Scientific Papers. Series A. Agronomy, Vol. LXIII, No. 1, 2020
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

ALLELOPATHIC ACTIVITY OF SUNFLOWER BROOMRAPE (*Orobanche cumana* Wallr.) ON SUNFLOWER (*Helianthus annuus* L.) VARIETIES

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Abstract

It has been found that dry biomass of sunflower broomrape (Orobanche cumana Wallr.) has an inhibitory effect on seed germination and the initial development of five tested sunflower varieties. Sunflower broomrape with the highest overall allopathic potential (OAP) can be identified with originating in Tyulenovo and Radnevo with average OAR 0.22. Sunflower broomrape with the lowest overall allopathic potential (OAP) can be identified with originating in the ecological areas of Senokos and Shumen with average OAR 0.10. High allelopathic tolerance was observed in the seed germination and in the initial development of the sunflower varieties San Luka and Diveda. Their index of initial plant development (GI) ranged from 72.9 to 127.0 at higher studied concentrations 16.00% w/v and 32.0% w/v. The lowest allelopathic tolerance was observed in the seed germination and in the initial development of the sunflower variety Enigma. Its index of initial plant development (GI) ranged from 39.4 to 68.5. It has been found an equivalent between the allelopathic effect of dry biomass of Orobanche cumana Wallr. and the allelopathic tolerance of Helianthus annuus L. varieties, according to the common allelopathic potential of Orobanche cumana Wallr. and the Initial Development Index (IDI) of the sunflower plants.

Key words*: allelopathic activity, allelopathic effect, sunflower broomrape, sunflower.*

INTRODUCTION

Weed species are a constant and ubiquitous companion of agricultural production, inflicting huge damage on it, which often exceeds the total losses caused by diseases and pests (Kubiszewski and Cleveland, 2012).

Research on weed species in recent years has focused mainly on development of highly efficient systems for their control (Rubiales, 2012).

Synthetic herbicides are an essential means of combating weeds in sunflower, but their intensive use is a prerequisite for creating resistance to some weeds and for a wider spread of the sunflower broomrape (*Orobanche cumana* Wallr.) (Mitkov et al., 2019; Masliiov et al., 2018; Molinero-Ruiz et al., 2015; Shindrova, 2006; Venkov and Shindrova, 2000; Venkov and Bozoukov, 1994).

Generalized studies of (Habimana et al., 2014; Plakhine et al., 2012; Runyon et al., 2009) show that the technological solutions and the effective ways to control of the sunflower broomrape by applying conventional methods are limited, due to the nearby physiological link between the parasite and the host plant (Matusova, 2014; Abbes et al., 2008).

Currently, there is a growing interest to alelopathy in agriculture, because alelopathy could offer promising alternative methods to control of weeds and to help for reduce of the application of synthetic herbicides (Singh et al., 2003).

Alelochemicals could replace partially used synthetic herbicides or to be used as a prototype for the synthesis of biodegradable herbicides. Due to their biological nature, they will be safer for the environment than synthetic herbicides (Takemura et al., 2013).

In recent decades, research has focused on the detection of species and varieties with allelopathic tolerance to typical weed flora. Chou (1999), Chon and Nelson (2010) have found that there are varietal differences in allelopathic tolerance with respect to the allelopathic effect of some typical weeds in different crops. According to summary studies of Parvatha (2017) and Jabran (2017) in breeding programs, allelopathy can be considered as a means of biological control against weeds.

Scientific reportings of Belz (2007), Ferguson et al. (2013) and Fragasso et al. (2013) to determine the allelopathic effect of parasitic weed species are controversial. Concerning allelopathic tolerance, specific data are lacking.

The purpose of the study is through use of standard methods in laboratory conditions to be determined and compare the allelopathic tolerance of sunflower varieties to the allelopathic effect of aboveground biomass of *Orobanche cumana* Wallr. as components in future breeding programs.

MATERIALS AND METHODS

During the period 2017-2018 at the Institute of Forage Crops - Pleven, Bulgaria using standard methods in laboratory conditions the allelopathic tolerance of five sunflower varieties (Enigma, Favorit, San Luka, Markesa and Diveda) to the allelopathic effect of aboveground biomass of *Orobanche cumana* Wallr. was studied and compared.

The following factors have been studied: Factor A - sunflower varieties (*Helianthus annuus* L.), selected in Dobroudja Agricultural Institute, General Toshevo, Bulgaria (DAI): a_1- Favorit; a_2 - San Luka; a_3 - Enigma; a_4 – Diveda; a_5 – Markesa; Factor B - concentrations: b_1 - 0.08% w/v; b_2 - 0.16% w/v; b_3 - 0.32% w/v of parasitic weed biomass of sunflower broomrape (*Orobanche cumana* Wallr.). The aboveground biomass of sunflower broomrape (*Orobanche cumana* Wallr.) is collected in phenophase flowering (BBCH 65-69) in the infectious field of Dobroudja Agricultural Institute, General Toshevo. The plant material of *Orobanche cumana* Wallr. is dried to constant dry weight at temperature 50^0C ± 5^0C.

To evaluate the allelopathic tolerance of the tested sunflower varieties to the allelopathic effect of the aboveground biomass of *Orobanche cumana* Wallr. under laboratory conditions was used the adapted method of (Fujii et al., 2005) “Rhizosphere Soil Method” (RSM). A dry parasitic weed biomass of sunflower broomrape (*Orobanche cumana* Wallr.) was placed in Petri dishes (90 mm), according to factor B, into which 20 ml (0.8%) agar were pipetted, as added 1 ml/l $C_{10}H_{14}O$. The thus prepared Petri dishes were placed in a thermostat in the dark for 72 h at a temperature of 18°C ± 2°C.

In each Petri dish, 10 numbers of seeds of *Helianthus annuus* L. were seeded according to factor A, and then they was incubated in the dark into a thermostat at temperature 23°C ± 2°C for five days. Each experimental option is pledged in seven repetitions. 0.8% agar whit added 1 ml/l $C_{10}H_{14}O$ was used for control.

The percentage of germinated seeds (GR%) and the length in cm of the sprouts for each variant of the experiment was determined.

Dynamic Development Index (DDI):

$$DDI = \left\{\frac{t\log^2}{\log b - \log a}\right\}$$

where: a is the sprouted seeds in %; b is the length of the sprouts in the control and the experimental variants in cm; t is duration of the exposure in days.

Allelopathic Effect Index (RI):

The Percent of Inhibition (IR) is determined by the formula:

$$IR\% = \frac{C-T}{C} x100$$

where: C is an indicator, reported in the control variant and T is an indicator, reported in the treated variants.

The rate of growth and accumulation of fresh sprout biomass is determined by the adapted formula of Dauta et al. (1990):

$$\mu = \left\{\frac{\ln N_t - \ln N_0}{t}\right\}$$

where: Nt e is the length of the sprouts in the experimental variants in cm; No is the length of the sprouts in the control variant in cm; t is duration of the exposure in days.

The Development Index (GI) is determined by the formula of Gariglio et al. (2002):

$$GI = \left[\left(\frac{G}{G_0}\right) \cdot \left(\frac{L}{L_0}\right)\right] . 100$$

where: G is the percentage of germinated seeds for the treated variants; G_0 is the percentage of germinated seeds for the control variant; L is the length of the sprout in the treated variants, in percentage; L_0 is the length of the sprout in the control variant accepted for 100%.

The viability of a sprout (SVI) is determined by the formula of Islam et al. (2009):

$$SVI = \left(\frac{S.G}{100}\right)$$

where: S is the length of the sprout in cm; G is the percentage of germinated seeds.
The mathematical and statistical processing of the experimental data has been done after preliminary transformation of the percentage of germinated seeds by the formula: Y= arcsin√(x%/100).
The data from the laboratory experiment are processed mathematically and statistically with the software products Statgraphics Plus for Windows Version 2.1 and Statistica 10.

RESULTS AND DISCUSSIONS

The laboratory germination of seeds in the tested varieties (*Helianthus annuus* L.) in the control varieties ranged from 56.8% to 90.0%. The highest percentage of germinated seeds was reported for the Enigma variety, and relatively lower for the San Luka and Diveda varieties. The Favorit and Marкesa varieties occupy an intermediate position, the differences between them being statistically proven at (P = 0.05) compared to Enigma variety.
The applied aboveground dry sunflower broomrape biomass (*Orobanche cumana* Wallr.) has a low stimulating effect with IR of 4.2% to 9.7% and/or an inhibitory effect with IR of 18.1% to 24.5% on the laboratory seed germination of the tested sunflower varieties. Depending on the degree of inhibition (IR), the tested sunflower varieties can be conditionally divided into three groups: I. Group with stimulating effect (IR ≤ -10%) varieties Diveda and Marкesa; II. Group with inhibitory effect (IR ≤ 20%) variety Favorit; III. Group (IR ≥ 20%) varieties San Luka and Enigma (Table 1).

Table 1. Allelopathic effect of sunflower broomrape (*Orobanche cumana* Wallr.) on the germination and the initial development of sunflower (*Helianthus annuus* L.) varieties

Variety	Concentration, % w/v	Germination				Seedling length			
		GR%	*IR*	*DDI*	*μ*	*cm*	*IR*	*DDI*	*μ*
Favorit	Control	63.4c				8.7b			
	0.08	60.0c	5.4	-20.41	-0.01	8.1b	6.4	-16.89	-0.01
	0.16	50.8b	19.9	-5.08	-0.04	7.8b	10.2	-10.41	-0.02
	0.32	45.0a	29.0	-3.28	-0.07	7.2a	17.5	-5.85	-0.04
Average		54.8	18.1	-9.59	-0.04	8.0	11.4	-11.05	-0.02
San Luka	Control	56.8b				8.8b			
	0.08	56.8b	0.0	0.00	0.00	9.0c	-2.4	47.65	0.01
	0.16	39.2a	31.0	-3.03	-0.07	7.8a	11.3	-9.41	-0.02
	0.32	39.2a	31.0	-3.03	-0.07	7.9a	10.1	-10.54	-0.02
Average		48.00	20.67	-2.02	-0.05	8.38	6.33	9.23	-0.01
Enigma	Control	90.0c				10.8b			
	0.08	63.4a	29.6	-3.21	-0.07	8.8a	18.6	-5.46	-0.04
	0.16	77.1b	14.3	-7.27	-0.03	9.3a	14.0	-7.49	-0.03
	0.32	63.4a	29.6	-3.21	-0.07	9.0a	16.3	-6.33	-0.04
Average		73.48	24.50	-4.56	-0.06	9.48	16.30	-6.43	-0.04
Diveda	Control	56.8ab				10.8b			
	0.08	63.4c	-11.6	10.23	0.02	14.4c	-33.4	3.904	0.058
	0.16	63.4c	-11.6	10.23	0.02	15.4c	-42.9	3.154	0.071
	0.32	50.8a	10.6	-10.08	-0.02	8.7a	19.7	-5.137	-0.044
Average		58.60	-4.20	3.46	0.01	12.33	-18.87	0.64	0.03
Markesa	Control	63.4a				8.6a			
	0.08	77.1b	-21.6	5.75	0.04	9.3b	-8.2	14.229	0.016
	0.16	71.6b	-12.9	9.25	0.02	8.5a	1.5	-74.115	-0.003
	0.32	60.0a	5.4	-20.41	-0.01	8.5a	1.5	-74.115	-0.003
Average		68.03	-9.70	-1.80	0.02	8.73	-1.73	-44.67	0.00

Legend: GR% - seed germination; IR - percent of inhibition; DDI -dynamic index development

A specific varietal response to the allelopathic effect of the sunflower broomrape (*Orobanche cumana* Wallr.) has been established. Relatively high allelopathic tolerance to the allelopathic effect of *Orobanche cumana* Wallr. to the seed germination was reported in the varieties Diveda and Marкesa, relatively lower in the variety San Luka and variety Enigma.

The applied concentrations have a significant effect on the laboratory germination of the sunflower seeds. By increasing of the applied concentration of *Orobanche cumana* Wallr. there is a general tendency to decrease the laboratory germination of the seeds from 5.40% to 31.0% for the Favorit, San Luka and Enigma varieties.

The differences in the laboratory germination of the seeds were statistically proven at (P = 0.05) only at the higher concentrations of 0.16% v/w and 0.32% v/w.

The lower concentrations 0.08% v/w and 0.16% v/w of *Orobanche cumana* Wallr. have a statistically proven stimulating effect on the laboratory germination of the seeds of the varieties Diveda and Marкesa.

Significant differences in the dynamic index (DDI) and the rate of seed germination (g) were found, depending on the manifested allelopathic tolerance of the sunflower varieties to the allelopathic effect of the sunflower broomrape.

These differences can be explained by the diffusion of the soluble allelochemicals from the aboveground of sunflower broomrape biomass (*Orobanche cumana* Wallr.) into the carrier - agar and with the allelopathic tolerance of the tested sunflower varieties, because the comparisons between them were made under controlled conditions (Sangeetha and Baskar, 2015).

Similar specific variety reaction with respect to the allelopathic interference has been found in other cultures (Kruse et al., 2000; Wu et al., 1999).

Data from the biometric measurements of the length of the sprout (cm) make it possible to objectively compare and evaluate the allelopathic tolerance of the tested sunflower varieties to the allelopathic effect of the sunflower broomrape (*Orobanche cumana* Wallr.).

The attached parasitic weed biomass from *Orobanche cumana* Wallr. has no a statistically proven inhibitory effect on the

sprouting and the growth of the Enigma, Marкesa, and Favorit varieties. In the Diveda variety, the applied parasitic weed biomass of *Orobanche cumana* Wallr. elicited a statistically proven stimulating effect at the lower concentrations 0.08% v/w and 0.16% v/w, respectively IR - 33.4% and IR - 42.9% (Table 1).

Regarding to the concentration dependences, it is evident that with an increase of the content of the parasitic weed biomass from 16.0% w/v to 32.0% w/v, there is no disproportionate reduction of the length of the sprout in the tested sunflower varieties except Enigma. The differences were statistically unproven at (P = 0.05) compared to the lowest concentration of 0.08% v/w. Similar results have been reported by (Labrousse et al., 2001), according to the authors the allelopathic tolerance is species and variety specific.

The results for the growth rate (μ) and the development index (DDI) of sprouts in the studied sunflower varieties are similar. They also depend on the applied concentrations of the parasitic weed biomass of *Orobanche cumana* Wallr.. The relatively lowest allelopathic effect (RI) was found from the lowest concentration of the parasitic weed biomass of *Orobanche cumana* Wallr. - 0.08% w/v. With increasing concentration of the parasitic weed biomass of *Orobanche cumana* Wallr. to 32.0% w/v, RI and μ increase respectively from 0.4 to 1.3 times and from 0.3 to 1.3 times. The Dynamic Plant Development Index (DDI) decreases from 0.7 to 2.9 times only in the Favorit and Enigma varieties. An exception to the described dependence is found in the varieties San Luka, Diveda and Marкesa, where lower concentrations have a stimulating effect up to 42.9%.

The results for determining of the viability (SVI) in cm of the tested sunflower varieties are similar (Figure 1). Relatively most sensitive are the Favorit and San Luka varieties. In these, the viability (SVI) in cm is in the range from 3.10 to 5.51, followed by Enigma and Marкesa, with (SVI) in cm from 5.10 to 9.68 and relatively least sensitive is Diveda with (SVI), in cm in the range from 6.12 to 9.76.

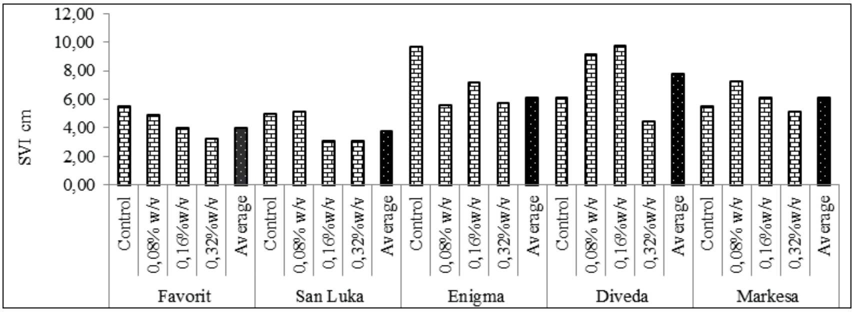

Figure1. Allelopathic effect of sunflower broomrape (*Orobanche cumana* Wallr.) on seedling vigour index $SVI_{(cm)}$ of sunflower (*Helianthus annuus* L.)

The plant development index (GI) depends on the same factors and follows the observed dependencies in terms of the laboratory germination and the dynamics of sprouting in the tested sunflower varieties (Figures 1 and 2). Depending on the GI values, they can be grouped conditionally in the following ascending order: Enigma → Favorit → San Luka → Markesa → Diveda. Therefore, the observed differences with respect to the viability SVI (cm) of the sprouts and the plant development index (GI) can be explained by varietal differences, since the comparisons between them are made under the same conditions, which also determine the allelopathic tolerance of the sunflower varieties to the allelopathic effect of the sunflower broomrape.

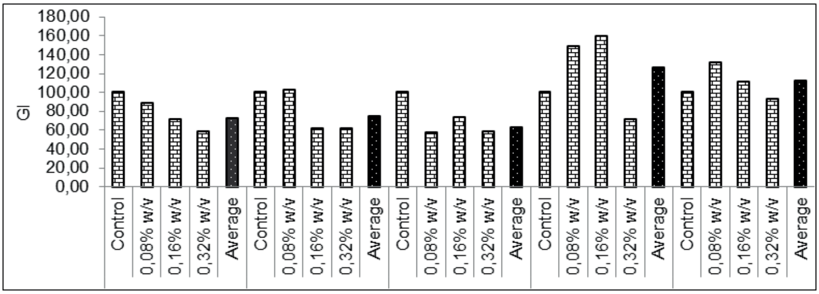

Figure 2. Index of development (*GI*) on the seed germination and the initial development of sunflower (*Helianthus annuus* L.) depending on the allelopathic effect of (*Orobanche cumana* Wallr.)

CONCLUSIONS

Concentrations from 0.08% w/v to 0.32% w/v of the aboveground biomass of the sunflower broomrape (*Orobanche cumana* Wallr.) exert an inhibitory effect on the seed germination and the initial development of the plants of the tested sunflower varieties (*Helianthus annuus* L.). They can be conditionally grouped into three groups: I. Group with IR ≤ -10% for Diveda and Markesa varieties; II. Group with IR ≤ 20% for Favorit variety; III. Group with IR ≥20% for San Luka and Enigma varieties.

The relatively high allelopathic tolerance to the allelopathic effect of *Orobanche cumana* Wallr. on the seed germination and in the initial development of the tested sunflower varieties has been reported in the Diveda and Markesa varieties, which may be used as components in future breeding programs. Relatively lower allelopathic tolerance was reported in the San Luka and Enigma varieties.

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