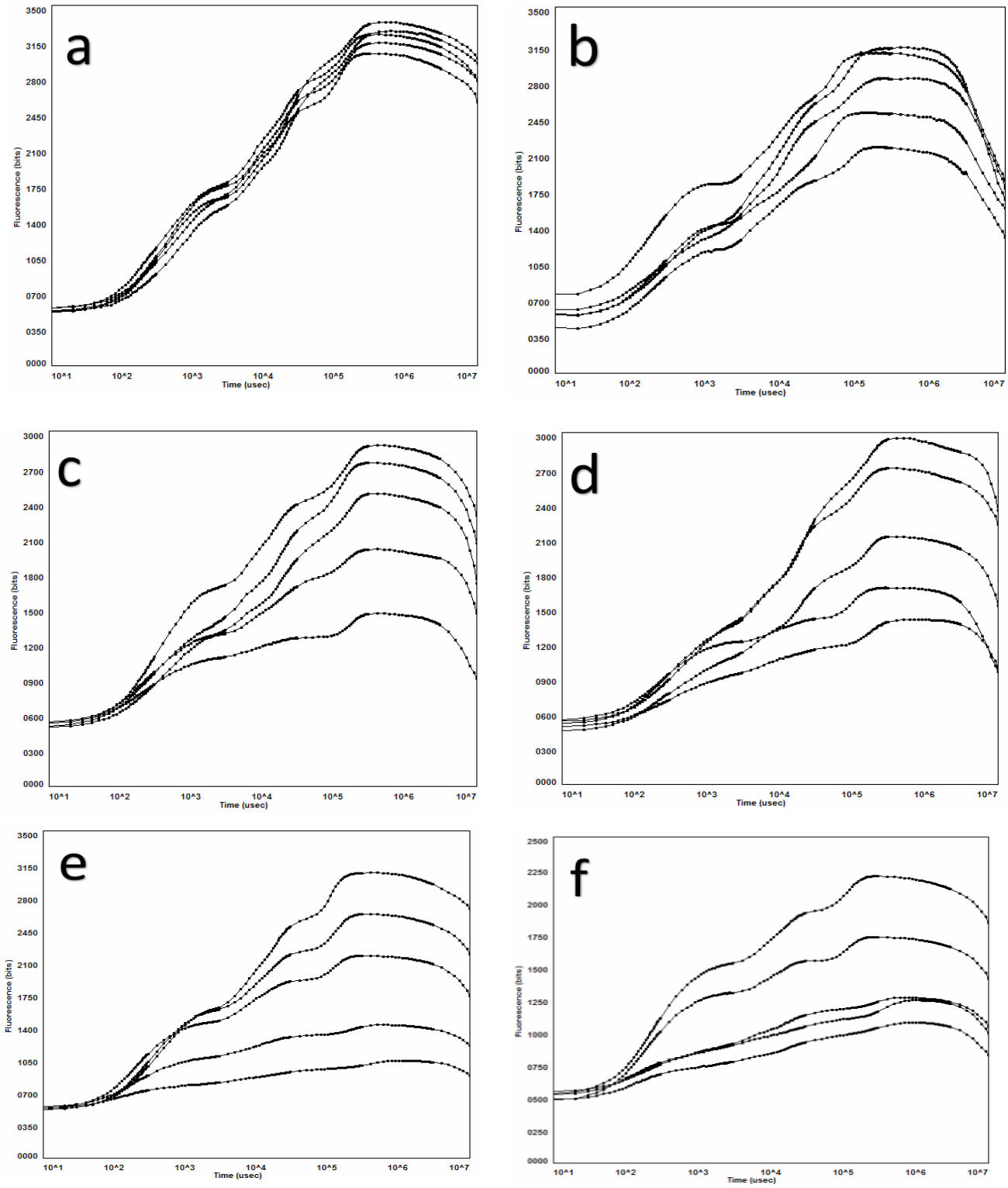

Fig. 3. Effect of sethoxydim plus turnip oil on the shape of the katusky curve in wild oat at 1, 2, 3, 5 and 7 days after treatment: a; 0, b; 45, c; 94, d; 187, e; 281 and f; 375 gr a.i. /ha

were between 0.835 and 0.846 at all times of measurement. By increasing the dose of the sethoxydim Fv/Fm-values were decreased whereas after 7 DAS Fv/Fm of 0.846 (control) get to 0.479 and 0.547 at the recommended dose (375 g. ai. ha^{-1}) with and without emulsifiable turnip oil respectively. Rate of decrease Fv/Fm-values for applied emulsifiable turnip oil as adjuvant was higher compared sethoxydim alone. Fv/Fm decreased with past time as 7 DAS at least this parameter was observed (Fig. 4).

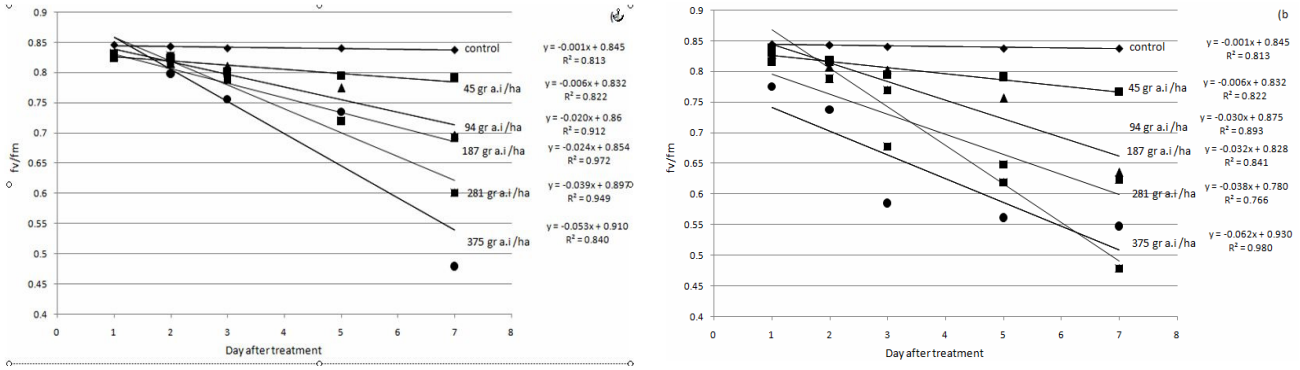

Fig. 4. Relationship between Fv/Fm parameter and days after treatment: a; sethoxydim only and b; sethoxydim plus turnip oil

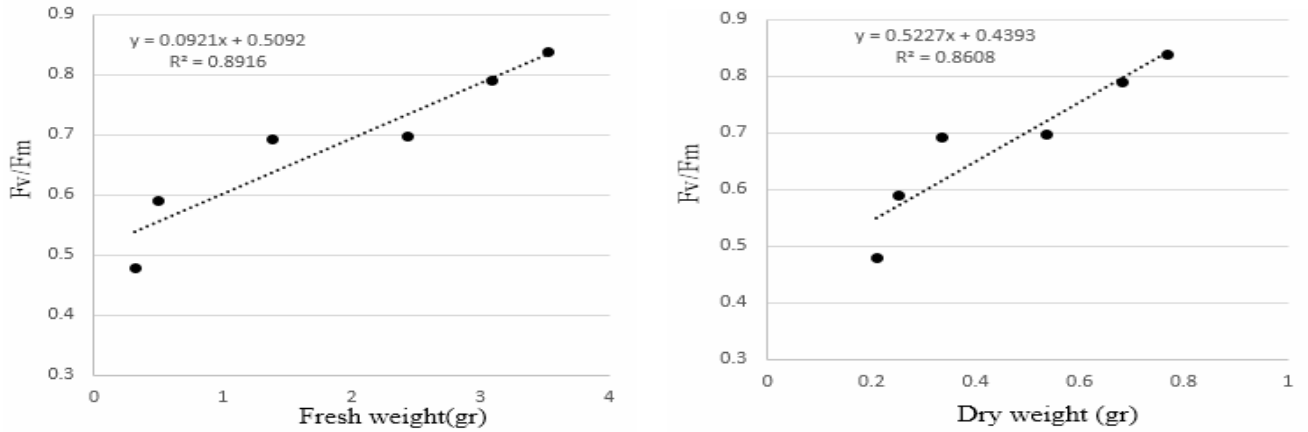

Fig. 5. Relationship between Fv/Fm parameter and fresh and dry weight at 7 days after spraying on sethoxydim only

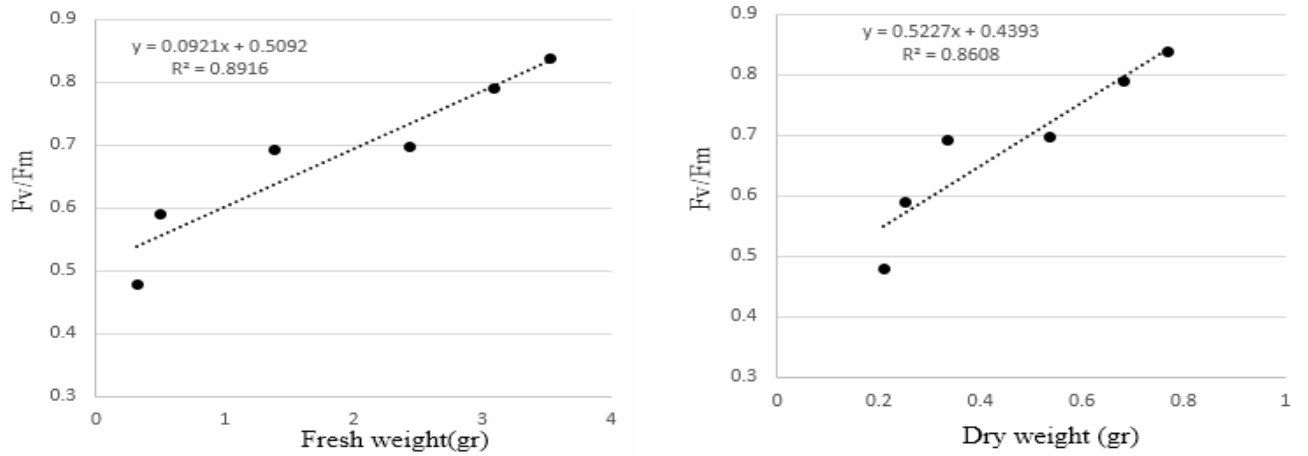

Fig. 6. Relationship between Fv/Fm parameter and fresh and dry weight at 7 days after spraying on sethoxydim plus emulsifiable turnip oil

The relationship between fresh and dry weight with fluorescence parameter

Figs. 5, 6 illustrate the relationships between fresh and dry weight (taken at 28 DAS) with fluorescence parameter (Fv/Fm) taken at 7 DAS for sethoxydim and sethoxydim plus turnip oil, respectively. The slope of the curves is steeper for sethoxydim plus turnip oil compared sethoxydim alone. A linear relationship between Fv/Fm with fresh and dry weight is evident. Other researchers, also, used relationship between fluorescence parameter and dry weight for bentazone (Christensen *et al*., 2003), metamitron and terbuthylazine (Abbaspoor *et al*., 2006), clodinafop (Abbaspoor and Streibig, 2005), desmedipham and phenmedipham (Abbaspoor and Streibig, 2007) and fresh weight (Elahifaru *et al*., 2013) in their experiments.

Conclusions

Based on available information and experimental evidence classical screening methods can be replaced by Chlorophyll Fluorescence studies. Because Chlorophyll Fluorescence method is a non-destructive, high sensitive

and fast compared to classical screening methods. The findings of this study revealed that Fv/Fm is a good parameter for evaluating the effect of herbicide shortly after spraying. Also this research showed that 4 folds more time for classical screening methods compared to chlorophyll fluorescence method. So the use of chlorophyll fluorescence method may be increased in herbicide bioassay studies in the future. Based on conducted studies by various researchers due to the production oxidative stress, numerous damaging reactions are initiated that cause an interruption of the electron transport chain from PSII to PSI, so chlorophyll fluorescence method can be used for herbicides with various mode of action. Finally, the linkage between fresh and dry weight and the fluorescence parameters may be used to shorten the screening experiments times for ACCase inhibitors.

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