ECOLOGIA BALKANICA
2019, Vol. 11, Issue 1 June 2019 pp. 167-177

A Case Study of Allelopathic Effect of Parsley, Dill, Onion and Carrots on the Germination and Initial Development of Tomato Plants

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Abstract. Vegetables are important worldwide, but their production faces many problems. One of them is the yield decline due to soil sickness, allelopathic effects of other crops, weeds and trees, and also the autotoxicity when grown continuously for several years. For the purpose of organic farming, allelopathy can be an important element in balancing the relationship between density and weeds, pests, diseases and cultivars. Relationships among the vegetable species and varieties in particular mixed stand are not sufficiently explored, which is a sufficient reason to conduct such a study. The present study focuses on the allelopathic relationships in agrophytocenoses in order to assess the possibilities of mixed crop cultivation of tomato plants with other vegetable crops and spices. Vegetable species may have a negative, neutral or positive effect one to another when grown in mixed agrophytocenoses. To explore the extent of this impact, test plants from tomatoes have been treated directly with plant extracts from other vegetable plants - parsley, dill, onion and carrots. The study showed that the applied concentrations of parsley, carrot, dill and onion extracts had stimulating, inhibiting or indigenous effect on tomato seed germination, growth and accumulation of dry biomass. Seedling vigor index of plant development (SVIcm) and biomass synthesis (SVIg) depended on the type of the extract applied more than the concentration applied ($p<0.05$). Seed germination was less affected than root and shoot growth in all species ($p<0.05$). Length of the seedlings was significantly influenced (positively or negatively) by the allelopathic plants and the effect was stronger with the increment of the extract concentration ($p<0.05$). Most pronounced negative effect was found at the 1% extract of fresh onion biomass – 34% reduction against the control ($p<0.001$). Stimulatory effect was strongest at the 1% extract of fresh carrot biomass – 37% increment against the control ($p<0.001$).

Key words: allelopathy, mixed crops, organic farming, vegetables.

Introduction

Some plants have allelopathic potential by releasing allelochemicals to their surroundings that have either deleterious or beneficial effects on other plants in the vicinity. The term allelopathy includes both harmful and beneficial biochemical interactions between all types of plants but also with microorganisms, through the release of chemicals from plant parts by leaching, root exudation, volatilization, residue decomposition and other processes in both natural and agricultural systems (Molish, 1937). Allelopathy can affect many

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aspects of plant ecology including occurrence, growth, and plant succession, the structure of plant communities, dominance, diversity and plant productivity (RICE, 1984). Allelopathic interactions are complex and can involve different classes of allelochemicals which are present in all plant tissues. Many of the chemicals may be released together and may exhibit additive or synergistic effects (QASEM, 1995).

Some of the major problems of vegetables production are the yield decline due to autotoxicity and soil sickness (when vegetables are grown continuosly for several years) and the low yield of vegetables in monoculture and cropping systems due to allelopathic effects of crops, weeds and trees (JOHN *et al*., 2010).

Tomato (*Lycopersicon esculentum* Mill.), when grown in monoculture for long periods leads to soil sickness (ZHENG *et al*., 2004). Autotoxicity and soil problems in tomato production have been reported (LUO *et al*., 1995; ZHOU *et al*., 1997). Continuous cropping reduces the root vitality and fruit quality of tomatoes (WU *et al*., 1997). Soil sickness is complex phenomenon due to several factors involved and autoxicity is major one. ZHOU *et al*. (1997) reported the presence of di-iso-octyl phthalate, di-iso-butyl phthalate, tannic acid and salicylic acids in tomato plants.

In mixed cropping systems, where numerous crops and trees are grown together, vegetables are essential components and allelopathic interactions arise. BHATT & DHAWAN (1980) reported that addition of crushed seeds of carrots and coriander (*Coriandrum sativum* L.) gave significantly higher grain yield of wheat. KIM & KIL (1985) reported that the aqueous extract of leaves, stem and root of tomato heavily inhibited the seed germination and growth of lettuce and tomato. In general, the phytotoxicity of the tomato extracts increased in the following order: time for extracting 25 h < 48 h < 72 h. Tomato exhibited auto inhibition. Four phytotoxic phenolics, salicylic acid, gallic acid, protocatechuic acid and vanillic acid were obtained from the aqueous extracts of tomato leaves by paper chromatography. The aqueous leachates of tomato significantly inhibited growth of certain crops in pot culture.

Mixed crop cultivation based on allelopathic relationships is a relatively new direction in the development of organic farming and vegetable production. When the crop combination was correctly done, plants in mixed agrophytocenoses obtain some benefits as: *i*) full utilization of soil nitrients; *ii*) to support each other through the release into the atmosphere and soil substances from roots or leaves and thereby be protected from attack by pests and diseases; *iii*) improvement of taste; *iiii*) "space exploitation", soil cover provided and better income generated (JOHN *et al*., 2010).

The aim of the present study was to investigate the allelopatic effect of parsley (*Petroselinum crispum* (Mill.) Nyman. ex A.W.Hill.), dill (*Anethum graveolens* L.), onion (*Allium cepa* L.) and carrot (*Daucus carota* L.) on seed germination, growth and development of tomato plants in order to assess the possibilities for mixed crop cultivation. Application of this allelopathic effect based method in organic farming would allow the cultivation of healthy and sustainable plants and the maintenance of relatively clean fields for the next crops in vegetable crop rotation.

Material and Methods

Plant interactions occurs primarily on the basis of the released allelochemicals in natural and agrophytocenoses or on the basis of allelochemical exudates from plant residues in crop rotation. Cultural plants may also have a negative, neutral or positive effect each other when they are grown in mixed agrophytocenoses. To explore the extent of this impact, test plants from tomatoes have been treated directly with plant extracts from other vegetable plants. The effect of the allelopathic influence of these extracts was used as a basis for tracking the interactions between studied species. Even in a relatively early phase (seedlings ready for field), some tendencies have been noticed and considered as characteristic of the more mature phases of development.

The study to establish the allelopathic interactions among four leguminous plants (parsley, dill, onions, carrots) and tomatoes (both seeds and seedlings) was carried out in the laboratory of the Department of Agroecology and Environment Protection, Agricultural University – Plovdiv. Detailed description of the method for preparation of seed extracts and fresh biomass was described below.

Aqueous extracts from seeds

Aqueous extract from each one of studied species (parsley, carrots, onion or dill) have been prepared using 1 g seeds, crushed in a mortar and pestle with quartz sand and distilled water and filtered through filter paper. The filtrate was made up to 100 ml with distilled water (stock solutions) (Popov *et al.*, 2009).

Experimental solutions have been prepared from the stock solution as follows: 1% seed extract = 10 ml stock solution; 0.3% seed extract = 3 ml stock solution + 7 ml distilled water; 0.1% seed extract = 0.1 ml stock solution + 9.9 ml distilled water.

Aqueous extracts from fresh biomass

Parsley, onion, carrot and dill seeds have been sown in plastic trays (about 0.5 L volume) containing a compacted and well-watered soil. Plant seedling have been grown in lab condition for about 28-day period. Then plants have been removed from the soil, their roots have been cleared from the adhering soil particles. Aqueous extract from each one of studied species (parsley, carrots, onion or dill) have been prepared using 25 g of fresh biomass (fb) (including roots, stems, leaves), cut at small pieces and flooded with 250 ml distilled water. After 24 hours stay they were filtered through filter paper (stock solutions).

Experimental concentrations have been prepared as follows: 1% fb extract = 10 ml stock solution + 90 ml distilled water; 0.3% fb extract = 3 ml stock solution + 97 ml distilled water; 0.1% fb extract = 1 ml stock solution + 99 ml distilled water (Popov *et al.*, 2009).

Bioassay with tomato seeds

Experiment was carried out in petri dishes (10 cm diameter), each one containing 10 tomato seeds sown on filter paper (Fig. 1A). The paper is wetted with 10 ml seed extract of parsley, carrots, onion or dill in different concentration (1%, 0.3% and 0.1%, respectively). Control was made with pure distilled water. All tested variants were made in three replications. Petri dishes were left in germination chamber at 22°C for 14 days (Schwarz *et al.*, 2014). After that, the number of germinated seeds, % germination, length of aboveground and underground plant parts (cm) have been observed. Length of the shoots and roots of experimental plants was recorded using graph paper.

Bioassay with tomato seedlings

Tomato seeds have been sown in plastic trays (about 0.5 L volume) containing a compacted and well-watered soil (Fig. 1B). Tomato seedling have been grown in lab condition for about 28-day period. The experimental design consisted in adding 50 ml fresh biomass extract of parsley, carrots, onion or dill in different concentration (1%, 0.3% and 0.1%, respectively) to the grown media at the 1-st and 3-th week and irrigation with 50 ml distilled water at the 2-nd and 4-th week. Control plants have been irrigated with distilled water only. All tested variants were made in three replications (Schwarz *et al.*, 2014). After that, the length of aboveground parts (cm) and dry biomass weight (g) per tray have been measured. Biomass was measured with analytical balance (Model H440-21N, Kern & Sohn Gmb, Germany).

Allelopathic effect assessment

The index of plant development (GI) was assessed by the formula of GARIGLIO *et al.* (2002):

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

where: G – germinated seeds (%) in each treatment; G_0 – germinated seeds (%) in the control treatment; L – average length (cm) of seedlings in treatment transformed into percentage in relation to the control treatment; L_0 – average length (cm) of the seedlings in the control treatment taken as 100%.

Seedling vigor index (SVI) was determined using the equation poposed by ISLAM *et al.* (2009):

$$SVI=\left(\frac{S\times G}{100}\right)$$

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 calculated by the formula of NASR & MANSOUR (2005):

$$CA=\frac{L_s}{L_r}$$

where: Ls is shoot length and Lr is root length, cm.

Percent inhibition (IR) was found according to the adapted formula of SURENDRA & POTA (1978):

$$IR=\frac{C-T}{C}\times 100,\%,$$

where: C – parameter (length or biomass of shoot/root) in control; T – same parameter in experimental treatment.

Overall allelopathic potential (OAP) was determined by the equation of TIQUIA *et al.* (1996):

$$OAP=\frac{IR_{average}}{IR_{min}+IR_{max}}$$

where: IR_{min} - percent inhibition of the seedling growth at the lowest applied concentration, compared to the control; IR_{max} - percent inhibition of the seedling growth at the highest applied concentration, compared to the control.

A score between 0.0 and 10.0 and more was obtained and the data were ranked according to this score. A maximum score of 10.0 and morewould indicate that the test samples 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√(x%/100), forwarded by HINKELMANN & KEMPTHORNE (1994).

Fig. 1. Experimental design of bioassays: A – tomato seeds; B – tomato seedlings

Statistical evaluation

Raw data from all analyses were processed using statistical software package Statistica 7.0. for Windows (StatSoft Inc., 2006). ANOVA and Student/Fisher test were used for testing the differences of allelopathic effect, both between different aqueous extracts and also between tomato seed and seedling reaction ($p<0.05$).

Results and Discussion

Allelopathic effect on tomato seeds

Results of the effect of seed extract of parsley, onion, carrot and dill in tested concentrations on germination, length and biomass of aboveground and underground plant parts of tomato are presented in Fig. 2-4.

Seed germination is a critical phase in the life cycle of most plant species, especially the cultural ones. Unfavorable conditions of the environment have a strong impact on the germination processes. Roots of the plants first come into contact with the environmental pollutants. The suppression of the growth is a consequence of that of the root as it limits the intake of water and mineral substances, and also because of the direct effect of inhibitors on the cellular metabolism (Vince & Zoltan, 2011). In our study, most pronounced and significant was the negative effect of seed extract from carrot and dill, especially at highest test concentration (1%) ($p<0.05$). We found no statistical differences in the rest of experimental variants although the observed inhibition of tomato seed germination (Fig. 2).

Lowest concentration of all four test plants (0.1% seed extract) exhibited a light stimulating effect on tomato shoot elongation but no statistical differences have been found. Increment of the test concentrations led to an inhibitory effect to the length of aboveground plant parts of tomato (Fig. 2). Strongest negative effect on the shoot length was proven to the experiments with 1% seed extract of onion (56% compared to the control, $p<0.001$) and dill (49% in comparison with the control, $p<0.01$), followed by parsley and carrot – 39-42% when compared to the control ($p<0.05$).

Data obtained showed that the growth inhibiting effect of the treatments was more expressed on root length (Fig. 4). Growth stimulating allelopathic effects are rare. In our study this effect was found only with the lowest concentrations of dill and parsley seed extract (0.1%), but even the small difference with the second test concentration of 0.3% led to the drastic reduction of tomato roots ($p<0.05$). Strong negative effect was proven for the carrot seed extract in all studied concentrations ($p<0.001$) and for the 1% solution of all four plant seeds ($p<0.01$).

As a whole, all seed experiments showed that parsley, onion, carrot and dill seeds possess some allelochemicals that affect in greater extent next stages of tomato vegetation than germination of seeds ($p<0.05$). Our results were in agreement with the findings of Kocacë-Aliskan & Terzi (2001), where seed germination was less affected than root and shoot growth in tomato, cucumber, garden cress and alfalfa. Growth stimulation in shoots and roots was observed in 0.1% solutions from parsley and dill. The higher concentrations revealed an inhibitory effect (Fig. 4). Similar results were found by Dhima *et al.* (2009) in relation to sweet basil, dill, parsley and mint, where the inhibition of maize root elongation ranged from 5% to 39%, while the corresponding inhibition of fresh weight ranged from 0% to 15%.

Allelopathic effects on tomato seedlings development

Results of the effect of fresh biomass extract of parsley, onion, carrot and dill in tested concentrations on length (cm) and dry biomass (g) of aboveground plant parts of tomato are presented in Figures 5-6. Biometric measurements of the seedlings growth and biomass synthesis gave a possibility for objective estimation of the differences at the initial developmental stages of tomato depending on the type and the concentration of the applied plant biomass extract (parsley, onion, carrot, dill).

Length of the seedlings was significantly influenced (positively or negatively) by the allelopathic plants and the effect was stronger with the increment of the extract concentration ($p<0.05$). Most pronounced negative effect was found at the 1% extract of fresh onion biomass – 34% reduction against the control ($p<0.001$). Stimulatory effect was strongest at the 1% extract of fresh carrot biomass – 37% increment against the control ($p<0.001$). Inhibitory effect was due both to the differences in the growth media and the allelochemicals type and concentration – seed extract or fresh biomass extract. Similar results were reported by FERNÁNDEZ-APARICIO *et al.* (2013), QASEM (2010), LEV-YADUN (2013), according to whom allelopathic effect was species specific and depended on the applied concentrations. The data obtained corresponded with the findings of ASHRAFI *et al.* (2007), OTHMAN *et al.* (2012), VLAHOVA (2014) and VLAHOVA & YOVEVA (2014) that the effect of the allelochemicals is manifested already during the seed germination, but it is more pronounced during the growth of primary seedlings of plants. Some exceptions were found in the aqueous extract of fresh dill biomass where length of seedlings in all experimental treatments was quite similar with the control (Fig. 5).

Stimulating effect on the biomass synthesis was observed in all experimental variants with onion extract and in the experiments with lowest concentration of the rest three species (Fig. 6). Most pronounced stimulation on the dry biomass weight exhibited the 0.1% carrot extract, reaching 136% when compared to the control ($p<0.05$). But two higher concentrations revealed to the significant decrement of dry biomass – up to the 46% of the control ($p<0.05$). VLAHOVA (2014) and VLAHOVA & YOVEVA (2014) have found a positive impact on the length of the pepper root upon the application of an extract of zinnia roots in low concentrations but no in higher concentrations studied. A leading effect of positive allelopathic influence have been observed on the height, the number of leaves and the root length of pepper plants for the extracts of roots by marigold and basil VLAHOVA & YOVEVA (2014).

Data on the tomato biomass per pot revealed a negative relationship between shoot length and dry biomass of tomatoes under the carrot extract treatment ($p<0.01$) and onion treatment ($p<0.05$). This fact could be explained with the development of longer but thinner stems in tomatoes, irrigated with these extracts (Fig. 5-6). A positive relationship was proven only for the effect of 0.1% extract of carrot, 0.1% extract of parsley, and 0.1%-0.3% extract of dill ($p<0.05$). The analysis showed that the applied concentrations of parsley, carrot, dill and onion extracts had stimulating, inhibiting or indigenous effect on tomato seed germination, growth and accumulation of dry biomass (Table 1). Seedling vigor index of plant development (SVI_{cm}) and biomass synthesis (SVI_g) depended on the type of the extract applied more than the concentration applied ($p<0.05$). Lowest SVI values were found under the experimental treatment with carrot (from 2.5 to 6.6) and onion extracts (from 2.3 to 8.6) while the highest SVI values were observed in the plants under parsley (from 3.1 to 10.8) and dill extracts treatment (from 2.1 to 10.0). When the concentrations raised up to 1% extract, there was an inhibition of SVI_{cm} in the range of 13.6-83.3% and of SVI_g in the range of 1.8-70.9% in comparison with the control plants. On the contrary, some stimulation on the process of biomass accumulation was observed in the tomato plants under the 0.1% onion extract treatment (Table 1).

The values of the Coefficient of allometry (CA) of tomato plants in different experiments varied between 0.7 and 4.7 as a consequence of the allelopathic effect of studied water extracts. On this basis they could be arranged in the ascending order as follows: parsley = 1.8 (0.7-2.8) > dill = 2.4 (0.8-4.5) > onion = 2.6 (0.7-4.7) > carrots = 2.9 (1.7-2.8). Statistical evaluation showed that an increment of the extract concentrations revealed a reduction of the CA in test tomato plants from 161% to 674% in comparison with the control ones ($p<0.05$).

Complex assessment of the studied water extracts effect using the plant development index (GI) showed that the average values altered between 33.2% (carrot) and 52.2% (parsley) depending mainly on the type of donor ($p<0.05$). Stronger inhibition on the tomato development exerted the water extracts from carrot and onion where GI varied in the range of 19.7%-52.7% and 18.2%-69.0%, respectively. Maximal development value was observed in the tomato plants influenced by the 0.1% extract of parsley (GI=86.4%) and 0.1% of dill (GI=80.1%), hence the allelopathic effect in these cases could be defined as poor (Tiquia *et al.*, 1996). But the allelopathic effect of all studied water extracts in 1% concentration was strong as the GI of the test plants was under 25% compared to the control. In the course of the experiments, a reduction of the GI from 1.4 to 4.8 fold was observed when regarding the tomato plants under the 0.1% and 1% extract influence.

Overall allelopathic potential (OAP) of the tested water extracts in three concentrations (0.1%, 0.3%, 1%) on tomato plants had values from 0.1 up to 9.8. Bioassay with tomato seeds revealed that relatively low allelopatic potential on the germination process had carrot and onion (OAP=0.1), while in the next stages of initial development this allelopathic effect increased (OAP of carrot = 3.8; OAP of onion = 4.6) (Table 1). Higher allelopathic potential was found for dill and parsley where even in the germination stage OAP values were 0.3 and reached up to 9.8 (dill) and 6.0 (parsley) at the seedling length suppression (Table 1). When regarding the OAP values concerning thy dry biomass accumulation in tomato, strongest inhibition was observed in the experiment with water extract of carrot (OAP=1.5), in the experiment with extract of parsley OAP was 0.1, and in two other variants – onion and dill, no OAP could be calculated due to the stimulatory effect. However, on the basis of all studied coefficients, water extract of onion was found to be highly allelopathic to the tomato plants and not suitable for mixed planting.

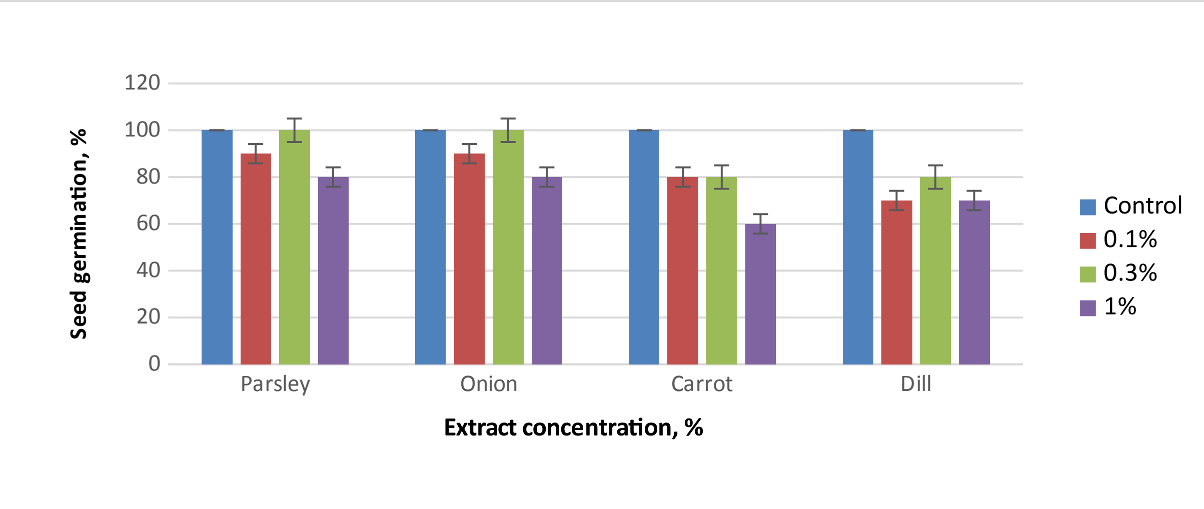

Fig. 2. Allelopathic effect of seed extract on tomato seeds germination.

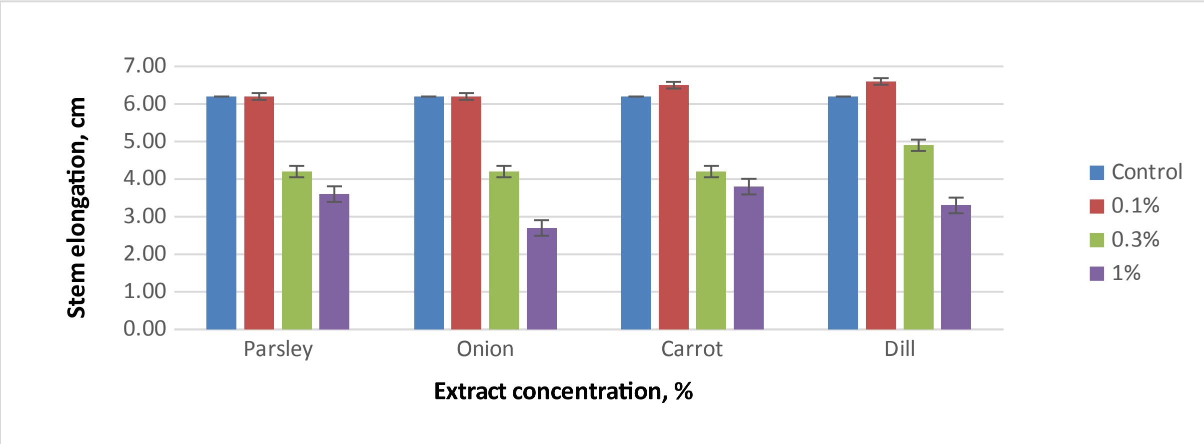

Fig. 3. Allelopathic effect of seed extract on stem elongation.

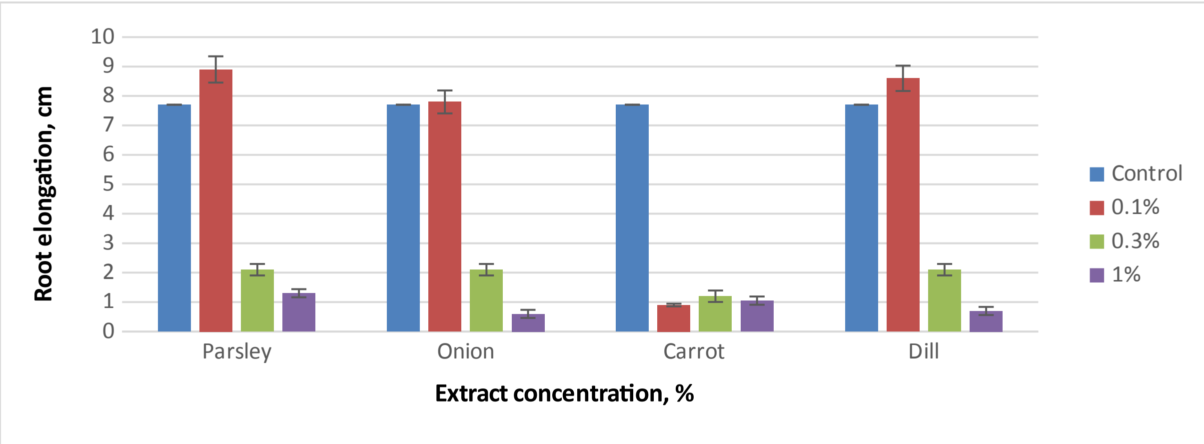

Fig. 4. Allelopathic effect of seed extract on root development

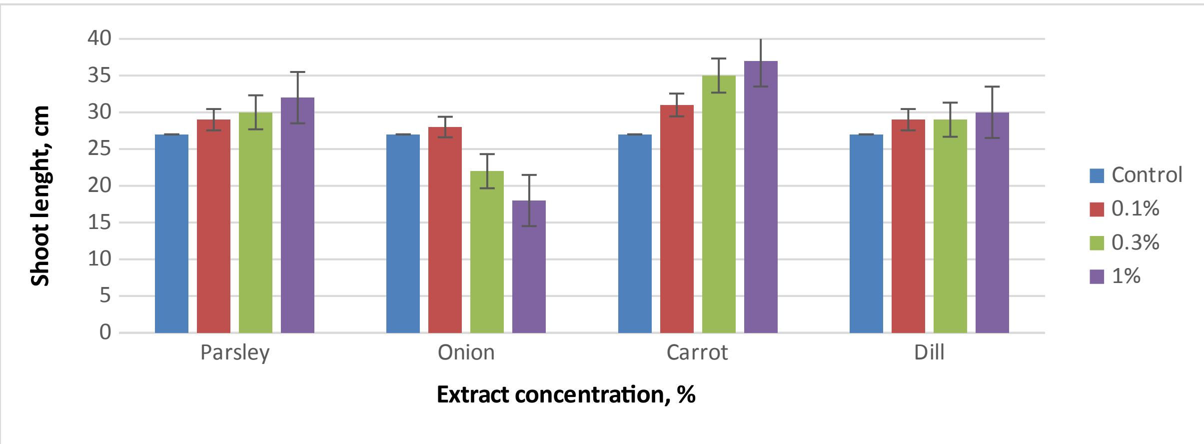

Fig. 5. Allelopathic effect of fresh biomass extract on shoot length.

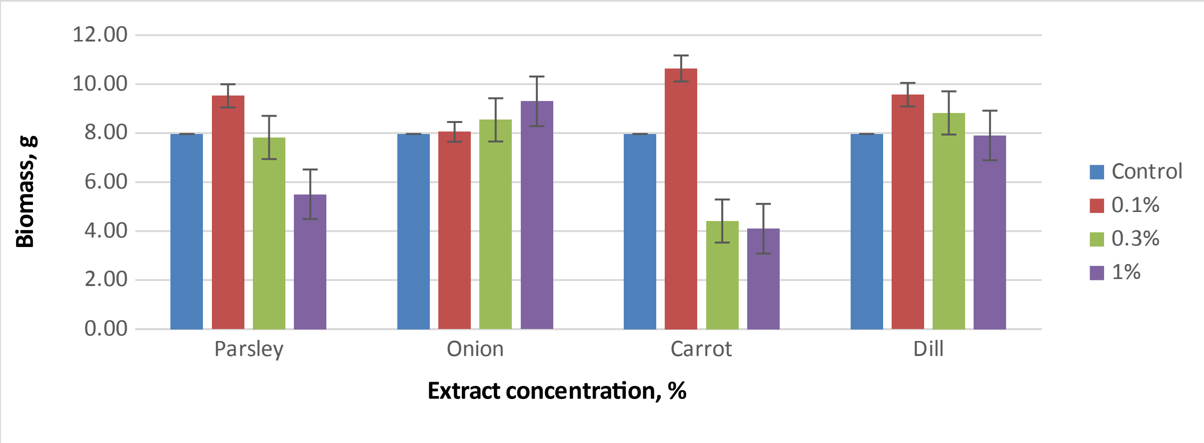

Fig. 6. Allelopathic effect of fresh biomass extract on biomass synthesis.

Table 1. Allelopathic potential indicated as GI (Index of plant development), SVI (Seedling vigor index), CA (Coefficient of allometry), OAP (Overall allelopathic potential) of cold aqueous extract from parsley, onion, carrot, dill on the germination and initial development of tomato.

Extract	Concentration (% w/v)	GI	SVI (cm)	CA (cm)	SVI (g)	*OAP* Germination seeds	Seedling length	Dry biomass
Control	0.0	100.0	12.51	0.8	7.2	-	-	-
Parsley	1.0	24.8	3.1	2.8	3.5			
	0.3	45.3	10.8	0.7	6.8	0.3	6.0	0.1
	0.1	86.4	10.8	0.7	6.8			
Dill	1.0	16.7	2.1	4.5	5.0			
	0.3	45.3	5.7	2.0	7.9	0.3	9.8	No OAP
	0.1	80.1	10.0	0.8	6.8			
	1.0	19.7	2.5	3.6	2.1			
Carrot	0.3	52.7	3.4	3.5	2.8	0.1	3.8	1.5
	0.1	27.4	6.6	1.7	6.7			
	1.0	18.2	2.3	4.7	5.3			
Onion	0.3	35.5	4.4	2.3	5.4	0.1	4.6	No OAP
	0.1	69.0	8.6	0.8	4.6			

Conclusion

The study showed that the applied concentrations of parsley, carrot, dill and onion extracts had stimulating, inhibiting or indigenous effect on tomato seed germination, growth and accumulation of dry biomass. Seedling vigor index of plant development (SVI_{cm}) and biomass synthesis (SVI_g) depended on the type of the extract applied more than the concentration applied ($p<0.05$). Seed germination was less affected than root and shoot growth in all species ($p<0.05$). Length of the seedlings was significantly influenced (positively or negatively) by the allelopathic plants and the effect was stronger with the increment of the extract concentration ($p<0.05$). Most pronounced negative effect was found at the 1% extract of fresh onion biomass – 34% reduction against the control ($p<0.001$). Stimulatory effect was strongest at the 1% extract of fresh carrot biomass – 37% increment against the control ($p<0.001$).

All data obtained through the experiment gave some information about the aspects of the vegetable interactions and amplified the scientific basis for mixed crop cultivation. This method is one of the most preferred in organic farming, aiming to improve yield quality, to minimize weed damages and to maintain biodiversity. We could recommend to plant tomato (*Lycopersicon esculentum* Mill.), parsley (*Petroselinum crispum* (Mill.) Nyman. ex A.W.Hill.) and dill (*Anethum graveolens* L.) as a mixed crop with expected higher yields.

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Received: 28.02.2019
Accepted: 31.05.2019