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Allelopathic activity of *Nepeta nuda* L. subsp. *nuda* water extracts

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Abstract: *Nepeta nuda subsp. nuda is a medicinal plant growing wild in Bulgaria. Different species of Nepeta genus have been reported to possess allelopathic potential. The present study was conducted to observe its phytotoxic effects on T. aestivum and C. sativus L. seeds in laboratory conditions. Nepeta water extracts (NWE) prepared from aerial parts of plants at concentrations 2, 4, 6, 8, 10, 12 and 14 g/l were tested. The rate of seed germination, the root and shoot length, fresh and dry weight of seedlings were observed after treatment with NWE. As a control served seeds treated with distilled water. Germination was not affected, but NWE showed deterioration in seedling growth. Roots were more affected than shoots. The fresh and dry weights were reduced upon treatment with the extracts tested. These negative effects were dose-dependent. The overall results indicate presence of water soluble allelochemicals in Nepeta nuda subsp. nuda.*

Keywords: *Nepeta nuda subsp. nuda, allelopathy, root and shoot growth inhibition, fresh and dry weight.*

Introduction

Secondary metabolites in plants have drawn great attention due to increasing public concern against synthetic agrochemicals [1, 2, 3]. Different medicinal plants from Lamiaceae family have been studied for their allelopatic potential. Allelopathy of essential oils has been documented [4, 5, 6, 7]. There are also data on presence of water soluble substances in plant tissues [8, 9, 10]. The effects of allelochemicals on sensitive plants can easily be tested in laboratory conditions: plants` responses such as changes in seed germination, plant height and root length, dry and fresh weight are estimated [1].

The genus *Nepeta* (Lamiaceae) includes approximately 250 species [11]. Various valuable constituents have been found in *Nepeta* essential oils – nepetalactones, germacrene D, β-caryophyllene; camphor, cineole, borneol, camphene etc. [12, 13, 14, 15]. Essential oil composition of *Nepeta* genus exhibits significant variations according to the geographic region, climatic conditions, time of plant collection etc. [15]. Different species of *Nepeta* genus are reported to possess some biological activity and are used in folk medicine [16, 17]. Allelopathic potential of this genus was also revealed. Phytotoxicity of *Nepeta* essential oils has been mainly tested [18, 19, 20, 21, 22, 23, 24]. Allelopathy of water extracts has been studied by very few authors [25, 26].

Nepeta nuda subsp. *nuda* L. (syn. *Nepeta pannonica* L.) is a valuable medicinal plant widespread in Europe [12, 27]. This plant is growing wild in Bulgaria [28]. To the best of our knowledge, there are no studies on phytotoxicity of *Nepeta nuda* subsp. *nuda* water extracts.

The aim of this study was to evaluate the allelopathic activity of water extracts made from the aerial parts of *Nepeta nuda* subsp. *nuda* in laboratory conditions using: 1) germination and root/shoot elongation and 2) fresh and dry weight assays.

2. Materials and methods

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2.1. Plant material

Aerial parts of plants cut about 30 cm from the top were collected in the summer of 2014 near Sofia and dried at room temperature. For allelopathic studies, seeds of *Cucumis sativus* L. cv. Gergana and *Triticum aestivum* L. cv. GTW were used.

2.2. Plant extracts. The air-dried and finely ground aerial parts of *Nepeta nuda* subsp. *nuda* were placed in distilled water and left to stay for 24 h at room temperature. The nepeta water extracts (NWE) were then filtered through filter paper. The test extracts were prepared at concentrations 2, 4, 6, 8, 10, 12 and 14 g/l.

2.3. Phytotoxicity testing. Twenty seeds of *C. sativus* and *T. aestivum*, respectively, were placed on filter paper in each of Petri dishes (11 cm in diameter). 5 ml of each extracts or distilled water, as a control, was applied to the seeds. The dishes were sealed and incubated at 25±1°C for 72 h. Three replications of each treatment were done.

Germination assay. Germination was determined by counting the number of germinated seeds. Final germination was expressed as percentage after statistical analyses were performed on the raw data.

Root/shoot elongation assay. The length of the roots and shoots of germinated seeds was measured. Seeds that did not germinate were not included in the test.

Fresh and dry weights assay. The fresh weight of seedlings was determined and the averages were calculated. To measure the dry weight, the seedlings were oven dried at 75^{o} C until a constant weight was obtained. In this experiment, the total weight of the seedling has been considered as the seedling weight.

2.4. Statistical analysis

Experimental data were processed by Student's t-test.

3. Results and discussion

Germination assay. Allelopathic effect of NWE on germination percentage of *T. aestivum* L. and *C. sativus* L. is shown in Table 1. The inhibition of wheat germination by the extracts ranged from 0% (4 g/l) to 26% (14 g/l). There was no clear dose-dependent effect: treatment with 2 g/l inhibited germination by 7.41%, but 4 g/l had no effect. Similarly the negative influence of 8 g/l was stronger than 10 g/l.

Water extracts had no signifficant effect on germination of cucumber seeds. A nonlinear effect also has been observed: the lowest concentration inhibited germination by 6.9% and the highest concentration – by 1.72%.

Table 1. Effect of NWE on germination of *T. aestivum* L. and *C. sativus* L. seeds

NWE, g/l	*T. aestivum*, %	*C. sativus*, %
0 g/l	90.00±0	96.67±0.58
2 g/l	83.33±0.58*	90.00±1.73
4 g/l	90.00±1.00	93.33±1.53
6 g/l	85.00±1.73	90.00±1.73
8 g/l	81.67±0.58**	91.67±1.53
10 g/l	85.00±1.00	93.33±1.53
12 g/l	81.67±1.53**	90.00±2.00
14 g/l	66.67±1.15**	95.00±1.00

Data are expressed as means ± SD (standard deviation),
*P ≤ 0.05, **P ≤ 0.01; NWE – *Nepeta* water extracts.

The results presented in Table 1 revealed that water soluble allelochemicals in *Nepeta nuda* have no significant influence on seed germination. The germination of wheat seeds was more affected than cucumber seeds. Moreover, the nonlinear effect was established. The inconsistent effects of extracts tested on germination are in accordance with data about *Nepeta meyeri* Benth. water extracts [25]. It is known that plant interaction is a complex phenomenon and even positive and negative effects could be observed at different concentrations of secondary metabolites [25, 10].

***Root/shoot elongation assay*.** NWE influenced significantly the growth of test species (Figures 1 and 2). The root and shoot lengths of 3-day-old treated seedlings of both species were much lower in comparison to the control. Unlike the germination, seedlings growth was affected in dose dependent manner.

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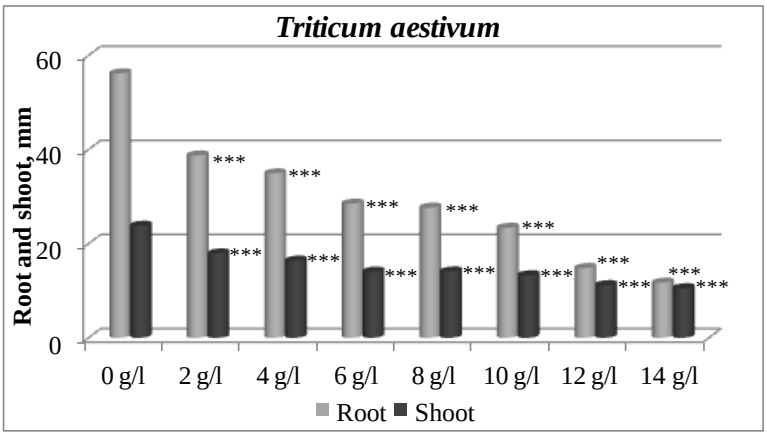

Figure 1. Effect of water extracts of *Nepeta* on root and shoot lenght of *T. aestivum* L.; ***P ≤ 0.001.

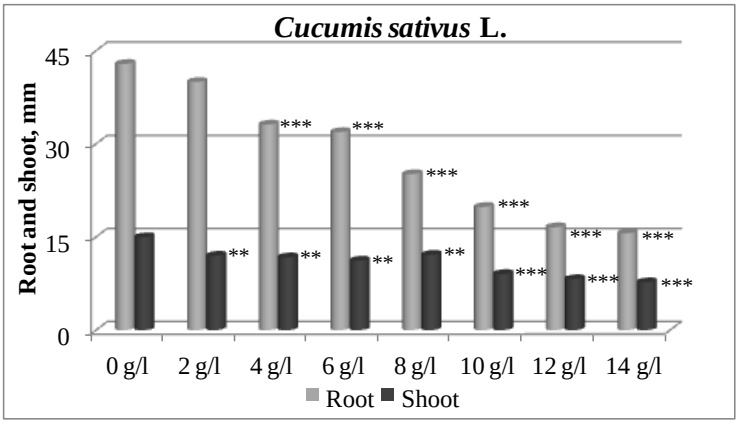

Figure 2. Effect of water extracts of *Nepeta* on root and shoot lenght of C. sativus L.; **P ≤ 0.01, ***P ≤ 0.001.

With all treatments, the lengths of roots showed more inhibition than the lengths of shoots. The inhibition of wheat root elongation by the extracts ranged from 31% (2 g/l) to 79% (14 g/l) and shoot elongation ranged from 25% (2 g/l) to 56% (14 g/l). The inhibition of cucumber root elongation by the extracts ranged from 7% (2 g/l) to 64% (14 g/l), and shoot elongation ranged from 20% (2 g/l) to 48% (14 g/l).

Germination is widely used parameter to detect the phytotoxicity, but the results of present study revealed that early seedling growth is influenced to great degree by extracts tested. Similar to our study, several researchers have reported that seedling growth is more sensitive to allelochemicals than germination [29, 30, 31]. When compared to the control, root growth was more inhibited than that of the shoots. Stronger inhibitory effect on root length as compared to shoot length was observed in other studies [32, 33]. A possible explanation is that direct contact of the roots with the filter paper leads to constant absorption of the water extract [34]. According to [35] the permeability of allelochemicals to root is greater than to shoot. Obviously, the root length reduction is a better indicator of allelopathic effect than shoot growth [36].

In comparative analysis between two test objects, *T. aestivum* were more sensitive to extracts tested than *C. sativum*. This observation confirmed other data that *T. aestivum* is appropriate test object in toxicity assays [37, 38].

Fresh and dry weights assay. As described by [1]: "Plant exposed to natural products can suffer modifications in pattern of biomass allocations between organs, rates of plant growth, water content and relative water content. These parameters are gravimetrically estimated through the dry and fresh weight of plant organs". The fresh weight (FW) and dry weight (DW) of 3-day old seedlings of both test objects were affected upon treatment with NEW (Tables 2 and 3). Both the FW and DW of treated seedlings were reduced

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in comparison to the control. These negative effect was dose-dependent. Similarly to growth inhibition, wheat seedlings were more sensitive than cucumber seeds.

Table 2. Fresh and dry weight of *T. aestivum* L.

Concentrations of NWE	Weight, g	
	Fresh	Dry
0 g/l	2.11±0.20	0.76±0.12
2 g/l	1.61±0.18*	0.74±0.25
4 g/l	1.67±0.21	0.72±0.11
6 g/l	1.38±0.23**	0.67±0.09
8 g/l	1.35±0.06**	0.68±0.09
10 g/l	1.37±0.02**	0.67±0.11
12 g/l	1.19±0.18**	0.56±0.02*
14 g/l	1.10±0.14**	0.51±0.01*

Data are expressed as means ± SD (standard deviation), *P ≤ 0.05, **P ≤ 0.01; NWE – *Nepeta* water extracts.

Table 3. Fresh and dry weight of *C. sativus* L.

Concentrations of NWE	Weight, g	
	Fresh	Dry
0 g/l	2.01±0.12	0.56±0.24
2 g/l	1.96±0.31	0.51±0.12
4 g/l	1.81±0.54	0.47±0.04
6 g/l	1.84±0.30	0.46±0.08
8 g/l	1.75±0.56	0.41±0.02
10 g/l	1.71±0.26	0.41 ±0.04
12 g/l	1.63±0.29	0.39±0.10
14 g/l	1.54±0.35	0.37±0.09

Data are expressed as means ± SD (standard deviation; NWE – *Nepeta* water extracts.

Conclusion

The results of present study revealed that *Nepeta nuda* subsp. *nuda* has allelopathic effect on the growth and development of *T. aestivum* L.and *C. sativus* L. Water soluble allelochemicals inhibited the seedling growth and reduced the fresh and dry weights of the tested plant species. These negative effects were dose dependent. Germination was not affected by water extracts. Further research is needed to identify specific allelochemicals presented in *Nepeta nuda* subsp. *nuda*.

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