

PHYTOTOXICITY ASSESSMENT OF POLYPHENOLIC EXTRACTS FROM *CARUM CARVI* L. FRUITS

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Abstract

The *in vivo* phytotoxicity of Romanian caraway (*Carum carvi*) fruits was assayed by *Triticum aestivum* L. test. We studied the effects of methanolic caraway fruit extracts upon germination, elongation and accumulation of dry biomass in the plants; in addition, it was investigated the influence of the extracts on mitosis in root tissues. The caraway extracts did not inhibit root and shoot elongation of *Triticum aestivum* plants, but enhanced seedling growth, as revealed by the increase of root and shoot length (up to 11.05%) and dry biomass (up to 15.81%). The frequency of chromosomal aberrations observed in root meristems varied slightly around the percentage of the control. The investigated caraway fruit extracts did not have phytotoxic effects but had a slight stimulating effect on germination and growth of *Triticum aestivum* L.

Rezumat

Toxicitatea fructelor de chimion (*Carum carvi*) de proveniență românească a fost determinată *in vivo* utilizând ca organism test *Triticum aestivum* L. A fost studiat efectul extractelor metanolice obținute din fructele de chimion asupra germinării, dezvoltării și acumulării de biomasă uscată în plantulele de grâu; de asemenea, a fost analizată influența acestor extracte asupra diviziunii celulare la nivelul meristemelor radiculare. Extractele de chimion nu au inhibat creșterea în lungime a rădăcinii și tulpinii plantulelor de *Triticum aestivum*, ci au stimulat dezvoltarea acestora, fapt demonstrat de o creștere a lungimii rădăcinii și tulpinii cu până la 11,05% și a acumulării de biomasă uscată cu până la 15,81%, comparativ cu martorul. De asemenea, frecvența abaterilor cromozomiale de la nivelul meristemelor radiculare a variat nesemnificativ, fiind similară cu cea a martorului. Analiza extractelor din fructele de chimion a relevat faptul că acestea nu prezintă efecte fitotoxice, ci au un ușor efect stimulator în ceea ce privește germinarea și creșterea plantulelor de *Triticum aestivum* L.

Keywords: *Carum carvi* L., *Triticum aestivum* L., polyphenols, phytotoxicity

Introduction

Ongoing research revealed that the medicinal use of several botanicals raises possible concern for human health that should be flagged and requires specific attention: plants or plants organs such as fennel fruits (*Foeniculum amari fructus*) or basil (*Ocimum basilicum*, *Ocimum sanctum*) were reported to contain large amounts of estragole, known for its high genotoxicity and carcinogenic potential [7].

Even before the establishment of the European Community there were a series of regulations of the World Health Organization which assessed the pharmaceutical use and safety for consumer exposure to products of plant origin [2]. These rules were subsequently developed and completed by the European Agency for the Evaluation of Medicinal Products. In 2009, the regulations of these organisations were resumed in a report of the Scientific Committee of the European Food Safety Authority (the authority responsible for the area of botanicals and botanical preparations widely used as food supplements) [1].

The fruits of *Carum carvi* L. (*Apiaceae*) have a long history of use in phytotherapy, but also as spice and flavoring agent in food products due to its pleasant aroma and pharmacological effects (digestive, anti-foaming, spasmolytic and galactagogue) [4].

Caraway fruits are well tolerated in medicinal doses and are safe for human consumption [3]. In our study we aimed to assess the potential risks associated with the frequent use of the Romanian caraway fruits as a galactagogue. We studied the cyto- and genotoxicity of caraway extracts upon *Triticum aestivum* L. (wheat) as test species.

Material and Methods

Plant material

Caraway fruits were collected during August 2011 from plants cultivated in the field of Agricultural Research and Development Center, Secuieni, Neamț (Romania) under the same environmental conditions, differing only by the size of the nutritional space (the plants were sown at 3 distances between planting rows: 30 cm, 50 cm and 70 cm). The voucher specimens of the experimental samples were deposited at the Department of Pharmacognosy, Faculty of Pharmacy, “Grigore T. Popa” University of Medicine and Pharmacy, Iași, Romania; the three experimental samples were encoded V1, V2 and V3.

Chemicals

All solvents, chemicals and reagents were of analytical grade and were purchased from Sigma-Aldrich (Steinheim, Germany) and Merck (Darmstadt, Germany).

Extraction

The methanolic extracts were obtained from delipidated caraway fruits with a drug:extract ratio of 2.5:100 g/mL. Briefly, 2.5 g dried caraway fruits were powdered in a knife mill and delipidated with dichloromethane for 40 h in a Soxhlet apparatus. The plant residue was further extracted with 30 mL methanol under reflux for 30 min.; this process was repeated twice (each time for 15 min.) and the combined methanolic extracts were added with the same solvent to 100 mL in a volumetric flask.

Total phenolic content

Total phenolic compounds in the methanolic caraway fruit extracts were determined by Folin-Ciocalteu method [6]. The methanolic extracts were concentrated under *vacuum* and the dry extracts were dissolved in dimethylsulfoxide (10 mg/mL) prior to use. To 40 μ L sample there were added 3.16 mL distilled water and 200 μ L of Folin-Ciocalteu reagent. After 5 min., 600 μ L of 20 % aqueous sodium carbonate were added and the mixture was vigorously shaken. After 2 h incubation at room temperature, the absorbance was measured at 765 nm. The total phenolic content was expressed as gallic acid equivalents (mg gallic acid/g extract).

Phytotoxicity assay

Toxicity assessment of caraway extracts (2011) used an *in vivo* phytotoxicity test; we studied the effects of its phenolic fraction upon *Triticum aestivum* L. as test species.

We used the same methodology as described in a previous publication for methanolic extracts of *Coriandrum sativum* L. fruits [8]. The experiment consisted of four treatments per sample with diluted methanolic extract (1%, 2.5% and 5%) and distilled water (control).

We studied the cyto- and genotoxicity of phenolic fractions upon wheat as test species, namely the effects upon germination, elongation and accumulation of biomass in the plants and the influence of extracts on mythosis in root tissues.

The phytobiological studies were carried out using grains of *Triticum aestivum* L., Dor variety. We used groups of 200 wheat grains for both the control and each dilution. Fruits of test plants were soaked for 24 hours in 50 mL of each treatment variant and in the same volume of distilled water for the control. 150 wheat seeds were washed and placed uniformly upon a system made of sieves, with plastic vessels filled with water placed below

[9]. The cultivation was performed in an air-conditioned chamber at an average air temperature of 22 ± 2 °C and constant light of 2500 lux. After 7 days, germination was abstained, seed germination rate was calculated and shoot and root length were measured. Seedlings having less than 50 mm in length (both root and stem) were excluded from the experiment.

Dry weight of roots and shoots for each treatment variant was determined, after oven drying at 40 °C for 24 h.

The remaining 50 pre-imbibed grains were placed in 10 cm diameter Petri dishes, on filter paper moistened with distilled water. After 72 h, the primary roots were cut off and a squash preparation stained with Schiff reagent was made [5]. Both normal ana- and telophases and those with different types of aberrations (bridges, fragments, fragments and bridges, tri- and tetrapolar anaphases, chromosomes in ring) were counted.

Results and Discussion

The amount of total phenolic compounds did not show any significant differences between individual extracts of caraway (92.99 mg/g; 90.73 mg/g; 95.07 mg/g); V3, possessing the largest nutritional space, showed the highest concentration of these constituents.

Seed germination was used to evaluate the effect of methanolic extracts of caraway fruits on physiological processes during initial seedling growth. Germination rate and the percentage of seedlings less than 50 mm in length are shown in table I.

Table I

The effect of caraway phenolic extracts on wheat plants germination

Treatment variant		Germination rate (%)	Seedlings < 50 mm (%)
Control		97.97	0
V1	1%	96.75	1.30
	2.5%	96.67	2.00
	5%	97.33	6.00
V2	1%	95.33	3.33
	2.5%	96.69	2.65
	5%	98.21	1.79
V3	1%	98.62	0
	2.5%	98.62	1.38
	5%	94.63	2.68

The effect of caraway fruits extracts on wheat plants germination was insignificant as at tested concentrations the differences in germination rate between the treatments were similar with those of the control (Figure 1).

The percentage of undeveloped seedlings (smaller than 50 mm) revealed that eight of a total of nine treatment variants determined normal growth while the plants treated with V1 (5%) dilution were relatively small.

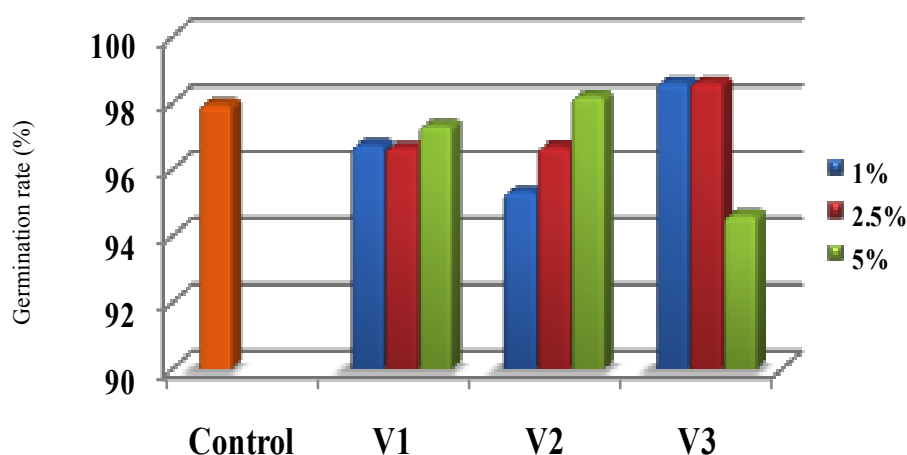


Figure 1

The influence of caraway fruit extracts treatment on germination rate of wheat seeds

The influence of the treatment with caraway fruit extracts on the growth of *Triticum aestivum* plants was studied by measuring the lengths of the roots and shoots and by determining the dry weight of seedlings (Table II).

Table II

Effect of caraway fruits extracts on root and shoot length and dry matter accumulation in wheat

Treatment variant		Root length (mm)±SD	Shoot length (mm)±SD	Dry seedlings weight (g)
Control		137.37±19.24	115.46±17.78	2.15
V1	1%	152.55±21.04 (+11.05)	127.36±18.06 (+10.31)	2.43 (+13.02)
	2.5%	146.82±15.96 (+6.88)	122.11±13.79 (+5.76)	2.30 (+6.98)
	5%	142.69±19.42 (+3.97)	127.29±16.74 (+10.25)	2.12 (-1.40)
V2	1%	135.19±16.10 (-1.59)	122.49±16.22 (+6.09)	2.25 (+4.65)
	2.5%	147.82±16.03 (+7.61)	124.09±17.51 (+7.47)	2.36 (+9.77)
	5%	148.67±20.84 (+8.23)	119.25±22.98 (+3.28)	2.49 (+15.81)
V3	1%	143.65±16.86 (+4.57)	117.80±13.39 (+2.03)	2.29 (+6.51)
	2.5%	148.40±17.62 (+8.03)	123.91±8.69 (+7.32)	2.43 (+13.02)
	5%	148.69±23.22 (+8.24)	126.1±22.56 (+9.22)	2.45 (+13.95)

Data presented as mean ±SD.

Figures in parenthesis indicate the percent of increase (+)/decrease (-) over the control.

As shown in table II, caraway extracts had a slight stimulating effect upon root and shoot elongation of *Triticum aestivum* plants; at a concentration of 1%, V2 determined a decrease in root length over the control (2.33%) while V1 1% exhibited the highest increase (11.05%). Likewise, shoot elongation was stimulated, the lowest value being calculated for V3 1% and the highest for V1 1% (10.31%).

The caraway treatment variants (except V1 5%) enhanced seedling growth as revealed by the increase in dry weight up to 15.81% for the V2 5% treatment in comparison with the control.

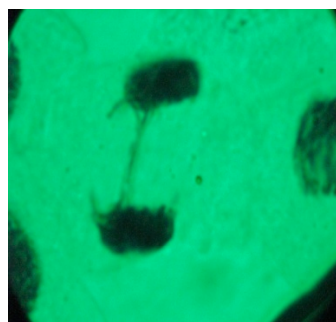
The cytogenetic analysis showed the presence of chromosomal aberrations with a frequency of 3.35% in the control, considered to be normal in this *in vivo* phytotoxicity test. Consequently, a certain genotoxicity induced by caraway extracts should be considered when the prevalence of chromosomal aberrations is higher than 6%. The highest percent of aberrant chromosomes (4.72%) was observed for V3 5% treatment; this value was below the accepted frequency of 6% (Table III).

Table III

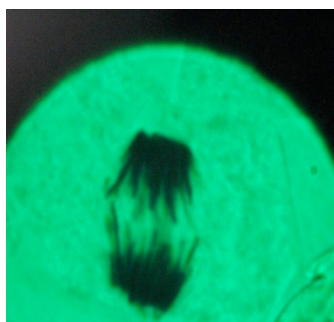
Frequency of caraway extracts-induced chromosomal aberrations in *Triticum aestivum* root meristem

Treatment variant		Number of analyzed anaphases and telophases	Aberrant anaphases and telophases	
			Number	%
Control		984	33	3.35 ± 0.01
V1	1%	847	37	4.36 ± 0.01
	2.5%	1087	36	3.31 ± 0.02
	5%	930	35	3.76 ± 0.01
V2	1%	744	30	4.03 ± 0.02
	2.5%	877	33	3.76 ± 0.01
	5%	658	20	3.14 ± 0.01
V3	1%	895	35	3.92 ± 0.02
	2.5%	810	25	3.08 ± 0.01
	5%	1036	49	4.72 ± 0.02

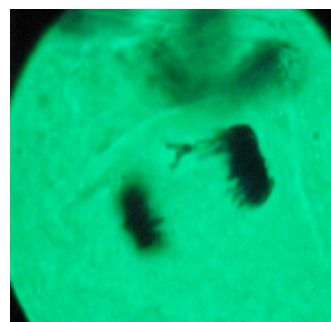
Using anaphase analysis, we could reveal the presence within accepted range of anaphase and telophase bridges as well as chromosome fragments, micronuclei and vagrant chromosomes (Figure 2).



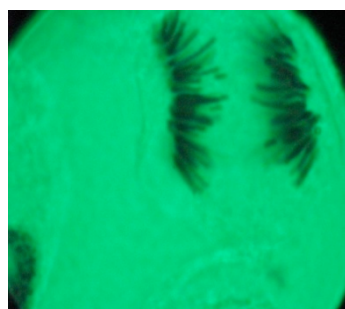
*Telophase bridge,
chromosome fragments and
micronuclei*



*Anaphase with multiple
bridges and chromosome
fragments*



*Ana-telophase with vagrant
chromosome*



*Anaphase with chromosome
fragments*



*Telophase bridge and chromosome
fragments*

Figure 2

Photomicrographs of caraway extracts effects on mythotic activity
in wheat root meristems cells

Conclusions

The caraway extracts did not inhibit root and shoot elongation of *Triticum aestivum* plants and enhanced seedling growth as revealed by the increase of root and shoot length and biomass in comparison with the control. Likewise, the frequency of chromosomal aberrations observed in root meristems varied slightly around the percentage of the control.

The *in vivo* phytotoxicity study of caraway methanolic extracts assayed by *Triticum aestivum* test revealed that the phenolic fraction did not possess genotoxic / clastogenic effects and had a slight stimulating effect on wheat germination and growth.

References

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