Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 31, issue 1, 2021, pp. 5 - 10

THE EVALUATION OF ALLELOPATHIC INTERACTIONS OF CUCUMBER ON MAIZE GERMINATION

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Abstract: During seeds germination, each plant species behave differently depending on certain external and/or internal factor of biological, physical and chemical origin. The presence in the seed's vicinity of other species germinating seeds, or some chemical substances, are acting either at the root or stem levels and furthermore can lead to important changes in plants development processes such as growth rate or biomass or on contrary may stop plant's development. In this study, the maize seeds germination was analyzed in the presence of cucumber seeds germinating to understand if certain effects can be recorded. Following the analysis of our data, the allelopathic effect of cucumber seed germination on maize seed germination was proven. Thus, all analyzed indicators were revealed to be lower compared to control seeds, excepting the number of roots, and highlighting the inhibitory effect of cucumber seed germination on maize seed germination and further development.

Keywords: allelopathy, germination, cucumber, maize, seeds.

INTRODUCTION

Allelopathy is today accepted as a biological phenomenon by which a plant that produces one or more biological compounds may act on different stages of other species development processes such as germination, growth, survival during pathogen attack, or reproduction. These active biological compounds may have either harmful either beneficial effects on a target organism and the community (Zeng *et al.*, 2008).

Data on the allelopathy phenomenon have been published since 1970, and after 1990 it becomes a research topic in the field of botany, ecology, horticulture, and other related research areas. The allelopathic phenomenon may be one of the most important and challenging factors that may contribute to the current distribution and abundance of plant species within certain established communities and may be a support mechanism in understanding the success of invasive alien species (Chou, 1999; Inderjit *et al.*, 2006). Inside natural ecosystems, such interference mechanisms of allelopathy may favor the production of specific active substances by certain plants species having major effects on other plants species in their immediate vicinity (Fitter, 2003). It is considered today that an allelochemical activity under research may vary depending on the applied techniques as well as on operational processes (Peng *et al.*, 2004). Moreover, certain laboratory tests have shown that the natural status of allelochemicals can be changed during the chemical extraction process (Li *et al.*, 2002).

Although several factors can be essential for synthesis and secretion outside the plant body, the genetic allelopathic capacity of any plant species and the existing environment is considered the most important factor. This is directly associated with all peculiarities related to the specific anatomy and physiology of plants organs and tissues directly connecting the plant body to the environmental factors. Thus, only the decrease in the conductance of stomata induced by certain biological compounds may cause further a decrease in the rate of assimilation of carbon dioxide and furthermore, a decrease in the photosynthetic rate. Different features of anatomy are closely connected to their tissues and organ functionality under the actions of certain allelopathic biological compounds. In cucumbers (*Cucumis sativus* L.), for example, it was proved that there is a direct relationship between the process of stomatal closure and the leaf water status or turgescence, followed by some stress symptoms and a reduction in the rate of net photosynthesis induced by allelopathic agents (Yu *et al.*, 2003).

One way to control weeds in crops culture by accessing competitiveness strategy between different plant species has been demonstrated by the allelopathic effect of cucumbers (Lockerman et al. 1979). The process was also studied by Putnam and Duke (1974), proving that the cucumber water extract as well as the powder, inhibited the germination and growth of the common weed barnyard grass *Echinochloa crus-gallii* (L.) P. Beauv., for laboratory and greenhouse conditions (Thi et al., 2008). It was considered that such inhibitory substances of phenolic origin with allelopathic effect are synthesized by the cucumber plants, transferred into the root system, and further may be leached outside into the rhizosphere giving them a way to defend themselves against other competitive plant species and acting as allelopathic substances.

Another species proved to exert allelopathic effects with high potential, used in crops' rotation, and studied under this perspective is garlic (*Allium sativum* L.). Its role in crops rotation has been proven to enriching certain soil physical and chemical properties thus reducing plant vulnerabilities towards pests and diseases (Cheng *et al.*, 2015). Through the research conducted by Xiao *et al.*, (2012) it has been proven that

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rotation of green garlic and cucumber crops had beneficial effects on soil fertilization, by increasing edaphic enzymatic activity of microbial origin, as well as the cucumber biomass. In other situations, such substances inhibited the activity of antioxidant enzymes, favoring the increase of radical's oxygen species (ROS), acting further on oxidation of membrane lipids. Moreover, green garlic produces volatiles that have significantly increased the content of hydrogen peroxide in cucumber leaves (Zhao *et al.*, 2017). It is well established the effect of ROS and secondary metabolites in different morphogenetic processes in plants for *in vitro* systems as well (Antofie and Mitoi, 2020).

Another interesting plant in terms of allelopathy is maize (*Zea mays* L.). The allelopathic potential of different parts of this plant was studied by Minorisky (2002). Maize has been proven to produce three phenolic acids, which are toxic and inhibit the activities of several enzymes (Iman *et al.*, 2006; El-Khawas *et al.*, 2005), really important in the process of nutrient complexes production in the soil and interfering with their root absorption (Kruse et al. al., 2000). The basic action of these compounds is to change the soil pH or furthermore are acting as chelating agents for soil nutrients (Marschner, 1998) making them not accessible for root plants of competitive species. Stimulating allelopathic effects could be observed between tobacco and maize so that Farooq *et al.* (2014) specified that when crops are grown by rotation, after a tobacco culture, maize has a more intense growth and development.

The allelopathy process was also studied in trees and shrubs species. Bughio *et al.* (2013) analyzed for laboratory conditions, the allelopathic effects of *Eucalyptus camaldulensis* Dehnh. on native trees of *Acacia nilotica* H.Karst., proving that eucalyptus leaf extract and leaf litter are reducing the germination rate, chlorophyll content, fresh and dry matters of *Acacia*. Studies show that *Acacia tortilis* Hayne (a not yet recognized subspecies *raddiana*) has an allelopathic potential, through the secreted allelochemical substances, having an inhibitory action on some species of trees and weeds, presenting benefits for crops species (Zouheir and Mohamed, 2011).

Regarding crop plants, the allelopathic effects are measurable especially at the margins of different crops areas, or in their immediate vicinity (Sava-Sand et al., 2013). This was the idea that support the scope of this article and analyse the possible existence of such a phenomenon from the very early stages of seeds germination in the case of crops species. The scope of this study was to select two different species well known, adapted, and integrated into the cropping system at the European level and moreover, well appreciated for their qualities, namely cucumber and maize. One of these species is of European origin (i.e. cucumber) and the other one is of American origin (maize). Also, this latter aspect was taken into consideration for the scope of this study. In this regard, the germination process as a very early event in plant development was studied for mutual allelopathic effects followed by the seedling stage.

MATERIALS AND METHODS

Plant material

The research was carried out with commercial seeds of maize (*Zea mays* L.), the hybrid 'Pioneer PR38A24' and cucumber, the hybrid 'Seminis - SV4097 CV' (*Cucumis sativus* L.). The seeds were carefully selected under the stereo-microscope to avoid the use of degraded seeds (i.e. dehydration or attacked by pathogens).

Experimental design

The seeds were placed into transparent plastic pans, with a length of 17 cm, a width of 10 cm, and a height of 3 cm. On the bottom of these pans, a layer of hydrophilic cotton wool with a thickness of about 1 cm as a vegetation substrate was used, over which an additional paper filter was placed and moistened with 50 ml of distilled water. For 20 days, the watering was performed daily with 10 ml of distilled and sterile water. 12 pots were prepared for sowing such as the following: three control pots with maize monoculture, three with cucumber monoculture, as well as other six mixed sowed pots, one half seeded with maize and the other half with cucumbers for each of the six pots. Each experimental pot contained 50 seeds. Thus, the control variant for maize contained a total of 150 caryopses, respectively 150 seeds of cucumber in case of monocultures (in three different pots for each species), respectively a total of 150 caryopses of maize along with 150 cucumber seeds, in the pots with sample variants (in six different pots). The encoding of the work variants was as follows:

- V0C - control - monoculture of *Cucumis sativus* L.
- V0Z - control - monoculture of *Zea mays* L.
- V1Cz - sample - mixed culture: values of *C. sativus* in the presence of *Z. mays*
- V1Zc - sample - mixed culture: values of *Z. mays* in the presence of *C. Sativus.*

Culture conditions

The research was conducted over a period of 20 days and during this time macroscopic observations were made at a frequency of 5 days.

Morphometry indicators and data interpretation

At 5, 10, 15 and 20 days, measurements were performed and the main analysed indicators in this experiment were as following: *germination capacity* (FG) (%), *number of roots* (pcs / plant), *root length* (mm / plant), *leaf length* (mm / plant) *and fresh matter* (mg / plant). In cucumbers, the *length of the hypocotyl* (mm / plant) and *the length of the epicotyl* (mm / plant) were analyzed, while in maize only the *length of the coleoptile* (mm / plant) was analyzed. The recorded results for control variants were considered 100%, for each date of the experimental observations, and all other marked values referring to the sample variants. V_0C and V_0Z were considered the control groups and the recorded values were taken as references for V_1Cz, respectively V_1Zc, experimental variants. All statistical analyses were made using Microsoft Excel; values are significantly different at P<0.05 according to the Student's t-test. The experiment was repeated three times.

RESULTS AND DISCUSSIONS

Agronomists claim that when a new seedling is developed from the seed and autotrophic feeding capacity is started, the germination process is ended. From a physiological point of view, the germination process is considered completed when the root penetrates the seeds' protective tissues towards the soils (Mazliak, 1982). Hereinafter, it will be presented the experimental results regarding allelopathic effects when cucumber and maize seeds are sown together, in the same incubation conditions from 5 days up to 20 days of cultivation.

The 5th day of incubation

No significant differences in absolute values between the experimental variants and the corresponding controls were noticed (tables 1 and 2).

The roots number and length in both experimental variants were lower than the corresponding control variants, the most affected being the maize seedlings in mixed culture with cucumber. The percentage differences reached values of 55% for the roots number and 57.26 % in the case of root length (i.e. statistically significant differences - table 1). Less intense influences were noticed in the seedlings of cucumber, where the presence of maize led to a decrease in the growth of the number and length of the root by 9.09% and 32.44% respectively (see table 2).

The inhibitory effects mutually induced by the two plants species were also noticed at the level of their aerial part. Thus, in the case of maize (V_1Zc), the length of coleoptiles was 50% lower compared to the control (V_0Z) (statistically significant difference), while in cucumbers (V_1Cz), the inhibition induced by maize led to lower growth increases of the corresponding control (V_0C) by 20.31% for the length of the hypocotyl and 81.81% for the length of the leaf.

The allelopathic influences induced by maize on those of cucumbers in mixed culture (V_1Cz) registered values lower by 20.31% of the length of the hypocotyls and the length of the epicotyls by 30% compared to the control V_0C, noting an inhibitory effect on the aerial organs as well.

In the first 5 days after germination, the maize seedlings did not have leaves, but in the control variant, in cucumber (V_1Cz), there was a slight decrease in the values of the leaf length parameter in front of control (V_0C), respectively the presence of an allelopathic pressure. The highlighting of the phytotoxic effect of allelopathic substances in aqueous horseradish extract on germinating cucumber seeds was also noticed before by Corbu *et al.* (2007), confirming that cucumber seedlings are sensitive from the first moments of their lives to inhibitory substances of plant origin released into the environment.

It is therefore noted that, as of the first 5 days of germination, the controls had lower parameters than the corresponding controls, both for corn and cucumber, highlighting the presence of the mutual inhibitory influences of the two species.

Table 1.

Statistical analysis of data measured during the germination process of *Zea mays* in monoculture (V_0Z) and co-culture with *Cucumis sativus* (V_1Zc) at 5, 10, 15, and 20 days

		V_0Z (control) (monoculture of *Zea mays*)		**V_1Zc** (values for *Zea mays* found in mixed culture with *Cucumis sativus*)				
No. of days	**Statistical data / Parameters**	**X ± Sx**	**s^2**	**X ± Sx**	**s^2**	**±d**	**%**	**Signi-ficance (*p*)**
5	Roots no.	1 ± 0	0	0.45 ± 0.51	0.26	-0.55	-55	***
	Roots length (mm)	12.05 ± 8.52	72.68	5.15 ± 7.08	50.23	-6.9	-57.26	***
	Coleoptile length (mm)	1.9 ± 1.11	1.25	0.95 ± 1.31	1.73	-0.95	-50	**
	Leaf length (mm)	0	n/a	0	n/a	0	0	n/a
10	Roots no.	2.25 ± 1.20	1.46	2.00 ± 0.85	0.73	-0.25	-11.11	ns
	Roots length (mm)	34.75 ± 8.91	79.46	28.95 ± 5.61	31.52	-5.8	-16.71	**
	Coleoptile length (mm)	6.5 ± 1.79	3.21	4.4 ± 1.69	2.88	-2.1	-32.3	***
	Leaf length (mm)	0.35 ± 1.08	1.18	0	n/a	-0.35	n/a	ns
15	Roots no.	2.85 ± 0.74	0.55	3.45 ± 0.75	0.57	0.6	21.05	**
	Roots length (mm)	72.65 ± 7.52	56.55	73.3 ± 6.24	38.95	0.65	0.89	ns
	Coleoptile length (mm)	18 ± 1.52	2.31	19.1 ± 3.98	15.88	1.1	6.11	ns
	Leaf length (mm)	12.85 ± 2.83	8.02	9.95 ± 3.99	15.94	-2.9	-22.56	**
20	Roots no.	4 ± 0.97	0.94	3.9 ± 0.55	0.3	-0.1	-2.5	ns
	Roots length (mm)	83.9 ± 7.67	58.93	82.9 ± 7.63	58.30	-1	-1.19	ns
	Coleoptile length (mm)	20.7 ± 2.36	5.58	19.9 ± 3.40	11.56	-0.8	-3.86	ns
	Leaf length (mm)	58.3 ± 4.25	18.11	46.3 ± 3.52	12.43	-12	-20.58	***

Note: X ± Sx [average (cm) ± standard deviation]; s^2 – variance; ±d – difference to the control lot in absolute values; % – difference to the control lot in percentage values; based on *p* values (significance of difference to control lot): ns – no significant difference (p>0.1), * - low significant difference (0.05<p≤0.1), ** - significant difference (0.01<p≤0.05), *** - very significant difference (p≤0.01); n/a – not applicable.

Table 2.
Statistical analysis of data measured during the germination process of *C. sativus* in monoculture (V_0C) and co-culture with *Z. mays* (V_1Cz) at 5, 10, 15, and 20 days

		V_0C (control) (monoculture of *Cucumis sativus*)		**V_1Cz** (values for *Cucumis sativus* found in biculture with *Zea mays*)				
No. of days	**Statistical data / Parameters**	**X ± Sx**	**s^2**	**X ± Sx**	**s^2**	**±d**	**%**	**Signi-ficance (*p*)**
5	Roots no.	1.1 ± 0.3	0.09	1 ± 0	0	-0.1	-9.09	ns
	Roots length (mm)	14.95 ± 6.82	46.57	10.1 ± 4.35	18.93	-4.85	-32.44	**
	Hypocotyl length (mm)	3.2 ± 1.39	1.95	2.55 ± 1.27	1.62	-0.65	-20.31	ns
	Epicotyl length (mm)	0.5 ± 0.76	0.57	0.35 ± 0.74	0.55	-0.15	-30	ns
	Leaf length (mm)	1.1 ± 1.37	1.88	0.2 ± 0.41	0.16	-0.9	-81.81	**
10	Roots no.	1.85 ± 0.93	0.87	1.4 ± 0.68	0.46	-0.45	-24.32	*
	Roots length (mm)	31 ± 5.16	26.63	34.85 ± 7.69	59.18	3.85	12.41	*
	Hypocotyl length (mm)	10.6 ± 4.42	19.62	6.45 ± 2.01	4.05	-4.15	-39.15	***
	Epicotyl length (mm)	0.9 ± 0.3	0.09	0.9 ± 0.3	0.09	0	0	ns
	Leaf length (mm)	4.1 ± 2.64	6.97	3.55 ± 1.73	2.99	-0.55	-13.41	ns
15	Roots no.	8.4 ± 1.53	2.35	8.3 ± 1.59	2.53	-0.1	-1.19	ns
	Roots length (mm)	53.75 ± 7.22	52.19	67.4 ± 7.3	53.41	13.65	25.39	***
	Hypocotyl length (mm)	16.6 ± 1.72	2.98	15.6 ± 1.93	3.72	-1	-6.02	*
	Epicotyl length (mm)	1.8 ± 0.41	0.16	1.6 ± 0.5	0.25	-0.2	-11.11	ns
	Leaf length (mm)	11.7 ± 1.71	2.95	11.85 ± 1.87	3.5	0.15	1.28	ns
20	Roots no.	9 ± 0.85	0.73	10.15 ± 2.68	7.18	1.15	12.55	*
	Roots length (mm)	76.35 ± 7.61	58.02	78.5 ± 9.82	96.47	2.15	2.81	ns
	Hypocotyl length (mm)	17 ± 1.91	3.68	15.9 ± 1.97	3.88	-1.1	-6.47	*
	Epicotyl length (mm)	1.9 ± 0.3	0.09	1.8 ± 0.41	0.16	-0.1	-5.26	ns
	Leaf length (mm)	15.55 ± 2.96	8.78	11.95 ± 2.56	6.57	-3.6	-23.15	***

Note: X ± Sx [average (cm) ± standard deviation]; s^2 – variance; ±d – difference to the control lot in absolute values; % – difference to the control lot in percentage values; based on *p* values (significance of difference to control lot): ns – no significant difference ($p>0.1$), * - low significant difference ($0.05<p\leq0.1$), ** - significant difference ($0.01<p\leq0.05$), *** - very significant difference ($p\leq0.01$); n/a – not applicable.

The 10th day of incubation

On the next experimental date, respectively 10 days of incubation, there was noticed an increased allelopathic effect, compared to that identified at 5 days, with some exceptions.

The mutual allelopathic effect was further observed in both control variants, compared to the corresponding controls, except for the root length in the V_1Cz variant, where the values of this parameter were higher by 12.41% than in the control (V_0C) (weakly significant values) (Table 2). This might be due to the negative chemotropism given by the presence of substances secreted at the root level by the maize seedlings and therefore new investigations should be considered. According to Majeed et al. (2012), the allelopathic compounds into the soil may interact with roots and furthermore, may alter its absorption capacity for water and minerals, cell division, and other physiological functions. As a result, the negative allelopathic effect increased, which was also highlighted in this case.

The height of the aerial parts of the two species subjected to this experiment was lower compared to controls and compared to the corresponding controls, noting that the largest differences were recorded in the length of the hypocotyls in cucumbers (where the negative increase was 38.15% - statistically significant values), while in the epicotyl a balance of the length values was found with that of the corresponding control (V_0C) (Table 2). For cucumber, the average length of the coleoptiles was also lower by 32.3% compared to control (statistically significant values), validating the observations recorded at 5 days (Table 1). As is known the inhibitory effect on the germination capacity of the maize seeds, but also on the growth of hypocotyls was noticed by using the concentrated extract obtained from the aerial organs of a weed (*Amaranthus retroflexus* L.), instead, the extract from the rhizosphere had inhibitory effect for the growth of maize epicotyls at all tested concentrations (Konstantinovic *et al.*, 2014).

The 15th day of incubation

After 15 days of germination experiment, the allelopathic effect of cucumber on maize became stimulating, possibly due to the negative chemotropic effect, noting an increased proliferation of roots number by 21.05%, but also an increase in length more intense of the sample group (V_1Zc), higher by 0.89% compared to the control V_0Z (monoculture of *Z. mays*); in parallel, the root elongation process was noted by 25.39% in cucumber seedlings in mixed culture, the data being statistically supported (Table 1 and 2).

Regarding the coleoptiles, there was an increase of this first leaf, with a protective role of the embryonic bud in a mixed culture of maize, by 6.11% higher compared to the control group (V_1Cz); a slight stimulation of the growth of the aerial part of the plant (stem) also recorded in the V_1Cz variant, where the leaves showed a reduced increase (of only 1.28%) compared to the control group (V_0C). Regarding all other monitored parameters, the recorded effects were mutually antagonistic. Similar results were obtained on the allelopathic effects of aqueous extract of *Conyza canadensis* (L.) Cronquist, on germination and growth rate of cucumbers and other plants and conducted by Xingxiang *et al.* (2009).

The 20th day of incubation

On the last experimental date, the inhibitory allelopathic effect of cucumber on maize became more conclusive, so that all the analyzed parameters were inferior to the control V0Z.

At the same time, an allelopathic pressure of maize on cucumber was observed, through the induced negative chemotropism, so that there was a stimulation of the proliferation and growth of cucumber roots (V_1Cz) compared to the corresponding control (V_0C) similar to that from 15 days for the maize seedlings (Tables 1 and 2).

Given that all experimental protocols were followed and that there were no other external influences on the two plant species put together or individually, the only explanation for the obtained results may be substantiated by the action of active biological compounds released by both plants species, which, as noted, have allowed either the stimulation either the inhibition of certain physiological parameters, leading to the above-mentioned result.

The allelopathic effects of some plant species on others have been recognized as a survival strategy, but self-poisoning has also been suggested as a useful mechanism to avoid competitors of some annual and perennial species (Canals et al. 2005). By using allelopathic plant species that selectively inhibit weeds and do not negatively influence crop growth, sustainable agriculture could be promoted (Singh et al. 2003, Sand et al., 2013).

CONCLUSIONS

The significance related to the ecological importance of allelopathic effects is difficult to be estimated since some of the active biological compounds released into the environment can be accessed and modified by edaphic microorganisms and thus, reducing their potential actions. However, it is obvious that there is a mutual antagonistic allelopathic effect between the seedlings of both species, *Z. mays* and *C. sativus* on stem growth and multiplication rate, and - with some exceptions – on the root development.

The purpose of this study is to highlight that exists a mutual influence between the two plant species, for laboratory conditions. Such effects can also be expressed in the field, but the myriad of other factors may either potentiate or on contrary decreased the expression of allelopathic effects. Moreover, based on similar experiments it is possible to define the subject of allelochemical residues that can be accumulated into the soil during a crop culture, and which could induce negative or positive effects over next year on the next crop species when it is the case of crops rotation. However, all these are subject of future studies for improving crops production for supporting food security under climate change.

AUTHORS CONTRIBUTION

Conceptualization, C.F.B.; methodology, C.F.B.; data collection, D.S.P..; data validation, C.F.B. and D.S.P.; data processing, C.F.B. and D.S.P.; writing - original draft preparation D.S.P.; writing - review and editing, C.F.B. and D.S.P.

FUNDING

This research received no external funding.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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