

Alelopathic effect of sunflower broomrape (*Orobanche cumana* Wallr.) on the development of *Helianthus annuus* L. under the conditions of the Republic of Bulgaria

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Abstract

The allelopathic effect of dry biomass of sunflower broomrape (*Orobanche cumana* Wallr.) collected from ten regions of the Republic of Bulgaria was studied in laboratory conditions at the Institute for Forage Crops - Pleven. The allelopathic effect of the experimental samples of sunflower broomrape on the seed germination and on the initial development of the sunflower, variety "Paredovik".

It was found that the studied concentrations (8.0, 16.0 and 32.0% w/v) dry weed biomass of sunflower broomrape showed a stimulatory effect (from -0.7 to -55.3%) or an inhibitory effect on the germination of seeds (from 1.8 to 43.1%) and on the initial development (from 2.4 to 21.0%) of sunflower, variety "Paredovik".

Depending on the allelopathic effect and the origin of the sunflower broomrape, they can be grouped in the following order: P₇(Selanovtsi) (86.9%) → P₉(Radnevo) (83.0%) → P₄(Kardam) (73.4%) → P₅(Tyulenovo) (70.7%) → P₆(Dyakovo) (61.9%) → P₁(Kardam) (54.8%) → P₂(DZI1, infection field) (50.6%) → P₃(DZI2, experimental field) (50.2%) → P₈(Svishtov) (46.0%) → P₁₀(Radnevo) (27.9%).

The highest overall allelopathic potential (OAP = 0.6) conditionally was determined for sunflower broomrape originating P₂ (DZI infection field) and P₃ (DZI experimental field). The lowest overall allelopathic potential (from 0.2 to 0.3) was determined for sunflower broomrape with the origin P₇ (Selanovtsi) and P₉ (Radnevo).

This information can be explained by the genetic differences of the studied origins.

Key words: *allelopathic effect, broomrape, sunflower, inhibition*

Parasitic plants are among the most problematic pests of agricultural crops worldwide (Runyon et al., 2009, Blagojević et al., 2014). The technological approaches and effective means for control against them are extremely limited, because the close physiological connection between the parasite plant and the host plant impedes the efficient control using traditional methods.

According to Macias et al. (2003), Reigosa et al. (2006) and Willis (2007), six families of parasitic weeds species (*Scrophulariaceae*, *Orobanchaceae*, *Santalaceae*, *Cuscutaceae*, *Viscaceae* and *Loranthaceae*) are a great economic importance and cause the highest loss of agricultural production.

The parasitic weed sunflower broomrape (*Orobanche cumana* Wallr.) is one of the major limiting factors in sunflower production in the Republic of Bulgaria (Encheva and Shindrova, 1993, Shindrova, 2006, Venkov and Shindrova, 2000, Shindrova 2006) and in many countries of the world (Miladinovic et al., 2012, Molinero-Ruiz et al., 2015, Pineda-Martos et al., 2014).

The broomrape in sunflower has a higher competitive ability than any other weed species as its invasion leads to an adverse effect on the natural weeds in sunflower crops. (Habimana et al., 2014).

Due to the competitive nature, the parasitic species – sunflower broomrape causes significant losses, expressed on the one hand in the reduction of yield and on the other - in the deterioration of the quality of the production obtained (Shindrova et al., 1994, Perez-de-Luque et al., 2001).

Scientific researches in recent years are focused mainly on creating resistant varieties and hybrids and on the development of highly effective systems for integrated control against parasitic weed species. (Chittapur et al., 2001, Rubiales, 2012; Joel et al., 2013).

In this respect, the search for alternative means of weed control is very important (Chauhan and Mahajan, 2014). There is a growing interest to the allelopathy in agriculture at present. This

phenomenon could provide perspective alternative methods of weed control and to help for reducing of the application of synthetic herbicides (Lopez-Raez, 2008).

Although allelopathy is under study by ecologists, chemists, soil scientists, agronomists, herbologists, biologists and plant physiologists the allelopathic interrelations in the "weed – plant" system are not fully understood and in the case of "hostplant–parasite" are extremely limited.

According to Jacobs and Rubery (1988), Serghini al., 2001, Perez-de-Luque et al., 2001, Qasem, (2006) Matusova, et al., 2005, Kalinova (2010) *Orobanche cumana* Wallr. has an allelopathic potential, probably due to the content of the allelochemicals (secondary metabolites - coumarins and others).

The discovery of main regularities in the allelopathic interaction in agrophytocenoses of *Helianthus annuus* L. and *Orobanche cumana* Wallr, appears to be a major element of the theoretical basis for sustainable plant-growing production.

The purpose of this study is to determine the allelopathic effect of some origins *Orobanche cumana* Wallr. distributed in the main agricultural areas of the country on the germination and growth of *Helianthus annuus* L.

MATERIALS AND METHODS

The study was conducted during the 2017-2018 period under laboratory conditions in the Institute of Forage Crops in Pleven, Bulgaria.

Two factors have been studied: Factor A - the location of the sunflower broomrape (*Orobanche cumana* Wallr.) (Table 1): a₁-Kardam; a₂ - DAI (infection field); a₃ - DAI (experimental field); a₄-Kardam; a₅-Tyulenovo; a₆ - Dyakovo; a₇ - Selanovtsi; a₈ - Svishtov; a₉ – Radnevo 2016 and a₁₀ – Radne 2017.

Factor B - parasitic weed biomass concentration: b₁- 0.0% (control); b₂-8.0%; b₃-16.0% and b₄-32.0% w/v. Sampling of sunflower broomrape is consistent with the factor A in a growth stage BBCH 65-69 (Hess et al., 1997).

Table 1. The race consists of sunflower broomrape (*Orobanche cumana* Wallr.)

Origin		Year	Race
Code	Location		
P ₁	Kardam	2016	H
P ₂	DAI(infection field)	2016	H
P ₃	DAI (experimental field)	2017	E
P ₄	Kardam	2017	H
P ₅	Tyulenovo	2017	H
P ₆	Dyakovo	2017	H
P ₇	Selanovtsi	2017	D
P ₈	Svishtov	2017	E
P ₉	Radnevo	2016	E
P ₁₀	Radnevo	2017	E

Legend: DAI- Dobroudja Agricultural Institute, General Toshevo, Bulgaria

To evaluate the allelopathic potential of tested samples of sunflower broomrape (*Orobanche cumana* Wallr.) was used the adapted method called "Rhizosphere Soil Method" (RSM) of Fujii et al. (2005) under laboratory conditions.

Parasitic weed biomass from tested samples sunflower broomrape (*Orobanche cumana* Wallr.), according to factor B is placed in petri dishes (90 mm).

On the parasitic weed biomass are pipetted 20 ml 0.8% strength agar supplemented with 1 ml/l thymol C₁₀H₁₄O, as a chemical preservative. (Marinov-Serafimov, Golubinova, 2015).

The samples are stored for 72 h at 18 ± 2°C, after then ten seeds of sunflower, variety "Paredovik" are added.

The prepared samples are placed in an incubator at 22 ± 2°C in the dark for five days.

Distilled water is used for control. Each variant is pledged in nine repetitions.

Effect assessment. For assessing the results of the experiments were used the following parameters.

Quantitative parameters. Number of germinated seeds in each treatment: percent of germination in each treatment (%).

Biometric parameters. Length of the root, stem and seedling, cm; fresh biomass in g per root, stem and seedling, g. Length was measured using graph paper and the weight was recorded on an analytical balance.

Statistical evaluation and calculated formulas.

Germination seeds (GS%) was determined by the Equation (1) prescribed according to ISTA (1985).

$$GS\% = (\text{Number of seed germinated}) / (\text{Total number of seed plated}) \cdot 100$$

(1)

Percent inhibition (IR) was determined by the equation (2) (Ahn and Chung, 2000).

$$IR\% = \frac{C-T}{C} \times 100 \quad (2)$$

Where, C – characteristic in the control treatment; T – characteristics in each treatment;

The index of plant development (GI) was determined by the Equation (3) (Gariglio et al., 2002).

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

(3)

Where, G – germinated seeds in each treatment, %; G₀ – germinated seeds in the control treatment, respectively %; L – average length (cm) of seedlings in treatment transformed into percentage as against the control treatment; L₀ – average length (cm) of the seedlings in the control treatment taken as 100%;

Seedling vigor index (SVI) was determined by the equation (4) (Islam et al. 2009).

$$SVI = \left(\frac{S \cdot G}{100} \right) \quad (4)$$

Where, S – seedling length in the treatments and control variant, cm; G – germinated seeds in the treatments and control variant, %;

Coefficient of allometry (CA) was determined by the equation (5) (Nasrand Mansour, 2005):

$$CA = \frac{L_s}{L_r} \quad (5)$$

Where,, L_s is shoot length and L_r is root length, cm.

Overall allelopathic potential (OAP) was determined by the equation (6) (Smith (2013):

$$OAP = \text{mean} (I_a + I_b) / 100 \quad (6)$$

where I_a, a percent inhibition of the seedling growth at the lowest applied concentration of 8.0% w/v and I_b percent inhibition of the seedling growth at the highest applied concentration of 32.0% w/v, compared to the control variants.

A score between 0.0 and 1.0 was obtained and the data were ranked according to this score. A maximum score of 1.0 would indicate that the test samples sunflower broomrape (*Orobanche cumana* Wallr.) had totally inhibited growth, while a score of 0.0 would indicate that no allelopathic inhibition had occurred.

The percentage of seed germination was calculated after preliminary arcsin-transformation following the formula, $Y = \arcsin \sqrt{(x\%/100)}$, forwarded by Hinkelmann and Kempthorne (1994).

The collected data were analyzed using the software Statgraphics Plus for Windows Ver. 2.1 and Statistica Ver. 10.

RESULTS AND DISCUSSION

The above ground dry biomass of samples sunflower broomrape (*Orobancha cumana* Wallr.) from different regions of Bulgaria manifests an inhibitory effect (IR from 5.0 to 55.2% w/v) on the seed germination of sunflower (*Helianthus annuus* L.) (Table 2).

By increasing the content of the parasitic weed biomass (from 16.0 to 32.0% w/v), the germinating seeds of test plant - *Helianthus annuus* L. decreased disproportionately compared to the control variant. The differences being statistically significantly reduced at $P=0.05$.

An exception to the described dependence was found in variant P7 at the lowest concentration (8.0% w/v). The differences being statistically unproven at $P = 0.05$ compared to the control variant (Table 2).

This relationship can be explained by the presence of allelochemicals (secondary metabolite, coumarins and others) in the studied samples from sunflower broomrape (Perez-de-Luque et al., 2001).

Depending on the origin and race of the tested sunflower broomrapes samples (Table 1), the degree of inhibition of *Helianthus annuus* L. (Table 2) germination seeds can be arranged in the following order: P_7 (IR_{average}17.1%) → P_6 (IR_{average}17.9%) → P_5 (IR_{average}20.7%) → P_8 (IR_{average}26.8%) → P_4 (IR_{average}28.0%) → P_1 and P_2 (IR_{average}30.2%) → P_{10} (IR_{average}30.6%) → P_9 (IR_{average}30.9%) → P_3 (IR_{average}43.1%).

The data of the biometric measurements of the length of the root, stem and seedling length growth (cm) give possibility for objective estimation of the differences at the initial growth and development stages of the *Helianthus annuus* L. depending on the type and applied concentration of the tested parasitic weed biomass from different tested sunflower broomrape (*Orobancha cumana* Wallr.) (Table 3).

The degree of reducing the length of the germ of *Helianthus annuus* L. is in the range of 5.5 to 68.5%. The most severe is the degree of inhibition of the root (from 2.9 to 71.5%), and relatively weaker of the stem (from 1.6 to 64.4%) compared to the control with distilled water (Table 3).

Concerning concentration dependencies, it is evident that with increase content parasitic weed biomass (from 16.0 to 32.0% w/v), the length of the seedlings decreased disproportionately in all treatments of *Helianthus annuus* L., compared to the control treatment. The differences are statistically proven reduced at $P=0.05$.

The lowest applied concentration (8.0% w/v) of parasitic weed biomass from all tested samples of sunflower broomrape has from relatively weak inhibitory to weak stimulating effect on the growth of sunflower seedling. The differences are statistically insignificant at $P=0.05$ (Table 3)

An exception is found at code P_9 where in all tested concentrations of parasitic weed biomass, the differences in the length of the root, shoot and seedling of the test variety Peredovik are statistically insignificant. At code P_2 and P_{10} the studied indicators are statistically significant decrease at $P=0.05$ compared to the control variant (Table 1 and 2).

The accumulation of fresh biomass in g for one root, one stem and one seedling at the early growth stages of test plant - sunflower depends of the same factors (origin of sunflower broomrape and applied concentrations) and follows the established dependencies on the growth of root, shoot and seedling of length with this difference they are less pronounced (Table 1 and 3).

Therefore, the observed differences at tested samples of *Orobancha Cumana* Wallr. in terms of their allelopathic potential versus test plants *Helianthus annuus* L. can be probably explained by biochemical differences, because the comparisons between them are performed at equal conditions. The variation analysis to determine the influence of the studied factors (η_2) on the seed germination and on the initial growth of *Helianthus annuus* L. shows that the largest share of the total variation was due to Factor A (η_2 from 11.8 to 34.1) - the races of *Orobancha cumana* Wallr.

The applied concentrations of parasitic weed biomass (Factor B) are factors with a significant impact on the variation of phytotoxicity (η_2 from 3.7 to 21.4).

The variants due to the "species sunflower broomrape – applied concentration" relationship are in the range from η_2 11.8 to 39.8.

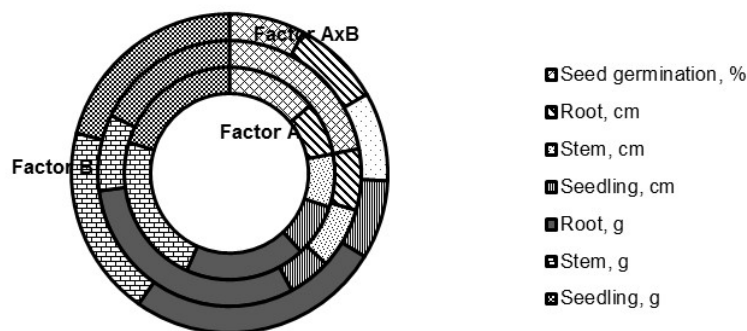


Figure 1. Projection of influence of factors on the factorial plane

The obtained experimental data confirmed the results of Ruiyu et al., 2007, Ali et al. (2013), Takemura et al. (2013), according to them the effect of the allelochemicals is manifested already during the seed germination, but it is more pronounced during the growth and accumulation biomass of seedlings of the test-plants.

The obtained results were analogous when determining seedling vigor index (SVI) and alometry coefficient (CA) of sunflower (*Helianthus annuus* L.) (Table 4). Tested sunflower broomrape samples provoked an inhibitory effect on sunflower vitality (from 5.2 to 82.6%) and reduces (from 0.5 to 2.8 times) alometry coefficient (CA), as compared to the control variant.

The index of plant development (*GI*) depends of the same factors and follows the observed relationship pattern with regard to laboratory seed germination, and accumulation of fresh biomass and growth of seedling of test plants - *Helianthus annuus* L.(Table 4).

The analyses indicated that the studied races sunflower broomrape (*Orobanche cumana* Wallr.) shows an allelopathic effect – *GI* varying from 27.9 to 144.4% and depending of the applied concentrations. It can be arranged in the following order: P₇(Selanovtsi) (86.9%) → P₉(Radnevo) (83.0%) → P₄(Kardam) (73.4%) → P₅(Tyulenovo) (70.7%) → P₆ (Dyakovo) (61.9%) → P₁ (Kardam) (54.8%) → P₂(DAI-infection field) (50.6%) → P₃(DAI-experimental field) (50.2%) → P₈(Svishtov) (46.0%) → P₁₀(Radnevo) (27.9%).

The overall allelopathic potential (OAP) of the studied races of *Orobanche cumana* Wallr. is in the range of -0.1 to 0.9.

With the highest overall allelopathic potential (OAP) can determine conditionally the races sunflower broomrape P₂(DAI-infection field), P₅(Tyulenovo), P₈(Svishtov) и P₁₀(Radnevo), with a value from 0.7 to 0.9 OAP. With the lowest OAP- 0,1 is P₉(Radnevo).

CONCLUSIONS

Dry weed biomass of sunflower broomrape (*Orobanche cumana* Wallr.) at concentrations of 8.0, 16.0 and 32.0% w/v has a stimulatory effect (from -0.7 to -55.3%) or an inhibitory effect on the germination of seeds (from 1.8 to 43.1%) and on the initial development (from 2.4 to 21.0%) of sunflower variety Peredovik

Depending on the allelopathic effect and the origin of the sunflower broomrape (*Orobanche cumana* Wallr.), they can be grouped in the following order: P₇(Selanovtsi) (86.9%) → P₉(Radnevo) (83.0%) → P₄(Kardam) (73.4%) → P₅(Tyulenovo) (70.7%) → P₆ (Dyakovo) (61.9%) → P₁ (Kardam) (54.8%) → P₂(DAI-infection field) (50.6%) → P₃(DAI-experimental field) (50.2%) → P₈(Svishtov) (46.0%) → P₁₀(Radnevo) (27.9%).

The highest overall allelopathic potential (OAP = 0.6) can be conditionally determined sunflower broomrape originating P₂(DAI-infection field) 2016), P₃(DAI-experimental field) and P₆ (Dyakovo) and with the lowest (OAP - from 0.2 to 0.3) originating from P₇(Selanovtsi) and P₉(Radnevo). This fact can be explained by the genetic differences of the studied origins.

Table 1. Allelopathic effect of a dry above ground biomass of different races *Orobanche cumana* Wallr. on seeds germination of sunflower variety Peredovik

Treatment	Origin		Concentration % w/v	Germination seeds, %	Germination in relative to the control variant, %	IR
	Code	Location				
1		Control	0.0	80.78d	100.00	0.0

2	P ₁	Kardam	8.0	61.17a-c	75.72	24.3
			16.0	53.78a-c	66.57	33.4
			32.0	54.22a-c	67.12	32.9
3	P ₂	DAI (infection field)	8.0	64.18bc	79.45	20.5
			16.0	57.10ab	70.69	29.3
			32.0	47.88ab	59.28	40.7
4	P ₃	DAI (experimental field)	8.0	53.78ab	66.57	33.4
			16.0	47.88ab	59.28	40.7
			32.0	36.22a	44.84	55.2
5	P ₄	Kardam	8.0	63.43b-c	78.53	21.5
			16.0	60.11ab	74.41	25.6
			32.0	50.89ab	63.00	37.0
6	P ₅	Tyulenovo	8.0	76.72c-d	94.97	5.0
			16.0	70.38b-c	87.13	12.9
			32.0	45.00ab	55.71	44.3
7	P ₆	Dyakovo	8.0	70.38bc	87.13	12.9
			16	67.50bc	83.56	16.4
			32	61.17a-c	75.72	24.3
8	P ₇	Selanovtsi	8.0	80.78d	100.00	0.0
			16.0	60.1a-c	74.41	25.6
			32.0	60.11a-c	74.41	25.6
9	P ₈	Svishtov	8.0	60.11a-c	74.41	25.6
			16.0	60.11a-c	74.41	25.6
			32.0	57.10ab	70.69	29.3
10	P ₉	Radnevo	8.0	61.17a-c	75.72	24.3
			16.0	55.40ab	68.58	31.4
			32.0	50.89ab	63.00	37.0
11	P ₁₀	Radnevo	8.0	56.79ab	70.30	29.7
			16.0	57.10ab	70.69	29.3
			32.0	54.22ab	67.12	32.9

Legend: Means with different letters differ at $P < 0.05$ level of probability by LSD test; IR – percent inhibitions; DAI- Dobroudja Agricultural Institute, General Toshevo, Bulgaria

Table 2. Allelopathic effect of dray aboveground biomass of different races of *Orobanche cumana* Wallr. on the seedling growth of sunflower variety Peredovik

Treatment	Origin		Concentration % w/v	Length, cm					
	Code	Location		root	IR	stem	IR	seedling	IR
1		Control	0.0	9.78j-m	0.0	4.50m	0.0	14.28i-j	0.0
2	P ₁	Kardam	8.0	8.25h-k	15.6	4.58m	-1.8	12.83h-j	10.2
			16.0	6.21e-g	36.5	2.79d-h	71.5	9.00e-g	37.0
			32.0	5.88ef	39.9	2.94b-i	69.9	8.81ef	38.3
3	P ₂	DAI (infection field)	8.0	6.14e-g	37.2	2.36a-e	75.9	8.50b-e	40.5
			16.0	5.13c-e	47.5	2.31a-e	76.4	7.44b-f	47.9
			32.0	5.25c-f	46.3	2.83d-i	71.1	8.08c-f	43.4
4	P ₃	DAI (experimental field)	8.0	6.57e-h	32.8	2.37a-e	75.8	8.94e-g	37.4
			16.0	8.00g-j	18.2	3.50i-k	64.2	11.50gh	19.5
			32.0	5.20c-f	46.8	3.00d-g	69.3	8.20c-f	42.6
5	P ₄	Kardam	8.0	10.38m	-6.1	2.76d-g	71.8	13.14h-j	8.0
			16.0	9.31j-m	4.8	3.44g-	64.8	12.75h-	10.7

			32.0	7.07f-i	27.7	k 3.00d- k	69.3	i 10.07f- h	29.5
6	P ₅	Tyulenovo	8.0	10.19lm	-4.2	2.29d- e	76.6	12.48 h- i	12.6
			16.0	4.20a-d	57.1	2.00a- c	79.6	6.20a-c	56.6
			32.0	2.90ab	70.3	1.60a	83.6	4.50a	68.5
7	P ₆	Dyakovo	8.0	9.50j-m	2.9	2.92d- j	70.1	12.42g- i	13.0
			16.0	8.75i-m	10.5	2.58d- e	73.6	11.33g- i	20.7
			32.0	3.90a-c	60.1	3.45h- k	64.7	7.35-e	48.5
8	P ₇	Selanovtsi	8.0	10.00k- m	-2.2	3.50a- e	64.2	13.50c- f	5.5
			16.0	9.38j-m	4.1	3.75i- k	61.7	13.13h- i	8.1
			32.0	5.72c-f	41.5	2.33de	76.2	8.06e-g	43.6
9	P ₈	Svishtov	8.0	8.57i-l	12.4	4.43lm	54.7	13.00h- j	9.0
			16.0	2.79a	71.5	3.71k- m	62.1	6.50a-d	54.5
			32.0	2.81a	71.3	2.69c- e	72.5	5.50ab	61.5
10	P ₉	Radnevo	8.0	10.44i-l	-6.7	4.75m	51.4	15.19h- j	-6.4
			16.0	8.50m	13.1	4.58m	53.2	13.08g	8.4
			32.0	8.25h-k	15.6	3.67j- m	62.5	11.92gh	16.5
11	P ₁₀	Radnevo	8.0	5.60d-f	42.7	3.25h- l	66.8	8.85b-f	38.0
			16.0	5.79d-f	40.8	1.93a	80.3	7.70ef	46.1
			32.0	4.86b-e	50.3	2.71c- f	72.3	7.57b-e	47.0

Legend: Means with different letters differ at $P < 0.05$ level of probability by LSD test; IR – percent inhibitions; DAI- Dobroudja Agricultural Institute, General Toshevo, Bulgaria

Table 3. Allelopathic effect of dray above ground biomass of different races *Orobancha cumana* Wallr. on the dynamics of the accumulation of fresh biomass of sunflower seedlings variety Peredovik

Treat ment	Origin		Concentration, % w/v	Fresh biomass, g					
	Code	Location		root	IR	stem	IR	seedling	IR
1		Control	0.0	0.115i-k	0.0	0.340mn	0.0	0.455mn	0.0
2	P ₁	Kardam	8.0	0.128k- n	-	0.365o	-7.4	0.493q	10.2
			16.0	0.089de	11.3	0.319kl	6.2	0.408jk	37.0
			32.0	0.094e-g	18.3	0.263de	22.6	0.357g	38.3
3	P ₂	DAI (infection field)	8.0	0.086de	25.2	0.323e	5.0	0.409jk	40.5
			16.0	0.089de	22.6	0.307i-k	9.7	0.396ij	47.9
			32.0	0.085de	26.1	0.295ij	13.2	0.380h	43.4
4	P ₃	DAI (experimental field)	8.0	0.136mn	-	0.323e	5.0	0.459no	37.4
			16.0	0.105g-i	8.7	0.307jk	9.7	0.411kl	19.5
			32.0	0.085de	26.1	0.236c	30.6	0.321e	42.6
5	P ₄	Kardam	8.0	0.167o	-	0.279f-h	17.9	0.446mn	8.0
			16.0	0.123g-	45.2	0.285f-i	16.2	0.408jk	10.7

			32.0	n 0.078cd	32.2	0.275e-g	19.1	0.353fi	29.5
6	P ₅	Tyulenovo	8.0	0.157o	-	0.285f-i	16.2	0.443m	12.6
			16.0	0.061b	47.0	0.215b	36.8	0.277c	56.6
			32.0	0.045a	60.9	0.181a	46.8	0.225a	68.5
7	P ₆	Dyakovo	8.0	0.098e-h	14.8	0.288g-i	15.3	0.386hi	13.0
			16.0	0.109g-i	5.2	0.279fg	17.9	0.388hi	20.7
			32.0	0.045a	60.9	0.205b	39.7	0.250b	48.5
8	P ₇	Selanovtsi	8.0	0.128k-n	-	0.349n	-2.6	0.477p	8.1
			16.0	0.120g-n	-4.3	0.338mn	0.6	0.458no	43.6
			32.0	0.090de	21.7	0.249cd	26.8	0.339f	5.5
9	P ₈	Svishtov	8.0	0.094e-g	18.3	0.330lm	2.9	0.424e	9.0
			16.0	0.066bc	42.6	0.293h-j	13.8	0.359j	54.5
			32.0	0.065bc	43.5	0.239c	29.7	0.303d	61.5
10	P ₉	Radnevo	8.0	0.120j-l	-4.3	0.326lm	4.1	0.446mn	-6.4
			16.0	0.140mn	-	0.345n	-1.5	0.485pq	8.4
			32.0	0.125f-i	-8.7	0.392p	15.3	0.517r	16.5
11	P ₁₀	Radnevo	8.0	0.104g-i	9.6	0.271ef	20.3	0.375h	38.0
			16.0	0.133mn	-	0.338mn	0.6	0.471op	46.1
			32.0	0.079a	31.3	0.307j-k	9.7	0.386hi	47.0

Legend: Means with different letters differ at $P < 0.05$ level of probability by LSD test; IR – percent inhibitions; DAI- Dobroudja Agricultural Institute, General Toshevo, Bulgaria

Table 4. Developmental index in the early stages of the development of sunflower (*Helianthus annuus* L.) in dependence on the sunflower broomrape (*Orobanche cumana* Wallr.)

Treatment	Origin		Concentration, % w/v	CA	SVI	GI	OAP		
	Code	Location					Germination seeds	Seedling length	Fresh biomass on sunflower seedling
1		Control	0.0	2.2	11.5	100.0	-	-	-
2	P ₁	Kardam	8.0	1.8	7.9	72.2	0.6	0.5	0.1
			16.0	2.2	4.8	46.5			
			32.0	2.0	4.8	45.7			
3	P ₂	DAI (infection field)	8.0	2.6	5.5	49.5	0.6	0.8	0.3
			16.0	2.2	4.2	49.7			
			32.0	1.9	3.9	52.5			
4	P ₃	DAI (experimental field)	8.0	2.3	6.2	62.3	0.9	0.6	0.3
			16.0	2.8	4.3	55.1			
			32.0	1.7	3.0	33.2			
5	P ₄	Kardam	8.0	3.8	8.3	75.9	0.6	0.4	0.2
			16.0	2.7	7.7	70.9			
			32.0	2.4	5.1	73.3			
6	P ₅	Tyulenovo	8.0	4.5	9.6	144.4	0.5	0.8	0.5
			16.0	2.1	4.4	46.9			
			32.0	1.8	2.0	20.7			
7	P ₆	Dyakovo	8.0	3.3	8.7	77.5	0.3	0.6	0.6
			16.0	3.4	7.7	63.8			
			32.0	1.1	4.5	44.4			

8	P ₇	Selanovtsi	8.0 16.0 32.0	2.9 2.5 2.5	10.9 7.9 4.8	85.7 84.3 90.8	0.3	0.5	0.2
9	P ₈	Svishtov	8.0 16.0 32.0	1.9 0.8 1.0	7.8 3.9 3.1	72.3 36.2 29.5	0.5	0.7	0.4
10	P ₉	Radnevo	8.0 16.0 32.0	2.2 1.9 2.3	9.3 7.2 6.1	73.6 100.7 74.7	0.6	0.1	-0.1
11	P ₁₀	Radnevo	8.0 16.0 32.0	1.8 2.9 1.8	5.1 4.3 4.1	53.8 16.8 13.2	0.6	0.9	0.3

Legend: CA - Coefficient of allometry; SVI - Seedling vigor index; GI - index of plant development; OAP - Overall allelopathic potential; DAI- Dobroudja Agricultural Institute, General Toshevo, Bulgaria.

REFERENCES

- Ahn, J.K., I.M.Chung, 2000. Allelopathic potential of rice hulls on germination and seedling growth of barnyard grass. *Agronomy Journal*, 92: 1162-1167.
- Ali, H.H., A. Tanveer, M.A.Nadeem, M.M.Javid, M.S.Kashif, A.R.Chadhar, 2013. Allelopathic effects of *Rhynchosia capitata* on germination and seedling growth of mungbean. *Planta Daninha*, 31(3): 501-509.
- Blagojević, M., B. Konstantinović, N. Samardžić, M. Popov, B. Konstantinović, 2014. Biological characteristics of some parasitic flowering plants. *Herbologia*, 14(2):71-80.
- Chauhan, B. S. and G. Mahajan, 2014. Recent advances in weed management. SpringerLink, Bücher, pp. 411.
- Chittapur, B. M., C. S. Hunshal, H. Shenoy, 2001. Allelopathy in parasitic weed management: Role of catch and trap crops. *Allelopathy Journal*, 8:147-159.
- Encheva, V., P. Shindrova, 1994. Broomrape (*Orobanche cumana* Wallr.)—a hindrance to sunflower production in Bulgaria. Proceedings of the 3rd International Workshop on Orobanche and Related Striga Research;; Amsterdam, The Netherlands. Royal Tropical Institute; p. 619–622.
- Fujii, Y., A. Furubayashi, S. Hiradate. 2005. Rhizosphere soil method: a new bioassay to evaluate allelopathy in the field. Proceedings of the 4th World Congress on Allelopathy, "Establishing the Scientific Base", Wagga Wagga, pp. 490-492.
- Gariglio, N. F., M. Buyatti, R. Pillati, R. D. Gonzales M. Acosta, 2002. Use a germination bioassay to test compost maturity of willow (*Salix sp.*) sawdust. *New Zealand Journal of Crop of Horticultural Science*. 30:135 – 139.
- Hess, M., G.Barralis, H.Bleiholderà, L.Buhr, T.H.Eggers, H.Hack, R.Stauss, 1997. Use of the extended BBCH scale general for the descriptions of the growth stages of mono- and dicotyledonous weed species. *Weed Research*, 37(6): 433-441.
- Hinkelmann, K., O. Kempthorne, 1994. Design and analysis of experiments. New York: Wiley and Sons.
- Islam, A., N. Anuar, Z. Yaakob, 2009. Effect of genotypes and pre-sowing treatments on seed germination behavior of *Jatropha*. *Asian J. Plant Sci.*, 8, 433-439.
- ISTA - International rules for seed testing (1985). *Seed Science and Technology*, 13, p. 361-513.
- Jacobs, M, P.H Rubery, 1988. Naturally occurring auxin transport regulators. *Science*, 241: 346-349.
- Kalinova, J. 2010. Allelopathy and Organic Farming. In: Lichtfouse E. (eds) Sociology, Organic Farming, Climate Change and Soil Science. *Sustainable Agriculture Reviews*, vol 3. Springer, Dordrecht.

Lopez-Raez, J. A., R. Matusova, C. Cardoso, M. Jamil, T. Charnikhova, W. Kohlen, C. Ruyter-Spira, F. Verstappena, H. Bouwmeester, 2008. Strigolactones: ecological significance and use as a target for parasitic plant control. *Pest Management Science*, 64: 471-477.

Macias, F. A., J. C.G. Galindo, J. M. G. Molinillo, 2003. Allelopathy: Chemistry and Mode of Action of Allelochemicals. CRC Press, p. 392, ISBN 9780849319648

Marinov-Serafimov, Plamen, I. Golubanova, 2015. A study of suitability of some conventional chemical preservatives and natural antimicrobial compounds in allelopathic research. *Pesticide and Phytomedicine*, 30(4), 233–241.

Matusova, R., K.Rani, F. W. A Verstappen, M. C. R. Franssen, M. H. Beale, H. J. Bouwmeester, 2005. The Strigolactone Germination Stimulants of the Plant-Parasitic *Striga* and *Orobanche* spp. Are Derived from the Carotenoid Pathway. *Plant Physiology*, 139(2), 920–934.

Miladinovic, D, B, Dedic, F. Quiróz, D Alvarez, M. Poverene, M. Cantamutto, 2012. *Orobanche cumana* wallr. resistance of commercial sunflower cultivars grown in Argentina. *BAG. Journal of basic and applied genetics*, 23(1): 37-41.

Molinero-Ruiz, L., Ph. Delavault, B. Pérez-Vich, M. Pacureanu-Joita, M. Bulos, E. Altieri, J. Domínguez, 2015. Habimana, S, A. Nduwumuremyi, R. Chinama, 2014. Management of orobanche in field crops: A review. *Journal of soil science and plant nutrition*, 14(1): 43-62.

Nasr, M. and S. Mansour. 2005. The use of allelochemicals to delay germination of *Astragalus cyclophyllus* seeds. *Journal of Agronomy*, 4(2): 147-150.

Perez-de-Luque A., D. Rubiales, G. C. Galindo, F. A. Macias, J. Jorin, 2001. Allelopathy and allelochemicals within the plant – parasitic weed interaction. Studies with the sunflower – *Orobanche cumana* system. In: Fer A. Thalanarn P., Joel D. M., Musselman L. J., Parker C., Verkleij J. A. C. (eds.) Proc. of the 7th International Parasitic Weed Symposium, Nantes, France: 196-199.

Pineda-Martos, R., A. J. Pujadas-Salvà, J. M. Fernández-Martínez, K. Stoyanov, L. Velasco, B. Pérez-Vich, 2014. The Genetic Structure of Wild *Orobanche cumana* Wallr. (*Orobanchaceae*) Populations in Eastern Bulgaria Reflects Introgressions from Weedy Populations. *The Scientific World Journal*, 2014, 150432.

Qasem, J. R., 2006. Parasitic weeds and allelopathy: from hypothesis to the proof. In Reigosa MJ, Pedrol N, González L (eds) *Allelopathy: A physiological process with ecological implications*. Springer Verlag, Dordrecht, p. 565–637

Reigosa, M. J., N. Pedrol, L. González, 2006. *Allelopathy: A Physiological Process with Ecological Implications*. Springer Science & Business Media.

Ruiyu, L., R. Hong, Z. Junjian, Y. Cuiping, Y. Chenying, Ch. Liangsheng, L. Wenxiong, 2007. Impact of allelopathic rice seedlings on rhizospheric microbial populations and their functional diversity. *Acta Ecologica Sinica*, 27(9), 3644–3654.

Runyon, J.B, J. Tooker, M. Mescher, C. De Moraes, 2009. Parasitic plants in agriculture: chemical ecology of germination and host-plant location as targets for sustainable control. A review. In: Lichtouse E (ed) *Sustainable agriculture reviews*, Springer, 1: 123–136.

Serghini, K., A. Perez de Luque, M. Castejon-Munoz, L. Garcia-Torres, J.V. Jorin, A.P de Luque, 2001. Sunflower (*Helianthus annuus* L.) response to broomrape (*Orobanche cernua* Loefl.) parasitism: induced synthesis and excretion of 7-hydroxylated simple coumarins. *Journal of Experimental Botany*, 52 (2001), 2227-2234

Shindrova, P., 1994. Distribution and race complex of broomrape (*Orobanche cumana* Wallr.) in Bulgaria. Proceedings of the Third International Workshop on *Orobanche* and related *Striga* research, Amsterdam, pp.142-145.

Shindrova, P., 2006. Broomrape (*Orobanche cumana* Wallr.) in Bulgaria-distribution and race composition. *Helia*, 29(44): 111-120.

Smith, O.P., 2013. Allelopathic Potential of the Invasive Alien Himalayan Balsam (*Impatiens glandulifera* Royle). A thesis submitted to Plymouth University in partial fulfilment for the degree of Doctor of Philosophy.

Takemura, T., E. Sakuno, T.Kamo, S. Hiradate, Y. Fujii, 2013. Screening of the Growth-Inhibitory Effects of 168 Plant Species against Lettuce Seedlings.*American Journal of Plant Sciences*.4(5):1095-1104.

Venkov, V., P. Shindrova, 2000. Durable resistance to broomrape (*Orobanche cumana* Wallr. /*Orobanche cernua* Loefl.) in sunflower,*Helia*, 23(33): 39-44.

Willis, R.J., 2007. Justus Ludewig von Uslar, and the First Book on Allelopathy. Springer Science & Business Media