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Investigation of the allelopathic effects of lyophilized ethanol extract of *Xanthoparmelia somloensis* (Gyelnik) Hale lichen on tomato plant

Ömer BİNGÖL[1]*, Abdulhamit BATTAL[2], Mehmet Emre EREZ[3], Ali ASLAN[4,5]
[1]*Faculty of Education, Van Yüzüncü Yıl University, Van, Turkiye*
[2]*Faculty of Pharmacy, Van Yüzüncü Yıl University, Van, Turkiye*
[3]*Faculty of Science, Van Yüzüncü Yıl University, Turkiye*
[4]*Faculty of Pharmacy, Van Yüzüncü Yıl University, Van, Turkiye*
[5]*Faculty of Arts and Science, Kyrgyz-Turkish Manas University, Bishkek, Kyrgyzstan*
**emreerez@hotmail.com, [1]narsisus@hotmail.com, [2]hamitbattal@gmail.com, [4-5]aliaslan@yyu.edu.tr*

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Xanthoparmelia somloensis (Gyelnik) Hale likeninin liyofilize etanol ekstraktının domates bitkisi üzerindeki allelopatik etkilerinin araştırılması

Abstract: All organisms in nature interact and compete with each other. Various groups of organisms such as algae, lichens, crops and weeds have been found as allelopathic interaction and it is known that lichens have many potent secondary metabolites. The aim of this study was to determine the allelopathic effects of lyophilized ethanolic extract of *Xanthoparmelia somloensis* (Gyelnik) Hale lichen (XS) on tomato (*Lycopersicum esculentum* L.) germination and early development stage. Obtained lyophilized ethanolic extracts of XS lichen at concentrations of 50 ppm (XS-50), 100 ppm (XS-100), 200 ppm (XS-200) and 400 ppm (XS-400) were applied to tomato seeds and seedlings. In order to determine the effects caused by the extracts, germination rate and seedling growth parameters (vigor index, length, fresh weight, relative water content and pigment contents) were analyzed. According to the obtained data, there were significant decreases in germination rate and vigor index values depending on the increase in lichen extract concentration. Similar effects were also observed in root and shoot length and pigment contents. However, results of the lowest application (XS-50) were similar to control except root length and carotenoid content. The data obtained from this study exhibited that the lichen *Xanthoparmelia somloensis* has allelopathic effects and has the potential to be used for agricultural purposes.

Key words: Allelopathy, germination, lichen, tomato, vigor index

Özet: Doğadaki tüm organizmalar birbirleriyle etkileşim ve rekabet halindedir. Algler, likenler, kültür bitkileri ve yabani otlar gibi çeşitli organizma gruplarının allelopatik etkileşime sahip oldukları ve likenlerin birçok güçlü sekonder metabolite sahip olduğu bilinmektedir. Bu çalışmanın amacı, *Xanthoparmelia somloensis* (Gyelnik) Hale liken (XS) liyofilize etanolik ekstraktının domates (*Lycopersicum esculentum* L.) çimlenmesi ve erken gelişme aşaması üzerindeki allelopatik etkilerinin belirlenmesidir. 50 ppm (XS-50), 100 ppm (XS-100), 200 ppm (XS-200) ve 400 ppm (XS-400) konsantrasyonlarında XS likeninin elde edilen liyofilize etanolik ekstreleri domates tohumlarına ve fidelerine uygulandı. Ekstraktların neden olduğu etkileri belirlemek için, çimlenme oranı ve fide büyüme parametreleri (canlılık indeksi, uzunluk, taze ağırlık, bağıl su içeriği ve pigment içeriği) analiz edildi. Elde edilen verilere göre liken ekstraktı konsantrasyonundaki artışa bağlı olarak çimlenme hızı ve vigor indeks değerlerinde önemli düşüşler tespit edildi. Benzer etkiler kök ve sürgün uzunluğu ve pigment içeriklerinde de gözlendi. Ancak en düşük uygulamanın (XS-50) sonuçları kök uzunluğu ve karotenoid içeriği dışında kontrole benzerlik gösterdi. Bu çalışmadan elde edilen veriler, *Xanthoparmelia somloensis*'in allelopatik etkilere neden olduğu ve tarımsal amaçlı kullanım potansiyeline sahip olduğunu göstermiştir.

Anahtar Kelimeler: Allelopati, çimlenme, liken, domates, vigor indeksi

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1. Introduction

Allelopathy is known as a biological phenomenon caused by biochemicals that affect the growth, existence, development and reproduction of other organisms. These biochemicals can have beneficial or harmful effects on target organisms, depending on their content and type. Allelopathic effects are often modified by additional biotic and abiotic stress factors, uncertain meteorological events, or physical, chemical, and biological soil factors, all of which can influence the residence time, persistence, concentration, and fate of allelopathic compounds in the environment (Belz and Hurle, 2005).

Lichens produce over a thousand different extracellular secondary metabolites known as lichen substances/compounds (Hauck and Huneck, 2007). Secondary metabolites produced by lichen-forming fungi have attracted attention of investigators for over 100 years. Approximately 500 compounds have been reported from lichens, of which about 350 appear to be unique. Most of these compounds are weak phenolic acids, which are produced by the fungal partner and accumulate on the outer walls of fungal hyphae. Concentrations vary considerably within and among species (Lawrey, 1995). Lichen secondary metabolites can function as allelochemicals and affect the development and growth of neighboring bryophytes, fungi, vascular plants, microorganisms, and even other lichens (Goga et al., 2018).

Lichens are compound organisms formed from a partnership of fungi living in a symbiotic relationship with a green algae or cyanobacteria. This interrelationship of lichens has been extensively studied (Green, 2008). Certain secondary metabolites of lichens appear to have allelopathic effects on vascular plants (Lechowski et al., 2006). Although it is known that lichens produce a wide variety of secondary metabolites chemically, studies on their allelopathic properties are limited.

Lichens are known to produce acid derivatives such as terpenes, terpenoids, and steroids originating from the mevalonic acid pathway and vulpinic and pinastric acids via the shikimic acid pathway (Shukla et al., 2010). All known lichen secondary metabolites have been confirmed by usnic acid and atranorine applications to have phytotoxic effects on lichen photobionts. Both metabolites have been found to exhibit phytotoxicity by causing growth inhibition, inhibition of chlorophyll and fluorescence, decreased plant viability, and induction of oxidative stress in plant cells (Bačkor et al., 2010).

In most cases, lichens used as growth substrates positively affect plant growth and are not much affected by lichen overgrowth, but there are not many studies of negative associations, including lichen parasitism and allelopathic interactions. Extraction with acetone and water was generally used in the studies. Secondary metabolites extracted from different lichen species showed inhibitory effects on the germination of *Funaria hygrometrica* Hedwig and *Ceratodon purpureus* C. Müller spores (Frahm et al., 2000). In this manner, the aim of the study was to investigate the effects of ethanol extracts of *Xanthoparmelia somloensis* lichen on the germination and seedling growth of tomato seeds.

2. Material and method

2.1. Preparation of lichen extract

Lyophilized hydro alcoholic extract of *Xanthoparmelia somloensis* (Gyelnik) Hale lichen was prepared with 80 % of ethanol. Ground lichen was shaken in 80 % ethanol (1:20 w/v) at 300 rpm for 3 hours. After vacuum filtration, ethanol was evaporated via rotary evaporator (Buchi) and water was removed by lyophilization. Finally, fine powder lyophilized lichen extract was stored at a freezer. Lichen extract was tested at different concentrations: 0 ppm (Control), 50 ppm (XS-50), 100 ppm (XS-100), 200 ppm (XS-200) and 400 ppm (XS-400).

2.2. The effect of lyophilized lichen extract on tomato germination

Ten tomato seeds were planted in a petri dish with bi-layer filter paper moistened with hydroponic solution. Germination was followed for 6 days. Germinated seeds having at least 0.5 mm radicle were counted and noted for every day.

Germination rate (Germinated seed number/Planted seed number x 100) and Vigor Index (Seedling length (cm) x germination rate (%)) were calculated.

2.3. Growth of tomato seedlings

Tomato seeds were surface sterilized with 3 % of sodium hypochlorite for 10 minutes and washed with sterile distilled water for several times. Seeds were planted on plastic pots filled with hydroponic culture (Hoagland and Arnon, 1950). 10 days old seedlings were incubated with different lichen concentrations for 5 days to determine allelopathic effects of XS lichen at early developmental stage of tomato plants. Length, fresh and dry weight, turgid weight and relative water content were calculated for shoot and root tissues. (RWC %) = [FW-DW] / [TW-DW] x 100 (Smart and Bingham, 1974).

2.4. Chlorophyll and carotenoid contents

A piece of tomato leaf was weighted and incubated in absolute acetone for 48 hours. Samples were read at 661.6 nm, 644.8 nm and 470 nm against absolute acetone as a blank. Pigments were calculated as following formula according to Lichtenthaler (1987):

Chlorophyll a (μg/mL) = Ca = (11.24 * OD 661.6) – (2.04 * OD 644.8)

Chlorophyll b (μg/mL) = Cb = (20.13 * OD 644.8) – (4.19 * OD 661.6)

Chlorophyll a+b (μg/mL) = Ca+b = (7.05 * OD 661.6) + (18.09 * OD 644.8)

Carotenoids = Cx+c = [(1000 * OD 470) – (1.9 * Ca) – (63.14 * Cb)]/21

2.5. Statistical analysis

For statistical analysis, groups that were higher than the normality test were compared. The samples were randomly selected with at least three replications. One Way Anova and Tukey tests were used using GraphPad Prism 8 program.

3. Results

In this study, the effects of four different lichen concentrations were evaluated. It was determined that the applications inhibited the germination of the seeds depending on the increase in the concentration. While the inhibition effect caused by the extracts was not prominent at 50 ppm, the effects were started in 100-200 ppm however the main and significant effect was in the application of 400 ppm compared to control. Germination percentage declined by 76 % at 400 ppm concentration (Fig. 1).

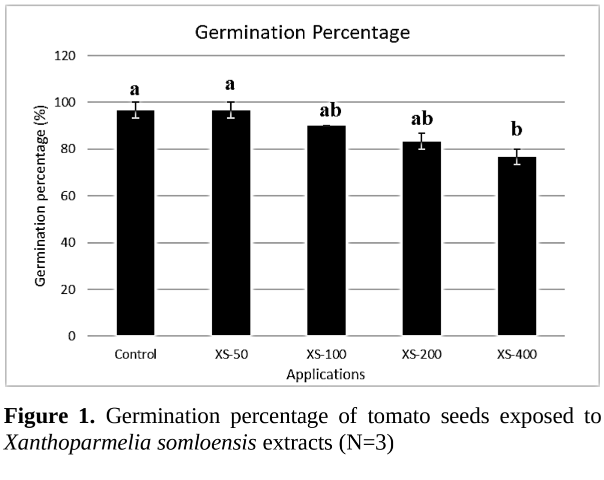

Figure 1. Germination percentage of tomato seeds exposed to *Xanthoparmelia somloensis* extracts (N=3)

Seedling vigor index was found significantly different in all extract except in 50 ppm. The 200 and 400 ppm concentrations have the higher reductions in seedling vigor index (Fig. 2).

While XS-50 caused significantly longer root length according to control, XS-400 application resulted with significantly shorter root length compared to control (Table

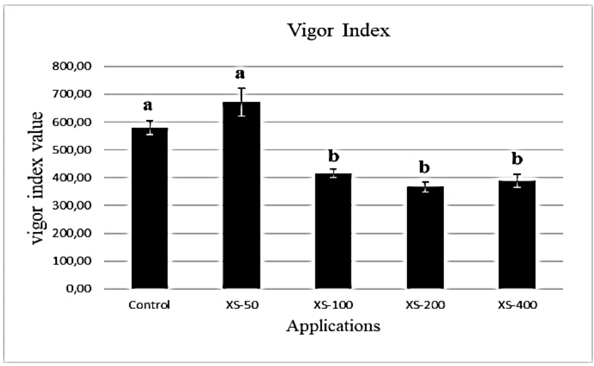

Figure 2. Vigor index parameters. Different letters on column indicates significant difference (P<0.05) (N=6)

1). According to shoot length results, the significant decrease in shoot length was observed in XS-100, XS-200 and XS-400 applications relative to control (Table 2). Although XS-50 application resulted with shoot length decrease compared to control, it was not significant (Table 2).

Table 1. The effects of lichen *Xanthoparmelia somloensis* extracts on root parameters

	Root length (cm)	Root weight (g)	RWC (%)
Control	14.95±0.40[b]	0.087±0.003[ab]	119.9±2.50[ab]
XS-50	17.66±0.48[a]	0.101±0.007[a]	125.4±4.05[a]
XS-100	13.31±0.54[bc]	0.070±0.007[bc]	120.5±2.44[a]
XS-200	13.33±0.36[bc]	0.053±0.003[c]	116.4±2.32[ab]
XS-400	11.89±0.28[c]	0.050±0.004[c]	108.6±2.69[b]

Different letters in the same column indicates significant difference (P<0.005) (N=8)

The highest root fresh weight was at XS-50 application and significantly higher than other lichen applications. Additionally, XS-200 and XS-400 caused significantly decrease in root fresh weight according to control. These two high concentrations negatively affected root fresh weight. On the other hand, while there was no significantly difference in shoot fresh weight parameter between control and XS-50, the higher concentrations of lichen extract (XS-100, XS-200 and XS-400) caused significantly decrease compared to control and XS-50. Application of the highest lichen extract concentration (XS-400) resulted with significantly decrease in relative water content (RWC) in the both of the root and shoot according to XS-50 application. However, it was not significantly different from control for RWC parameter. Growth parameters like root elongation, are even more sensitive to allelopathic effects than seed germination (Peres et al., 2009). Especially the effect on shoot was more evident with increasing concentration compared to root growth (Tables 1,2).

Table 2. The effects of lichen *Xanthoparmelia somloensis* extracts on shoot parameters

	Shoot length (cm)	Shoot weight (g)	RWC (%)
Control	8,08±0,21[a]	0,26±0,007[a]	98,30±1,92[ab]
XS-50	7,69±0,13[a]	0,24±0,0012[a]	106,70±2,92[a]
XS-100	6,43±0,13[b]	0,18±0,0012[b]	102,20±2,38[ab]
XS-200	6,30±0,15[b]	0,16±0,008[b]	102,50±1,92[ab]
XS-400	6,05±0,17[b]	0,15±0,01[b]	93,30±3,04[b]

Different letters in the same column indicates significant difference (P<0.005) (N=8)

When the effects of XS ethanolic extracts on the photosynthetic pigments of tomato seedlings, lichen extract caused decrease in Chl-a content (Table 3). However, only the highest concentration (XS-400) was significantly different from control (Table 3). While there was no statistical difference between control and lichen applications for Chl-b, total chlorophyll content of XS-400 was significantly lower than control. According to Table 3, carotenoid content in tomato seedlings was obviously negatively affected by all treatment of the lichen extract (Table 3).

Table 3. The effects of lichen Xanthoparmelia somloensis extracts on pigment parameters (mg/g)

	Chl-a	Chl-b	Total Chl	Carotenoids
Control	1673,2±99,2[a]	609,9±30,1[a]	2283,1±128,7[a]	484,41±34,4[a]
XS-50	1595,7±32,4[ab]	623,5±16,4[a]	2219,2±47,8[ab]	400,23±4,4[b]
XS-100	1539,9±51,4[ab]	608,9±21,8[a]	2148,8±73,1[ab]	393,07±15,1[b]
XS-200	1445,1±58,1[ab]	575,1±29,8[a]	2020,2±87,3[ab]	366,05±13,3[b]
XS-400	1353,9±32,7[b]	529,4±25,4[a]	1883,3±88,1[b]	365,14±14,6[b]

Different letters in the same column indicates significant difference (P<0.005) (N=8)

4. Discussions

Water extracts of lichens, reduce the seed germination of several vascular plants, including *Pinus sylvestris* L. and angiosperms, trees, and shrubs and grasses (Sedia and Ehrenfeld, 2003). The different effects of lecanoric acid (*Parmotrema tinctorum* (Despr. ex Nyl.) Hale and its orsellinate derivatives on the germination and growth of *Lactuca sativa* L. and *Allium cepa* L. (strongly affected) have highlighted that the allelopathic action of certain metabolites, which can vary to a great extent between different species. As a result, in our study it is thought that the active compounds of the *Xanthoparmelia somloensis* lichen cause the germination inhibition due to the increase in concentration.

Allelochemicals were reported to influence several physiological processes during seed germination such as inhibiting amylase activity and delaying the translocation of food reserve (Kruse at al., 2000). Lowering germination rate as a result of allelochemical stress may be due to inhibition of water uptake and alteration in the activity of gibberellic acid (Tawaha and Turk, 2003).

One of the first effects of allelopathic compounds at the cell level is membrane depolarization. It causes disorders in the transport of anions and cations, and damage to membranous cell structures depends, among others on the concentration and solubility of allelopathic substances and the pH of the environment (Einhellig, 2004).

Application of evernic acid decreased total chlorophyll content and chlorophyll a content as well as chlorophyll a to chlorophyll b ratio Bačkor et al. (2010). Also plants cultivated with usnic acid (20 or 30 μM) have demonstrated lower photosynthetic (about -40%) and respiratory (down to -80%) activities than the controls and displayed a reduction in their chlorophyll and carotenoid contents (Latkowska et al., 2006). Reduction in Chl a, Chl b and total Chl were previously reported as a result of allelochemical stress (Singh and Singh, 2009). Root exudates from sorghum were reported to inhibit the activity of hydroxyphenyl pyruvate dioxygenase which resulted in plastoquinone deficiency and therefore, disrupt the biosynthesis of carotenoids (Meazza et al., 2002). Romagni et al. (2000) reported in their study that usnic acid is a potent inhibitor of phytoene desaturase, which converts phytoene to carotenoids.

As a conclusion, ethanolic XS lichen extract has dose dependent allelopathic effects on tomato germination and on physiological and pigment parameters. Especially, the highest XS extract concentration (XS-400) caused severe negative effects in the most of the parameters (germination rate, vigor index , root length and fresh weight, shoot length and fresh weight, Chl-a, total Chl and carotenoids) in this study. On the other hand, results for the lowest XS extract concentration (XS-50) were mostly similar to control. Furthermore, XS-50 application significantly promoted root length compared to control. However, carotenoids in the XS-treated tomato seedling was significantly lower than control. In the light of these results, the ethanolic lyophilized XS extract shows allelopathic effects on tomato plant at high concentrations by negatively affecting germination rate, physiological and pigment parameters. As a further study, the different concentrations of XS extract could be tested for weed studies, because of secondary metabolite potential of lichens.

Conflict of Interest

Authors declare that there is no conflict of interest.

Authors' Contributions

ÖB, AB and MEE contributed to the study conception and design. Material preparation, data collection and analysis were performed by ÖB, AB, MEE and AA. The first draft of the manuscript was written by ÖB and AB. MEE and AB commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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