© by PSP Volume 30– No. 01/2021 pages 707-715 Fresenius Environmental Bulletin

FLUORESCENCE AND CHLOROPHYLL CONTENT AS INDICATORS OF THE EFFICACY OF PLANT EXTRACTS OF *AMBROSIA ARTEMISIIFOLIA* AND *SORGHUM HALEPENSE* IN WEED CONTROL

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ABSTRACT

Efficient weed control is essential in agricultural production. However, bearing in mind the economic, environmental and health implications of classical chemical weed control, the aim of this study was to test the potential use of plant extract solutions for weed control in wheat. Plant extracts of two widespread and invasive weed species: *Ambrosia artemisiifolia* and *Sorghum halepense* were used to test the efficacy of these bioherbicides on three weed species common in wheat fields: *Avena fatua*, *Bromus rigidus* and *Convolvulus arvensis*. Parameters generally used for testing the efficacy of commercial herbicides: relative chlorophyll content, chlorophyll fluorescence and pigment content, were used to evaluate the efficacy of plant extract solutions, in a series of concentrations (1, 5, 10 and 20%). Results have shown that plant extracts of both tested species have negative effects on target weed species, with all tested concentrations of these solutions affecting the recorded parameters in *B. rigidus* and *C. arvensis*. However, as negative effects of *A. artemisiifolia* extract solutions were also documented on the crop plants, only plant extracts of *S. halepense* can be considered as a safe and efficient alternative option for weed control in wheat fields.

KEYWORDS:
Common ragweed, Johnson grass, Pant extracts, Plant pigments, Chlorophyll fluorescence, SPAD readings

INTRODUCTION

Wheat is the most important crop in food consumption worldwide and the second most widespread agricultural crop, behind maize [1]. Due to its nutritional value and large cultivation areas, weed control in wheat is a critical step in its production [2]. However, it is well-known that none of the weed control methods individually can ensure a completely weed-free environment, nor free the soil of the weed seeds and vegetative remains [3, 4]. Consequently, the idea of integrated weed control, based on complementary technologies, is increasingly prevalent in practice [5, 6, 7].

The most effective way for weed control is still chemical weed control. However, herbicide application, while reducing the weed population for a period of time, significantly increases the production price, exacerbates environmental pollution and the onset of herbicide resistance [8, 9, 10, 11]. Therefore, including bioherbicides in agricultural practice could result in a restoration of biodiversity in intensively cultivated conventional agroecosystems, while simultaneously resulting in higher yields [12]. The first idea of using bioherbicides in agricultural production appeared in the 1960s and 1970s, [13, 14, 15] while the first experiments in weed control (*Rumex* spp.) were performed in USA in the 1960s [13] and in Chile in the 1970s (*Rubus* spp.) [15]. Bioherbicides are produced using the whole plant, its parts, metabolites or microorganisms and their toxins [16]. Some bioherbicides can already be found on the market, e.g. the Corn gluten meal (CGM) [17], acetic acid in concentrations of 5-20% [18] and citrus oil (D-limonene) [19]. In general, bioherbicides have already found their application in organic agriculture systems [20, 21], achieving the expected results after a year or two, when a decrease in weed seedling density can be recorded, due to a reduction in the seed bank potential [12].

Consequently, bearing in mind the significance of finding an environmentally acceptable solution for food production, the possibility of using plant extracts for weed control in wheat was tested. Invasive weed species which cause significant problems in agriculture and the environment (*Sorghum halepense* (L.) Pers. and *Ambrosia artemisiifolia* L.) were selected for testing weed control by applying their aqueous plant solutions. This research could result in a valuable solution to the problem of weed infestations in wheat, and the agricultural production

overall, while simultaneously preserving the environment and reducing the harmful effects of chemicals on human health.

MATERIALS AND METHODS

Seeds of weed species *Avena fatua* L. (AVEFA)*, Bromus rigidus* Roth (BRORI) and *Convolvulus arvensis* L. (CONAR). were collected during July 2018 in Tarhouna (Libya). The seeds were cleaned and stored in an underground storage facility. Crop susceptibility testing plants (wheat) were grown from seeds of the Simonida variety (*NS SEME*, Serbia). Weed and wheat seedlings were obtained from seeds and grown in a growth chamber (Conviron, CMP 3032, S 10 H), under controlled conditions (day/night 12/12 h, temperature 25/22ºC) and watered as needed. The seeds were sown in pots (2 L volume), filled with a commercial growth substrate (Freepeat: NPK 1 kg/m^3, pH 5.2-6.0, salt content 0.6-1.1 g/l), and later hand-pulled to reach a two plants per pot density (a total of six plants per each treatment). CONAR plants were treated at the 4-6-8 leaves growth stage, grass weed species when they reached a 10-15 cm height and wheat at the growth stage 12-13, according to the BBCH scale. Application of herbicides and aqueous extracts was done using a hand sprayer (volume 500 ml). Quantities for herbicide application in the field were calculated for application per 1 m^2 (pyroxsulam 0.025 g/m^2, bentazone 0.4 ml/m^2). Aqueous extracts were made from weed species *A. artemisiifolia* (AMBEL) and *S. halepense* (SORHA). Plant material was crushed in liquid nitrogen and 20 g of each species was weighed. Following the addition of distilled water (100 mL), the vessels were placed in an ultrasonic bath (2 times for 30 min, 15 min apart). The solution was then filtered through filter paper (Whatman) to remove the plant material. Prior to application of the extract, solutions of 1, 5, 10 and 20% dilution were made (application rate calculated per 1m^2). Ratio of the maximum and variable fluorescence Fv/Fm and the photosystem efficiency (ΦPSII) were measured. Plants were kept in the dark (15 min) before measurement to ensure deactivation of the photosystem [22]. Chlorophyll extraction was performed from plant leaf material (0.5 g) which was mechanically grinded using liquid nitrogen in the dark. Methanol (5 mL) was added to each sample, centrifuged at 1500 rpm for 10 min before measurement and analyzed on a spectrophotometer (UV 2100, Shimadzu). The absorption of chlorophyll *a* was read at λ = 653 nm, chlorophyll *b* at λ = 666 nm and carotenoids at λ = 470 nm. The content of chlorophyll *a, b*, carotenoids as well as the total content of chlorophyll were calculated according to the formulas given in Wellburn [23]. The following parameters were measured: relative chlorophyll content (SPAD meter 502, Minolta), chlorophyll fluorescence (PAM 2100, Heinz Walz, GmbH, Effeltrich, Germany) and pigment content (chl *a*, *b* and carotenoids, spectrophotometer UV 2100, Shimadzu). Measurement of the relative content and fluorescence of chlorophyll was done prior to the application (0 DAT) and 6 DAT on the fourth leaf (in broadleaf weeds) and on the third leaf (in grass weeds) in six replications. Statistical analysis of the data was done using the analysis of variance (ANOVA, Duncan test), performed in Statistica 8.

RESULTS

This study was conducted with the idea of finding an adequate solution of organic origin (bioherbicides) for the control of weed growth and development in agroecosystems. Although these compounds usually have a lower efficiency when compared to synthetic herbicides, they can contribute to weed control if applied with other control measures such as thermal (application of hot water or flame), mechanical, mulch, cultivation of combined or competitive crops, etc. [24, 25]. Methods confirming the effectiveness of herbicides were used in order to determine the level of efficiency of solutions of different concentrations (1, 5, 10 and 20%) of AMBEL (AA) and SORAH (SH). The quickest (less precise, when compared to the other two methods) way to test the levels of efficacy of herbicides and plant extracts is by using a non-destructive method, the SPAD reading of the relative chlorophyll content (Table 1).

TABLE 1
Statistical analysis of the relative chlorophyll content 6 DAT herbicide and plants extracts

	herbicides		AMBEL (AA)				SORAH (SH)			
	CvsB	CvsP	Cvs1%	Cvs5%	Cvs10%	Cvs20%	Cvs1%	Cvs5%	Cvs10%	Cvs20%
AVEFA	0.036 ±6.99*	0.04 ±7.09*	ns	ns	ns	ns	ns	ns	ns	0.000 ±6.74**
BRORI	0.014 ±7.04*	0.01 ±6.44*	0.0001 ±8.71**	0.000± 9.53**	0.001 ±9.75**	0.000 ±9.34**	0.000 ±9.24**	0.000 ±10.22**	0.000 ±9.96**	0.000 ±10.41**
CONAR	0.000 ±15.11**	0.021 ±6.28*	0.001 ±7.93**	0.002 ±4.98**	0.001 ±7.87**	0.004 ±6.95**	0.001 ±5.77**	0.014 ±6.61*	0.000 ±6.37**	0.000 ±7.39**
Wheat	ns	ns	ns	ns	ns	0.000 ±8.39**	ns	ns	ns	ns

p<0.05*, p<0.01**, ns-not statistically significant, B-bentazone, P-pyroksulam, C-control (0DAT)

Application of the tested herbicides had a statistically significant effect on the relative chlorophyll content in all the tested plants (except wheat), when compared to the initial values (Table 1). Contrary to this, the applied AA and SH solutions did not affect any significant changes in the relative chlorophyll content in AVEFA plants (except for the 20% SH solution, Table 1) and wheat (except for the 20% AA solution, Table 1). However, all of the tested solutions of AA and SH have yielded statistically significant differences in the relative chlorophyll content 6 DAT in BRORI and CONAR, when compared with their initial values (0 DAT) (Table 1). The weak effect of aqueous solutions of plant extracts on AVEFA could be related to epicuticular waxes on its leaves, [26] as McWhorter [27] considers that their presence on the leaf surface could affect the adsorption. A similar opinion is shared by Sanyal et al., [28] who claim that older plants contain more waxes. The results of our research indicate that all of the tested solutions of AA and SH can be used for the control of BRORI and CONAR in wheat (with the exception of a 20% AA solution, due to its effect on wheat, Table 1). The success and efficiency of plant extracts are still being tested because there are doubts about the product life, its stability during storage, the length of its effect on the target weeds and development of the market for their application [29, 30, 31, 32], as well as the possibility for developing a resistance/tolerance by weeds. Photosynthesis is a critical process in plants and any exposure to stress leads to changes in this process. Photosynthetic activity is based on the amount and activity of pigments (chlorophyll *a, b*, carotenoids). Consequently, monitoring of changes in their content and fluorescence may explain the response of plants to stress [33, 34, 35, 36]. Although numerous factors affect their content in plants, both biotic (leaf age and position, processes of chlorophyll synthesis and degradation [37, 38] and abiotic (temperature, herbicides, light, [37, 39]) this method is highly reliable.

Changes in the pigment content following the application of different solutions of AA (1, 5, 10 and 20%) are given in Table 2. Analysis of the obtained results has shown that the highest examined concentration (20%) of AA solution had a statistically significant effect on all measured parameters in all the studied weed species and wheat (except for chl *b* in AVEFA and BRORI and the caro content in AVEFA and CONAR, Table 2). Results have shown that the content of all pigments was lower, when compared to untreated plants in wheat (0 DAT, except for the content of chl *a,* data not shown), which confirms the sensitivity of wheat to a 20% solution of AA and therefore excludes its use in weed control. On the other hand, the lowest tested concentration (1%) only affected the parameters in BRORI (except for chl *b* content, Table 2). In general, it can be said that all of the tested concentrations affected all of the parameters BRORI (except on the content of chl *b* (except 10% AA solution)), while 1, 5 and 10% solutions had no effect on the pigment content of AVEFA (except 10% AA solution on the content of chl *b*, Table 2).

TABLE 2
Statistical analysis of the pigment content, fluorescence and photosystem efficiency in weeds and wheat 6 DAT by herbicide and aqueous plant extracts of AMBEL

		Chl *a*	Chl *b*	T Chl	Caro	Fv/Fm	ΦPSII
1 %AA	AVEFA	ns	ns	ns	ns	ns	0.000±0.03**
	BRORI	0.000±2.6**	ns	0.003±9.35**	0.005±0.19**	ns	ns
	CONAR	ns	ns	ns	ns	0.000±0.15**	0.000±0.12**
5 %AA	AVEFA	ns	ns	ns	ns	0.013±0.02*	0.000±0.03**
	BRORI	0.000±2.6**	ns	0.000±9.35**	0.000±0.19**	ns	0.013±0.03*
	CONAR	0.033±3.54*	0.000±1.83**	ns	0.000±0.36**	ns	ns
10 %AA	AVEFA	ns	0.024±0.44*	ns	ns	ns	0.000±0.03**
	BRORI	0.000±2.6**	0.02±3.62*	0.002±9.35**	0.000±0.19**	ns	ns
	CONAR	ns	ns	ns	0.000±0.36**	ns	0.016±0.12*
20 %AA	AVEFA	0.025±2.27*	ns	0.037±2.35*	ns	0.000±0.02**	0.000±0.03**
	BRORI	0.000±2.6**	ns	0.000±9.35**	0.000±0.19**	0.000±0.02**	ns
	CONAR	0.002±3.54**	0.011±1.83*	0.001±4.05**	ns	ns	0.047±0.12*
	Wheat	0.000±2.98**	0.000±1.54**	0.034±3.8*	0.000±0.43**	0.000±0.07**	0.000±0.06**
B	AVEFA	ns	0.000±0.95**	ns	ns	0.000±0.01**	0.000±0.02**
	BRORI	ns	0.000±3.45**	0.000±5.09**	ns	0.006±0.02**	0.023±0.02*
	CONAR	ns	0.000±2.52**	0.000±3.08**	0.000±0.27**	0.000±0.33**	0.000±0.28**
P	AVEFA	ns	ns	ns	ns	0.000±0.04**	0.000±0.06**
	BRORI	0.000±0.51**	0.000±3.45**	0.000±5.09**	ns	0.000±0.11**	0.000±0.11**
	CONAR	0.039±1.5*	0.025±2.52*	ns	0.000±0.27**	0.001±0.26**	0.001±0.22**

p<0.05*, p<0.01**, ns-not statistically significant, AA-*A. artemisiifolia,* Chl *a*-chlorophyll *a*, chl *b*-chlorophyll *b*, caro-carotenoids, T chl-total chlorophyll, Fv/Fm- chlorophyll fluorescence, ΦPSII-photosystem efficiency, P-pyroksulam, B-bentazone.

TABLE 3
Statistical analysis of the pigment content, fluorescence and photosystem efficiency in weeds and wheat 6 DAT by aqueous plant extracts of SORHA

		Chl *a*	Chl *b*	T Chl	Caro	Fv/Fm	ΦPSII
1 %SH	AVEFA	ns	0.04±3.26*	0.044±4.51*	ns	ns	0.000±0.03**
	BRORI	0.000±2.51**	0.000±2.89**	0.000±4.23**	0.000±0.35**	0.044±0.03*	ns
	CONAR	ns	0.000±1.44**	0.000±2.00**	0.000±0.24**	ns	0.044±0.12*
5 %SH	AVEFA	ns	0.000±3.26**	0.003±4.51**	0.000±0.95**	ns	0.003±0.03**
	BRORI	0.000±2.51**	0.000±2.89**	0.000±4.23**	ns	ns	0.03±0.04*
	CONAR	ns	0.000±1.44**	0.000±2.00**	0.000±0.24**	ns	0.047±0.12*
10%SH	AVEFA	ns	0.013±3.26*	0.018±4.51*	ns	ns	0.000±0.03**
	BRORI	0.000±2.51**	0.001±2.89**	0.000±4.23**	0.000±0.35**	ns	ns
	CONAR	0.035±1.24*	ns	ns	0.003±0.24**	0.016±0.17*	0.021±0.12*
20 %SH	AVEFA	ns	0.000±3.26**	0.016±4.51*	ns	0.000±0.02**	0.000±0.03**
	BRORI	0.000±2.51**	0.000±2.89**	0.000±4.23**	0.000±0.35**	0.000±0.03**	0.011±0.04*
	CONAR	ns	0.000±1.44**	0.031±2.00*	0.037±0.24*	0.044±0.17*	0.042±0.12*
	Wheat	0.000±1.77**	0.000±0.58**	0.000±1.66**	0.038±0.66*	ns	ns

p<0.05*, p<0.01**, ns-not statistically significant, *SH-S. halepense,* Chl *a*-chlorophyll *a*, chl *b*-chlorophyll *b*, caro-carotenoids, T chl-total chlorophyll, Fv/Fm- chlorophyll fluorescence, ΦPSII-photosystem efficiency.

Table 3 shows chages in the pigment content following the application of different concentration of the SH solution (1, 5, 10 and 20%). Analysis of the results has shown that all of the tested concentrations had a statistically significant effect on the content of chl *b* and total chlorophyll content 6 DAT, when compared with the initial values 0 DAT (with the exception of 10% SH solution in CONAR, Table 3). In general, all of the tested concentrations showed the weakest effect on the content of chl *a* (Table 3). If the effects on the tested species are observed, it can be concluded that all of the tested concentratoins have affected the changes in the pigment contents in BRORI. This suggests that SH solutions can be used in practice, as the tests conducted on wheat have shown that SH aqueous solutions are safe for use in wheat crops (Tables 2 and 3). Pigment content change analysis in wheat plants has shown that all of the analysed values increased (except caro), when compred with the values recorded prior to SH application (0 DAT). Contrary to this, the fact that all of the parameteres measured following the application of AA (all values lower than the initial values), leading to the conclusion that SH solutions can be used in practice in wheat crops. Changes in chlorophyll fluorescence (Fv/Fm) were statistically highly significant in all tested plants (weeds and wheat) after the application of the highest concentrations of both plant extract solutions (20%, except after 20% SH on wheat plants, Tables 2 and 3). The effects of the highest concentrations of both plant extract solutions on weeds can be theoretically linked to the mechanism of action of the tested herbicides, based on the Fv/Fm parameter (especially SH solution which had no negative effect on wheat plants). Lower concentrations of both solutions (AA, SH) did not have a statistically significant effect on the changes in Fv/Fm, when compared to the initial values (0 DAT) (except with 1% AA in CONAR and 5% AA in AVEFA; 1% SH in BRORI and 10% SH in CONAR, Tables 2 and 3).

Parameter ΦPSII is a direct indication of the photosynthesis I yield, which is why it is seen as an important indicator of the plant's metabolic activity (Figures 1, 2 and 3). AA solutions of 1, 10 and 20% showed no effects only on BRORI, and 5% on CONAR (Table 2; Figures 1a and 3a). Also, 1, 10 and 20% SH solutions had no effects on BRORI (Table 3; Figure 1b), unlike statistically significant changes recorded following the application of a 20% solution.

Use of chlorophyll fluorescence as an indicator of weed stress after herbicide application was confirmed by Pavlović [40]. In *C. album, A. theophrasti* and *A. retroflexus* plants, a low level of fluorescence (Fv/Fm<0.2) was documented following the application of atrazine (5 DAT). A comparison with the obtained results has also shown that lower values, when compared to untreated plants, suggest that damage occurred during the process of photosynthesis (caused by stress). In the experiments performed, the most pronounced changes were seen after the application of bentazone in CONAR plants which suffered a 100% damage. The values of Fv/Fm and ΦPSII parameters were not measurable in this case (Figure 4), which was further confirmed by the Duncan test (Table 2). These results confirm the reliability of this method, and thereby also of the values obtained when testing the effects of different concentrations of plant extracts on the tested parameters.

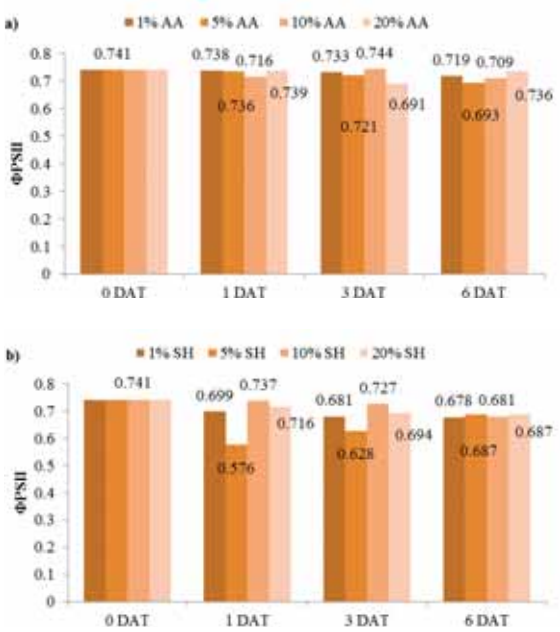

FIGURE 1
ΦPSII values in BRORI plants treated by aqueous solutions of (a) AMBEL (AA) (b) SORAH (SH) plant extracts

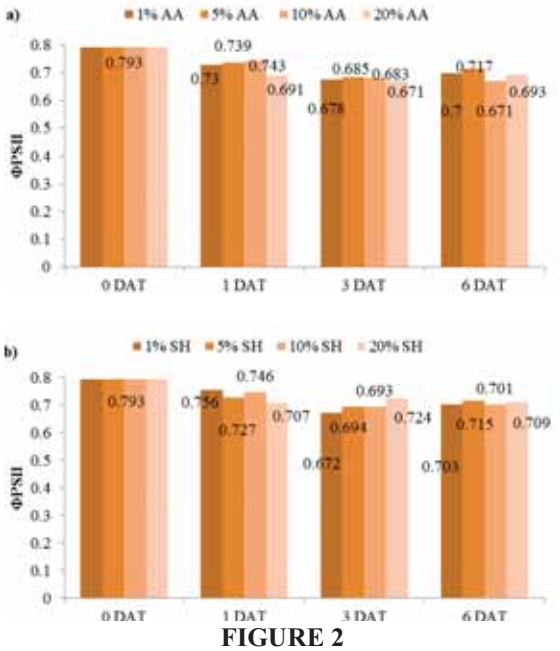

FIGURE 2
ΦPSII values in AVEFA plants treated by aqueous solutions of (a) AMBEL (AA) (b)SORAH (SH) plant extracts

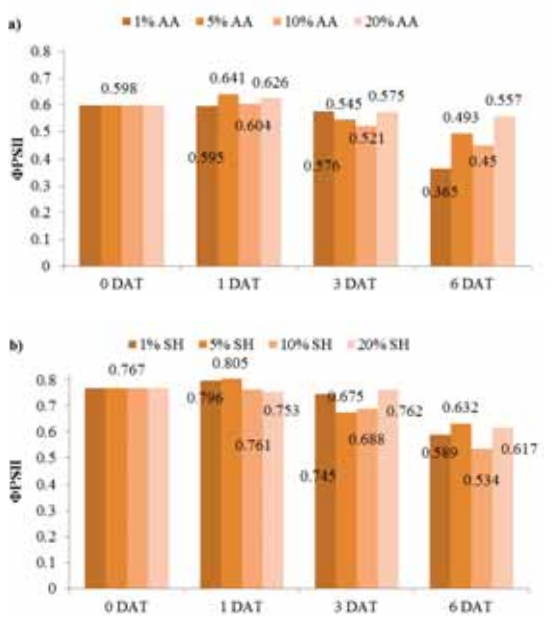

FIGURE 3
ΦPSII values in CONAR plants treated by aqueous solutions of (a) AMBEL (AA) (b) SORAH (SH) plant extracts

Quenching, i.e. the transformation of the absorbed energy, is also an important parameter for the analysis of the changes occurring during photosynthesis (qP photochemical, qN non-photochemical). The values recorded in the tested weed species prior to treatment (0 DAT) were: AVEFA qP 0.941 and qN 0.019; BRORI qP 0.914 and qN 0.010 and CONAR qP 0.754 and qN 0.0053 (data not shown). In the undamaged state, the qP values are high (max = 1), unlike the high values of qN in the damaged systems [41]. Measurements have shown that the treated plants have survived the stress conditions and that these values, in addition to the variations in ΦPSII, have also oscillated between 0 DAT and 6 DAT. These changes were especially evident in CONAR, following herbicide application (Table 4). The average of photochemical quenching (qP) decreased on the third day following the application, when compared to the initial values (0 DAT qP=0.835; 3 DAT qP=0.577, Table 4), while the non-chemical increased (qN) (0 DAT qN=0.012; 3 DAT qN=0.188, Table 4). This trend indicates that the process of photosynthesis is highly damaged, ending in a complete cessation of biochemical processes 6 DAT (Fv/Fm=0, ΦPSII=0 and quenching qP=0, qN=0 in CONAR plants, Figure 4, Table 4). Changes in qP and qN after the application of a.i. pyroxsulam (regardless of the variation) were not extreme and do not lead to the same state as after the application of a.i. bentazone, even though the changes in Fv/Fm and ΦPSII have shown that photosynthesis process is damaged to a degree (6 DAT: Fv/Fm =0.341; ΦPSII=0.281, Figure 4, Table 4). The somewhat weaker effect of pyroxsulam on the process of photosynthesis can be related to the fact

that the target site of action of pyroxsulam is primarily acetolactate synthetase and not protein D [42]. Analysis of the qP and qN values shown in Table 4 indicated that the solutions of AA and SH affect the photosynthesis process and that their action is similar to that of pyroxsulam on the tested parameters.

In general, measuring these parameters 6 DAT has shown that an increase in qN is weaker, when compared with the initial values, which points to the fact that the absorbed energy is getting dissipated somewhere, i.e. that the plant extract solution affects the process of photosynthesis in weed species (6 DAT 20% AA: AVEFA qN 0.010; BRORI qN 0.016; CONAR qN 0.008; 6 DAT 20% SH: AVEFA qN 0.019; BRORI qN 0.019; CONAR qN 0.007; data not shown). It is interesting to highlight that the visual changes in the treated plants were only visible in BRORI, CONAR and wheat plants after applying the 20% solution of AA. Moreover, a comparison of the effects caused by the highest tested concentrations (20%) on all of the tested parameters in the crop plants, has confirmed the safety of using the solutions of SH plant extracts in wheat fields (Table 5). Table 5 shows the average values of the parameters measured before the treatment (0 DAT) and 6 DAT. Statistical analysis of these values has demonstrated that the 20% concentration of the AA plant extract solution reduces the content of the chlorophylls *a* and *b* and the total chlorophyll content (with the exception of carotenoids, where these differences were not statistically significant). Furthermore, these differences were also highly statistically significant ($p<0.01$), when compared to the values obtained after the application of the SH plant extract solution on wheat. Changes in the values of the Fv/Fm and ΦPSII parameters, due to the effects of AA were also more pronounced, when compared with the effects caused by the SH solution (Table 5).

TABLE 4
Quenching average values 6 DAT in CONAR following the application of herbicides and different solutions of AMBEL (AA) and SORHA (SH)

	0 DAT		3 DAT		6 DAT	
	qP	qN	qP	qN	qP	qN
			herbicides			
bentazone	0.835	0.012	0.577	0.188	0	0
pyroxsulam			0.951	0.124	0.867	0.028
			AA			
1 %	0.835	0.012	0.806	0.005	0.789	0.017
5 %			0.732	0.003	0.719	0.002
10 %			0.736	0.003	0.678	0.007
20 %			0.755	0.014	0.781	0.008
			SH			
1 %	0.835	0.012	0.808	0.006	0.789	0.01
5 %			0.770	0.003	0.696	0.006
10 %			0.797	0.005	0.839	0.005
20 %			0.721	0.006	0.781	0.007

DAT-day after treatment, qP-photochemical quenching, qN-non-photochemical quenching

TABLE 5
Average values and statistical significance of the differences in Fv/Fm and ΦPSII values in wheat plants 6 DAT treated with 20% solutions of AMBEL (AA) and SORHA (SH)

	AA **vs** SH						
	Chl *a*	Chl *b*	T Chl	Caro	SPAD	Fv/Fm	ΦPSII
0 DAT	0.93	2.81	3.74	1.58	35.59	0.784	0.712
AA 20% 6 DAT	3.03	0	3.03	0.89	17.67	0.641	0.588
SH 20% 6 DAT	3.31	3.55	6.85	0.48	31.97	0.739	0.694
p (AA vs SH)	0.034^{*}	0.000^{**}	0.000^{**}	0.33^{ns}	0.000^{**}	0.001^{**}	0.000^{**}
mean	2.42	2.12	4.54	0.98	28.41	0.721	0.664
SD	1.12	1.62	1.76	0.63	8.26	0.073	0.069

$p<0.01$**, ns-not statistically significant, SD-standard deviation, DAT-day after treatment, Chl *a* - chlorophyll *a*, chl *b* - chlorophyll *b*, caro - carotenoids, T chl – total chlorophyll, SPAD reading - relative chlorophyl content, Fv/Fm – chlorophyll fluorescence, ΦPSII- photosystem efficiency.

CONCLUSION

In general, many studies have described the effects of allelopathy [43, 44, 45]. However, as majority of their conclusions has been reached in controlled (laboratory) conditions, this poses limitations (abiotic and biotic factors) for their application in the field [46, 47]. Assessing the chlorophyll content by fluorescence and documenting the changes in its content (SPAD-meter and methanol extraction) has shown that by applying different concentrations of plant extracts of AA and SH, other weed species can be controlled. The greatest effect was evident in *B. rigidus* and *C. arvensis* plants. The highest concentrations (20%) of these two solutions have impacted the Fv/Fm and ΦPSII parameters significantly, in all the tested weed species. However, testing the changes in chlorophyll parameters has shown that only the SH extract solution is safe for application. The most important issues pertaining to future studies and wider practical application are increasing their efficacy in comparison with commercial herbicides and achieving longer effect duration, while considering their quick degradation in the field conditions.

ACKNOWLEDGEMENT

DMP, AAA and SBĐ would like to acknowledge the financial support of the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 451-03-68/2020-14/200010).

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Received: **06.10.2020**
Accepted: **07.10.2020**

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