THE IMPACT OF *CASTANEA SATIVA* AND *AESCULUS HIPPOCASTANUM* AQUEOUS EXTRACTS ON *TRITICUM AESTIVUM* L. SEEDS GERMINATION

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Abstract

The aim of this study was to determine the influence of aqueous extracts (crude extract, 50%, 25%, 10%, 5%, 2.5% and 1% extract, respectively) prepared from the seeds of Castanea sativa (CS) and Aesculus hippocastanum (AH) on the germination of Triticum aestivum L. caryopsis. Germination characteristics were evaluated over 5 days. High concentrations of CS and AH had an inhibitory effect on the germination of Triticum aestivum L. caryopsis. The AH extract most strongly influenced the germination capacity of the seeds, therefore no sample reached the germination rate of 100% by the end of the experiment. The CS extract positively influenced the germination at low concentrations, the samples treated with 10%, 5%, 2.5% and 1% CS extract showing a germination percentage of 97-100%.

Key words: allelopathy, *Castanea sativa, Aesculus hippocastanum, Triticum aestivum,* extract

INTRODUCTION

Castanea sativa, the edible chestnut, grows in Romania in a discontinuous area represented by long strips located at the foot of the Carpathian Mountains. The most widespread culture is in the Baia Mare area and occupies the northeastern part of the European natural chain (Bolea et al., 2001). *Aesculus hippocastanum* is known as the wild chestnut. It is original from Southeastern Europe (Avtzis et al., 2002; Walas et al., 2018) and it has been planted in Central and Southern Europe since the 17th century (Ianovici et al., 2012; Datcu et al., 2018). Chestnut seeds are large and weigh about 13-20 g in fresh condition (Daws et al., 2004; Bonner et al., 2008).

Some plants release chemical compounds into their environment from different parts. Usually, these chemicals are secondary metabolites secreted in very small amounts of plants, but playing a very important role in defending plants against microorganisms or other abiotic factors (Reigosa et al., 2006). Allelopathy is a common biological phenomenon by which an organism produces biochemicals known as allelochemical substances. These compounds

influence the growth, survival, development and reproduction of other organisms. Specifically, these substances can stimulate or inhibit plant growth. Allelopathy can have beneficial effects such as weed control, crop protection or negative influence by stimulating autotoxicity or biological invasion. Therefore, allelochemicals can be used as growth regulators or herbicides (Chang at al., 1998).

Many authors indicate that the secondary metabolites of plants can stimulate the seed germination or plant growth, but this only happens at low concentrations (Khan et al., 2009; Kocira et al., 2019). The higher the concentration, the greater the toxic effect is (Sipos et al., 2012; Terzi et al., 2008; Turk et al., 2005; Walas et al., 2018; Ianovici et al., 2012). Chestnut seeds contain many biologically active compounds that influence the preservation and germination of seeds, as well as the growth and development of seedlings (Cheng et al., 2015). In this study we tried to find out how different aqueous extracts of *C. sativa* and *A. hippocastanum* influence the germination of *Triticum aestivum* L. seeds.

MATERIAL AND METHOD

The biological material consisted of carefully selected caryopsis of *Triticum aestivum* L., Trublion variety, C1, semi-rotten wheat, harvest year 2020. The two aqueous extracts were prepared from the seed of *C. sativa* (CS) and *A. hippocastanum* (AH) respectively, both varieties of chestnut being harvested in 2019. The seeds were stored and kept in a room with a humidity of 40% and a constant temperature of 24ºC until 2021 when they were used. 50 g of vegetable material, crushed to a size of 1 mm, were mixed with 100 mL of double distilled water. The mixture was left at room temperature for 24 hours and stirred from time to time, then filtered through 150 mm size filter paper. After filtration, the extract was made up to 100 mL with double distilled water (crude extract, 100% extract) and kept at 4ºC until the beginning of the experiment. Dilutions of 50%, 25%, 10%, 5%, 2.5% and 1%, respectively were made from the crude extract.

T. aestivum seeds were carefully selected to have the same size and not be physically damaged. The seeds were washed with running water for 30 minutes, then the caryopsis were immersed in 70º ethyl alcohol for 30 seconds and then rinsed for one minute with double-distilled water. This process was done 3 times. Ten caryopsis were placed on a filter paper in a Petri dish and 5 mL of seed extract was added. The control was prepared by adding 5 mL of

double distilled water. Each experiment was performed in triplicate for the extracts of every concentration. The readings were made for 5 days.

RESULTS AND DISCUSSION

The values of the parameters registered for the control (C) were considered as reference (100%), to which the values of the samples (V1-crude extract, V2-50% extract, V3-25% extract, V4-10% extract, V5-5% extract, V6-2.5% extract and V7-1% extract) were reported. From the first day of the experiment there is a significant difference between the two extracts (CS and AH) and the control. High concentrations of CS and AH extracts caused the germination of a smaller number of caryopsis (Tables 1 and 2).

Caryopsis treated with V5CS, V6CS and V7CS, respectively had a higher germination rate than the control of up to +57% (V5CS), while the caryopsis treated with V1CS and V2CS did not germinate at all on the first day. On day 2, sample V5CS maintains a higher germination rate than the control with a value of +28%. On the last day of the experiment, it can be seen that the seeds treated with V4CS, V5CS, V6CS and V7CS, respectively had a germination percentage of over 97%, very similar to that of the control (100%). The V1CS and V2CS were the ones that most inhibited the germination of caryopsis (inhibition of 76.7% and 73.4%, respectively on day 5) (Table 1, Fig. 1).

The seeds treated with V1AH (crude extract) and V2AH, respectively did not germinate at all until the end of the experiment. The V3AH sample strongly inhibited germination, only 30% of the seeds being germinated at the end of the 5 days (70% inhibition compared to the control). Similar to CS extracts, some dilutions produced a higher germination rate than the control in the early days. In this case there were V6AH (+45.8%) and V7AH (+28.5%). No samples reached the germination rate of 100% by the end of the experiment, however the V6AH and V7AH samples gave very good results with germination rates of at least 90% (Table 2, Fig. 2).

The results of the experiment show differences between CS and AH extracts. It can be seen that the *A. hippocastanum* (AH) extract more strongly inhibits the germination of wheat seeds compared to *C. sativa* (CS) extract, which suggests the different composition of the two extracts.

Table 1

The GF values for control (C) and GF values for samples treated with *Castanea sativa* (CS) extract

Parameters	Exp. V. / Day	V0 (C)		V1 (100%CS)		V2 (50%CS)		V3 25%CS)		V4 (10%CS)		V5 (5%CS)		V6 (2.5%CS)		V7 (1%CS)	
		Abs. val.	**% Val**	**Abs. val.**	**% Val.**	**Abs. val.**	**% Val.**	**Abs. val.**	**% Val.**	**Abs. val.**	**% Val.**	**Abs. val.**	**% Val.**	**Abs. val.**	**% Val.**	**Abs. val.**	**% Val.**
GF No	**1**	14	*100*	0	*0*	0	*0*	4	*28.6*	13	*92*	22	*157*	17	*121.4*	20	*142.9*
	2	21	*100*	2	*4.7*	2	*4.7*	15	*71.4*	24	*114*	27	*128*	23	*109*	21	*100*
	3	28	*100*	5	*17.9*	4	*14.3*	17	*60.7*	25	*89.3*	28	*100*	26	*92.9*	24	*85.7*
	4	30	*100*	6	*20*	6	*20*	18	*60*	26	*86.7*	30	*100*	28	*93.3*	27	*90*
	5	30	*100*	7	*23.3*	8	*26.6*	19	*63.3*	30	*100*	30	*100*	30	*100*	29	*96.6*

Table 2

The GF values for control (C) and GF values for samples treated with *Aesculus hippocastanum* (AH) extract

Parameters	Exp. V.** / Day	V0 (C)		V1 (100%AH)		V2 (50%AH)		V3 (25%AH)		V4 (10%AH)		V5 (5%AH)		V6 (2.5%AH)		V7 (1%AH)	
		Abs. Val.	**% Val.**	**Abs. Val.**	**% Val.**	**Abs. Val.**	**% Val.**	**Abs. Val.**	**% Val.**	**Abs. Val.**	**% Val**	**Abs. Val.**	**% Val.**	**Abs. Val.**	**% Val.**	**Abs. Val.**	**% Val.**
GF* No	**1**	14	*100*	0	*0*	0	*0*	0	*0*	4	*28.5*	10	*71.4*	20	*145.8*	18	*128.5*
	2	21	*100*	0	*0*	0	*0*	4	*19*	11	*52.4*	12	*57.1*	21	*100*	24	*114.2*
	3	28	*100*	0	*0*	0	*0*	8	*28.6*	11	*39.3*	13	*46.4*	23	*82.1*	25	*89.3*
	4	30	*100*	0	*0*	0	*0*	8	*26.6*	12	*40*	13	*43.3*	24	*80*	28	*93.3*
	5	30	*100*	0	*0*	0	*0*	9	*30*	14	*46.6*	16	*53.3*	27	*90*	28	*93.3*

*GF – Germinative faculty, **Exp. V. – experimental variant (V0, V1, V2, V3, V4, V5, V6, V7), Abs. val. - number of germinated seeds, % Val - % of germinated seeds relative to % Val of the control which is considered 100%

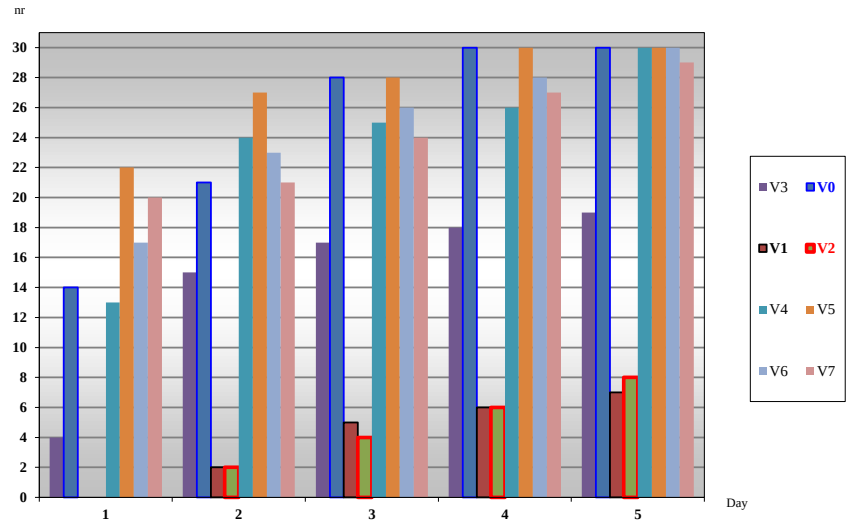

Fig.1. The influence of *Castanea sativa* extracts on the germination of *Triticum aestivum* seeds

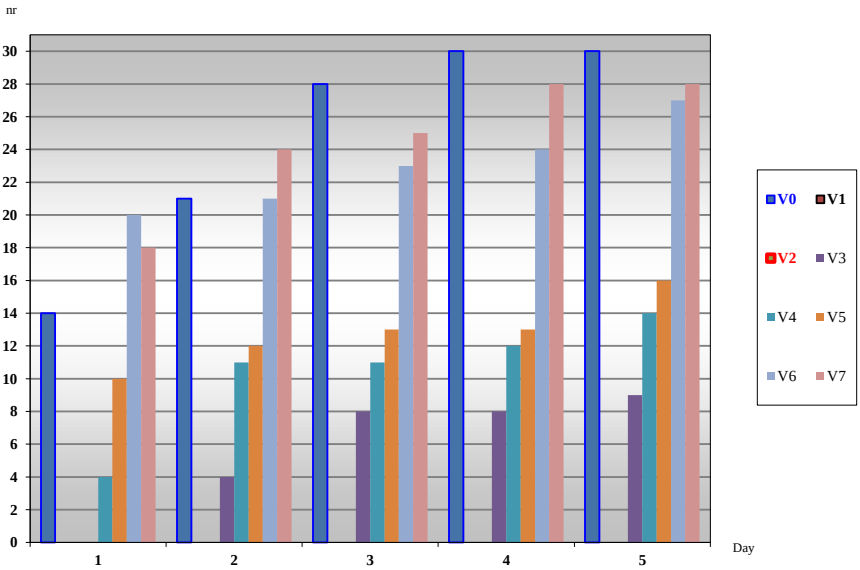

Fig.2. The influence of *Aesculus hippocastanum* extracts on the germination of *Triticum aestivum* seeds

CONCLUSIONS

Studies show that the percentage of germinated and non-germinated seeds is strongly influenced by the concentration of extracts used (Avtzis et al., 2002). As can be seen in the previous graphs, the extracts of *C. sativa* and *A. hippocastanum* greatly inhibited the seed germination in our study. The values obtained were directly proportional to the concentrations of the extracts used, respectively the higher the concentration of the extract, the more strongly the germination of *T. aestivum* L. caryopsis was inhibited. These results suggest a cytotoxic effect of the studied extracts and their potential as a natural herbicide. On the other hand, there are samples of *T. aestivum* seeds treated with *C. sativa* extracts where a higher germination capacity of the control in the first days of the experiment was observed. Similar results have been reported by other allelopathy studies (Melo et al., 2001; França et al., 2008; Silva et al., 2009; Silva-Candido et al., 2010; Silva et al., 2011).

Chiang (Chiang et al., 2003) mentioned that the effect of plant extracts on seed germination comes from different chemical constituents present in these plants. Chemical components (secondary metabolites) can influence seed germination and plant productivity. Inhibition effects usually result from a combination of allelochemicals that interfere with various physiological processes in the recipient plant (Mominul Islam et al., 2018). Some flavonoids have an allelopathic effect and are able to increase the levels of reactive oxygen species causing the inhibition of germination (Kumar et al., 2010). The allelopathic effect on germination rate is due to interference that blocks or delays the progress of metabolic processes during the germination process. The germination rate index measures how long it takes for caryopsis to germinate. The presence of allelochemicals can decrease the rate of development and translocation of endosperm nutrients to the embryo (Ferreira et al., 2004).

REFERENCES

1. Avtzis N., Avtzis D., 2002, The attack of *Aesculus hippocastanum* L. by *Cameraria ohridella* Deschka and Dimic (Lepidoptera: Gracillariidae) in Greece. Ecology, survey and management of forest insects GTR-NE-311, pp.1-5
2. Bonner F.T., Karrfalt R.P., 2008, The woody plant seed manual. Agriculture Handbook 727, United States Department of Agriculture
3. Bolea V., Chira D., 2001, Resistance of chestnut (*Castanea sativa* Mill.) to SO2 in comparison with other tree species. Forest Snow and Landscape Research, 76, pp.420-424

4. Chang S.M., Sung J.M., 1998, Deteriorative changes in primed sweet corn seeds during storage. Seed Science and Technololy, 26, pp.613-626
5. Cheng F., Cheng Z., 2015, Research progress on the use of plant allelopathy in agriculture and the physiological and ecological mechanisms of allelopathy. Frontiers in Plant Science, 6, article 1020, pp.1-16
6. Chiang W., Liu M.C., Lin C.C., 2003, In vitro antiviral activities of *Caesalpinia pulcherrima* and its related flavonoids. Journal of Antimicrobial Chemotherapy, 52, pp 194-198
7. Datcu A.D., Răcoiu V.A., Kolozsvari A.G., 2018, *Aesculus hippocastanum* L. - aspects regarding physiology and pharmaceutical properties. Biostudent, 1, pp.15-22
8. Daws M.I., Lydall E., Chmielarz P., Leprince O., Matthews S., Thanos C.A., Pritchard H.W., 2004, Developmental heat sum influences recalcitrant seed traits in *Aesculus hippocastanum* across Europe. New Phytologist, 162, pp.157-166
9. Ferreira A.G., Borghetti F., 2004, Germinacao: do basico ao aplicado. Porto Alegre: Artmed, pp 323
10. Franca A.C., Souza I.F., Santos C.C., Oliveira E.Q., Martinotto C., 2008, Atividades alelopaticas de nim sobre o crescimento de sorgo, alface e picao-preto. Ciência e Agrotecnologia, Lavras, 32, pp.1374-1379
11. Ianovici N., Ciocan G.V., Matica A., Scurtu M., Șesan T.E., 2012, Study on the infestation by *Cameraria ohridella* on *Aesculus hippocastanum* foliage from Timişoara, Romania. Annals of West University of Timişoara, Biology, XV, pp.67-80
12. Khan A.L., Hussain J., Hamayun M., Shinwari Z.K., Khan H., Kang Y., Kang S., Lee I., 2009, Inorganic profile and allelopathic effect of endemic *Inula koeilzii* from Hamalaya Pakistan. Pakistan Journal of Botany, pp.2517-2527
13. Kocira S., Szparaga A., Kubon M., Czerwinska E., Piskier T., 2019, Morphological and biochemical responses of Glycine max (L.) Merr. to the use of seaweed extract. Agronomy, 9, pp.93-115
14. Kumar D., Kumar N., Akamatsu K., Kusaka E., Harada H., Ito T., 2010, Synthesis and biological evaluation of indolyl chalcones as antitumor agents. Bioorganic and Medicinal Chemistry Letters, 20, pp.3916-3919
15. Melo J.T., Goncalves A.N., 2001, Inibidores de germinacao em frutos e sementes de pequi. Boletim de Pesquisa e Desenvolvimento. Embrapa Cerrados, Planaltina, pp.1-11
16. Mominul Islam A.K.M., Yeasmin S., Qasem J.R.S., Juraimi A.S., Anwar M.P., 2018, Allelopathy of medicinal plants: Current status and future prospects in weed management. Agricultural Sciences, 9, pp.1569-1588
17. Reichel T., Barazetti J., Stefanello S., Paulert R., Zonetti P.D.C., 2013, Allelopathy of leaf extracts of jatropha (*Jatropha curcas* L.) in the initial development of wheat (*Triticum aestivum* L.). Idesia, 31, pp.45-52
18. Reigosa M.J., Pedrol N., Gonzalez L., 2006, Allelopathy. A physiological process with ecological implications. Springer, pp. 229-267

19. Silva-Candido A.C., Schmidt V., Laura V.A., Faccenda O., Hess S.C., Simionatto E., Peres M.T.L.P., 2010, Potencial alelopatico da parte aerea de *Senna occidentalis* (L.) Link (Fabaceae, Caesalpinioideae): bioensaios em laboratorio. Acta Botanica Brasilica, 24, pp.235-242
20. Silva H.L., Trezzi M.M., Marchese J.A., Buzzello G., Miotto Jr. E., Patel F., Debastiani F., Fiorese J., 2009, Determinacao de especie indicadora e comparacao de genotipos de girassol quanto ao potencial alelopatico. Planta Daninha, 27, pp.655-663
21. Silva V.S., Silva Candido A.C., Muller C., Laura V.A., Faccenda O., Simionatto E., Hess S.C., Peres M.T.L.P., 2011, Potencial fitotoxico de *Dicranopteris flexuosa* (Schrad.) Underw. (Gleicheniaceae). Acta Botanica Brasilica, 25, pp.95-104
22. Șipos M., Blidar C.F., Bunta D., 2012, Allelopathic effects of aqueous extracts from horseradish (*Armoracia rusticana* L.) metamorphosed roots on several cereals. Romanian Agricultural Research, 29, pp.169-173
23. Terzi I., 2008, Allelopathic effects of juglone and decomposed walnut leaf juice on muskmelon and cucumber seed germination and seedling growth. African Journal of Biotechnology, 7, pp.1870-1874
24. Turk M.A., Lee K., Tawaha A.M., 2005, Inhibitory effects of aqueous extracts of black mustard on germination and growth of radish. Research Journal of Agriculture and Biological Sciences, 1, pp.227-231
25. Walas Ł., Dering M., Ganatsas P., Pietras M., Pers-Kamczyc E., Iszkuło G., 2018, The present status and potential distribution of relict populations of *Aesculus hippocastanum* L. in Greece and the diverse infestation by *Cameraria ohridella* Deschka and Dimić. Plant Biosystems, 152, pp.1048-1058