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Effect of oregano water on *Pythium* density in soil and damping-off disease on bean plants

Emine Demirbaş[1] **Gürsel Karaca**[1,*]

[1]Isparta Applied Sciences University, Faculty of Agriculture, Department of Plant Protection, Isparta, Turkey

[*]Corresponding Author: gurselkaraca@isparta.edu.tr

Abstract

Pythium genus includes significant and destructive soil borne plant pathogens causing losses on many plants, especially by root rot and damping-off diseases. Like other soil borne pathogens, control of the diseases caused by this group is rather difficult. Usually fungicides are used against *Pythium* species, but investigations on alternative methods and chemicals gained importance because of the harmful effects of pesticides on human health and environment. Plant extracts and essential oils are among the safer alternative chemicals. Since oregano oil has high fungitoxic effect even in low concentrations, effect of oregano water, by-product of oregano oil, was investigated in this study against *P. deliense*, one of the most common and virulent species in Turkey. Oregano water obtained from *Origanum onites*, diluted with distilled water in 100, 80, 60, 40, 20, 10 and 5% rates was applied to soil and its effects on *P. deliense* population density in soil and severity of damping-off disease on bean plants caused by the pathogen were determined. As a result, all solutions containing oregano water over 10% rate significantly decreased pathogen populations in the soil samples taken every three days after the applications, and the least pathogen density was obtained with undiluted oregano water. Similarly, undiluted oregano water increased emergence rates of the bean seedlings and significantly decreased root and hypocotyl rot severity by suppressing the effect of the pathogen. In addition, application of oregano water supported the shoot and root development of the plants by decreasing the negative effect of the pathogen on plant growth. This study showed that oregano water was an effective alternative against *Pythium* related damping-off disease.

Keywords: *Pythium* spp., Root rot, Control, Oregano hydrosol

Introduction

Pythium genus, belonging to Phylum Oomycota of the Chromista Kingdom, includes species, parasitic on different organisms such as fish, insects, fungi and even on human. There are 355 described species in the genus with many destructive plant pathogens causing root rot and damping-off diseases (Ho, 2018). *Pythium* species are especially common in rhizosphere soil, prefer young and watery plant tissues and cause pre or post-emergence damping-off symptoms on young plants. They can also decrease yield by causing root rot and suppressing growth of older plants. Leaf, stem or fruit rots may also be caused by *Pythium* species (Hendrix and Campbell, 1973). *P. deliense* is among the most common and virulent species of the genus. It is first isolated from tobacco plants showing stem blight symptoms in Sumatra. It is known as a pathogen living mostly in warmer soils with an optimum growth temperature of 36°C (Yu and Ma, 1989). It is also isolated from cabbage (Yu and Ma, 1989), tomato, mung bean, ginger, pawpaw, lettuce, bitter melon and cowpea (Lodha et al., 2004). It is reported as a common pathogen in vegetable and tobacco nurseries and that

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it also caused severe root rot on wheat and sugarbeet plants in Turkey (Hatat, 1995; Karabuğa and Karaca, 2011).

Human beings have been dealing with agricultural production for centuries and continuously increasing yields by modernizing the techniques. However, monoculture agriculture, wrong irrigation techniques, intensive soil tillage and pesticide and chemical fertilizer usage have some harmful effects. Growers generally prefer chemical control method to reduce the yield losses caused by pests, diseases and weeds. But pesticides have negative side effects on environment and human health (Arslan, 2016). Research showed that pesticides had serious dermatological, gastrointestinal, neurological, carcinogenic, respiratory, reproductive, and endocrine effects on human body depending on the pesticide type, duration and route of exposure (Nicolopoulou-Stamati et al., 2016). In addition, unconscious and excessive pesticide applications cause development of resistant pest and pathogen populations. Alternative methods and chemicals gained importance because of the negative effects of pesticides. Chemicals synthesized by plants are among those alternatives and they are thought as biologically effective, wide spectrum, easily decomposable, economical and safer pesticides (Alvarez-Castellanos, 2001).

Effects of plants on plant pests and pathogens depend on their secondary metabolites and azadirachtin, pyrethrin, rotenone, nicotine, ryania, sabadilla and thymol are some examples (Bayram et al., 2010). Recently significance of phytochemicals and studies on them increased with the development of sustainable and ecological agricultural systems (Boyraz and Koçak, 2006). Oregano comes first among the plants whose secondary metabolites have commonly been studied. It's also one of the medicinal plants with highest production in Turkey. It is reported that 60% of the 52 known *Origanum* species grow in Turkey and this is a strong proof that Turkey is the gene origin of this group of plants (Başer, 2001). Before, internal and external consumption was mainly provided with the naturally grown *Thymus* and *Thymbra* species. However, with the production of *Origanum* species, amount of yield increased in years and Turkey becomes the first country in terms of production (Bayram, 2018). More than 90% of oregano production is made in Turkey and *Origanum onites* has the biggest part in the export. It is the only species commercially cultivated besides natural production (Kapluhan, 2013). Oregano production in Turkey was 11 752 tonnes in 92 959 da area in 2014, while production increased to 15 752 tonnes in 139 061 da area in 2018. According to 2018 data, Denizli province comes first with 14 009 tonnes production, followed by Manisa (828 tonnes), Kütahya (475 tonnes), Uşak (262 tonnes), and Hatay (187 tonnes) provinces, respectively (TUİK, 2019).

Oregano is used as a medicinal plant for years in order to cure different diseases. Besides it is used in agriculture to prevent crops from pests and diseases. It is especially known as a repellent against storage pests (Altundağ and Aslım, 2005). It was reported in some studies that it was not phytotoxic when used as an insecticide or fungicide (Hayta and Arabacı, 2011). Extract or essential oils obtained from oregano species were found to have antifungal effects on many plant pathogenic fungi such as *Alternaria alternata*, *Botrytis cinerea*, *Fusarium* spp., *Macrophomina phaseolina*, *Penicillium* spp., *Phytophthora capsici*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* (Arslan and Karabulut, 2005). Antimicrobial effect of oregano essential oil is mainly because of its chemical constituents like thymol, carvacrol, p-cymene, terpineol, borneol, cymol and linalool (Altundağ and Aslım, 2005). Main constituents of oregano essential oil are thymol and carvacrol (Çoban and Patır, 2010) and amounts of these chemicals depend on the species. Carvacrol and thymol contents of *Origanum onites* are 72-89%, while those of *O. vulgare* are 23-79% (Başyiğit et al., 2017).

Oregano water is a by-product of oregano essential oil obtained under oil during distillation process. Active ingredients of oregano oil are also present in oregano water. Carvacrol is the main constituent of oregano water, while there is also about 10% p-mentendiol, which is rare in nature and not found in the oil (Başer, 2014). Oregano essential oil is expensive for usage in agriculture and it may have phytotoxic effects on plants. There are many studies on the effect of oregano oil against plant pathogens, but very less study on oregano water and those are mainly performed *in vitro* (Özcan and Karaca, 2016; Kaya et al., 2016).

In this study, effect of oregano water on *P. deliense* population density in soil and related severity of damping-off disease caused by the pathogen on bean plants were investigated.

Materials and Methods

Isolation and Selection of the *P. deliense* Isolate

Soil samples were taken from nurseries and greenhouses in Isparta and Antalya provinces, where seedlings showing damping-off symptoms were observed. Samples were taken to the laboratory and isolations were performed by SSDP (Surface soil dilution plate) method on VP3 selective medium. In this method, soil samples were first air dried on plastic trays for a few days and then sieved through 35 and 60 mesh screens. Twenty grams of soil retained on the 60 mesh screen was suspended in 100 ml of 0.2% sterile water agar and 1/50 dilution of the soil suspension was spread on the surface of the selective medium by using a sterile glass rod. After two days incubation at 21°C in the dark, growing mycelia were transferred to Potato carrot agar (PCA). Agar pieces taken from the growing edges of the isolates were then transferred to water culture plates made with corn and hemp seeds and sterile soil extract (Karaca et al., 2008). *P. deliense* isolates with toruloid sporangia, smooth walled oogonia, intercalary antheridia and aplerotic oospores (Figure 1), were identified according to related literature and keys (Plaats-Niterink, 1981; Yu and Ma, 1989; Dick, 1990). Isolates were grown on PCA and small agar pieces with pathogen mycelia were cut and transferred to small glass bottles with sterile distilled water. After a few days incubation, bean seedlings were dipped into the bottles after root tips were cut. After incubation for one week, bean seedlings were evaluated for damping-off symptoms and the most virulent isolate causing severe symptoms was selected for the rest of the study (Hatat, 1995).

Inoculation of Soil with *P. deliense*

Small agar pieces with virulent *P. deliense* isolate on PCA culture were cut and transferred to glass bottles with sand:water:corn meal in 9:2:1 rates and incubated at 21°C in the dark for 15 days. Soil sample used in the study was taken from an area where no chemical was used. Soil sample was autoclaved two times for 45 minutes. Some part of the soil was kept for control application. Other part was inoculated with the pathogen and checked by SSDP method if it contains at least 200 propagules per gram of soil. When inoculum concentration was lower than 200 propagules, some more inoculum was added to the soil, otherwise some sterile soil was added to dilute the concentration (Hatat, 1995).

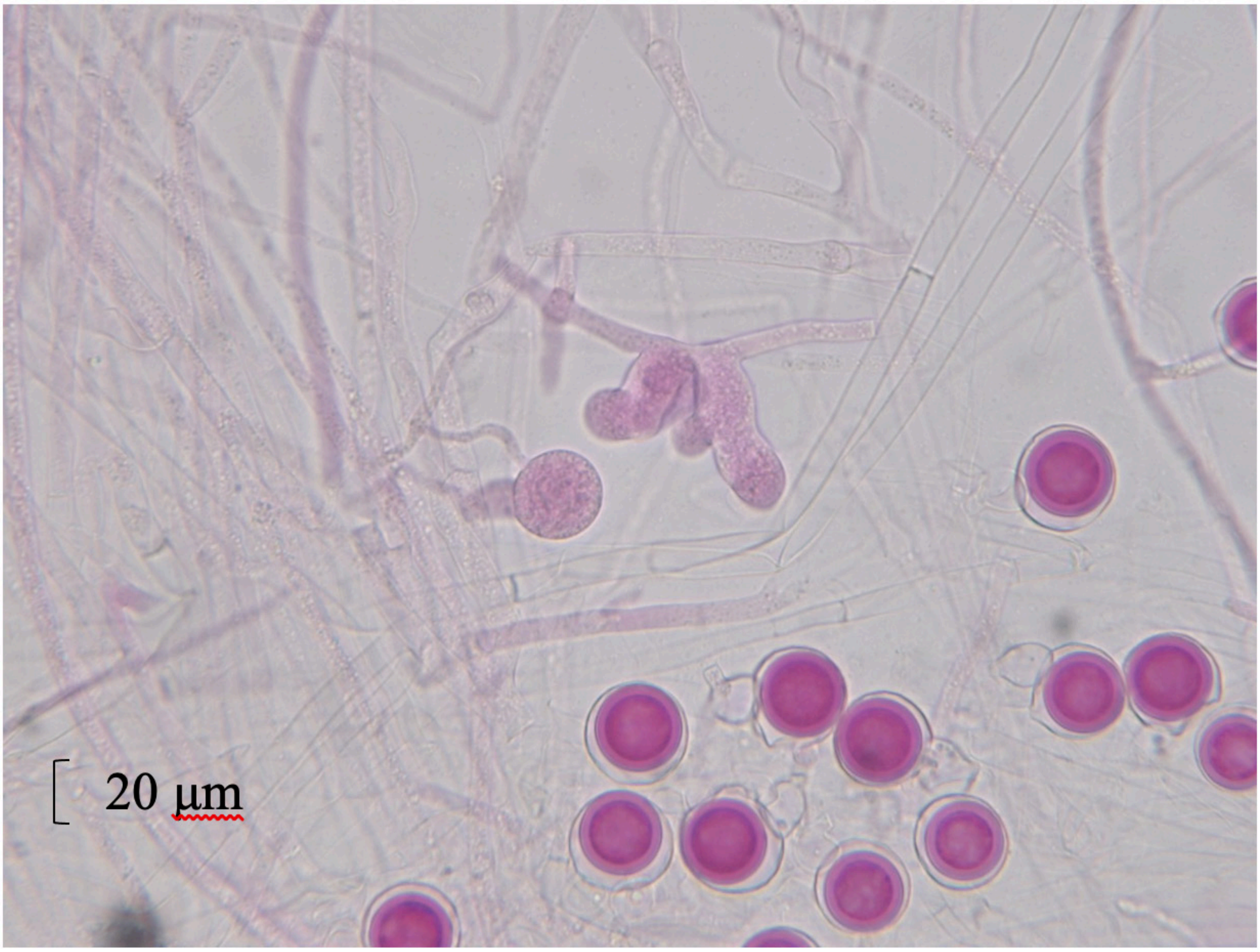

Figure 1. Distinctive features of *Pythium deliense* (toruloid sporangium, mostly intercalary antheridia and aplerotic oospores

Application of Oregano Water

Oregano water produced from *Origanum onites* by "Efecan Badem Aromatik Ürünler A.Ş." company in 2018, was used in the study. Main constituents of the oregano water determined by GC-MS analyses and their rates were given in Table 1.

Oregano water was diluted with distilled water in 5, 10, 20, 40, 60, 80 and 100% concentrations. Sterilized soil was separated into 5 kg parts and transferred into clean plastic containers. Each soil sample was sprayed with 100 ml of different oregano water dilutions. For comparison, one sample was sprayed with 1% NaOCl solution, and another one with sterile distilled water as control application. Applications were made with a hand sprayer and soil samples were mixed during spraying in order to ensure homogenized application (Özcan and Karaca, 2016).

Monitoring Pathogen Population Density in Soil

After the application of oregano water, containers with the soil samples were kept closed for three days to provide the distribution of volatiles of oregano water in the soil samples. Then necessary amounts from each soil sample were transferred to plastic pots for plant experiment, and remaining parts were kept for population monitoring. First soil samples were taken three days after the applications and sampling was continued every three days for 24 days. For each sampling, 20 g of soil were taken from the soil samples and population density of *P. deliense* was determined by SSDP method, using 5 replicate plates for each sample.

Table 1. Main chemical constituents of the oregano water used in the study and their rates

Chemicals	Rates (%)
Linalool	0.6
Terpinene-4-ol	0.6
a-Terpineol	0.5
Borneol	0.8
Thymol	1.1
Carvacrol	95.9

Determination of the Effects of Oregano Water Applications on Damping-off Disease of Bean Plants

Soil samples sprayed with different dilutions of oregano water, 1% NaOCl and sterile distilled water were transferred to plastic pots and 10 bean seeds were sown into each pot. Trial was performed with a randomized parcel design with three replications and pots were kept in a climatized room with 23±2°C temperature and 16 hours light-8 hours dark conditions. Seeds of Dermason cultivar were used and pots were irrigated with equal amounts of sterile water when necessary. One week after sowing, emergence rates were determined and four weeks after sowing, plant and root lengths, shoot and root fresh and dry weights were determined. Plants were dried in oven at 60°C for three days and weighed immediately for determination of the dry weights. In addition, disease severity rates were determined by using two scales, one for root rot and the other for hypocotyl rot (Li et al., 2014). In 0-4 root rot scale; 0=healthy roots, 1=less than 2% of roots rotted, 2=moderate rot on roots, 3=general rot on roots and less than 50% root loss, and 4=severe root rot and more than 50% root loss or dead plant. In the scale used for hypocotyl symptoms; 0=healthy hypocotyl, 1=less than 10% of hypocotyl with lesions, 2=10-25% of hypocotyl with lesions, 3=25-50% of hypocotyl with lesions, 4=50-75% of hypocotyl with lesions, and 5=more than 75% of hypocotyl with lesions or dead plant. Disease severity was calculated with the formula below, where n: number of plants with the scale value, v: scale value, N: total number of plants, and Z: highest scale value.

Disease severity rate (%)=Σ (nxv) x 100 / ZxN

After the evaluations, reisolations from the diseased plants were made in order to confirm the pathogen was *P. deliense*. All data were subjected to analyses of variance and means were compared with Tukey test by using JMP (Ver.9) program. Emergence and disease severity rates were subjected to arcsin transformation before the analyses.

Results and Discussion

Effect of Oregano Water Applications on *P. deliense* Density in Soil

It was found that the effect of soil applications of different concentrations of oregano water on *P. deliense* population density differed depending on the dilution rates. None of the applications totally destroyed pathogen propagules in soil, so the pathogen population increased again in time. *P. deliense* inoculum densities determined during the first four samplings, in the soil samples taken every three days after the applications, were given in Table 2. Pathogen inoculum reached 370 propagules per gram of soil in the samples taken from the containers sprayed with sterile distilled water representing control group, while the density of the pathogen decreased in the soil samples depending on the dilution rate of oregano water. The lowest inoculum density of the pathogen was obtained with undiluted oregano water application. Pathogen density increased in the soil sample where 5% oregano water was sprayed one week after the application and statistically arranged in the same group with control application. Samplings made 15 days after applications showed that the number of pathogen propagules per gram of soil gradually increased. However, pathogen populations were continuously lower than the others, in the soil samples taken from the containers sprayed with undiluted oregano water. Population densities obtained with the lowest concentration of oregano water were always statistically similar with the control, while all other dilutions were different in the samplings made 15 and 18 days after applications. But in the last two samplings, 10% dilution of oregano water also seemed to be ineffective to decrease the population. In a previous study, oregano water, added into culture medium in 5 and 10% concentrations, totally inhibited the mycelial growth of *Fusarium oxysporum* f. sp. *phaseoli*, *M. phaseolina*, *B. cinerea*, *R. solani*, *Alternaria solani* and *Aspergillus parasiticus* (Özcan and Boyraz, 2000). The cause of the difference between the results of two studies maybe the difficulty of the homogenous application of the solution into the soil. Another reason may be the susceptibility differences between the pathogens.

Effects of Oregano Water Applications on Growth and Damping-off Disease Severity of Bean Plants

It was found in the experiment that oregano water applications affected emergence rates and growth of bean seedlings. Emergence rates decreased in all applications, when compared to controls without pathogen inoculation. However, emergence rates of the bean seedlings in the pots with undiluted oregano water and solutions with 80 and 60% oregano water applications arranged in the same group with control. The lowest rate of emergence was found in pots where 5% oregano water was applied and this rate was statistically similar with that of pathogen inoculated positive control group (Table 3).

In the previous studies, it was found that oregano essential oil had negative effect on seed germination. Aydın and Tursun (2010) reported that germination rates of some weed seeds decreased with the increasing doses of onion, garlic and oregano (*Origanum dubium* L.) essential oils and higher doses totally inhibited the germination. In another study, doses over

5% of clove, mentha and oregano essential oils significantly decreased or totally inhibited the germination rates of wheat seeds (Karaca et al., 2017). Similarly, 2.5% dose of oregano oil totally inhibited the growth of seed-borne fungi but also decreased germination rates of rice seeds (Olgunsoy and Karaca, 2018). It is known that oregano water contains same chemical constituents of oregano oil in lesser amounts. Main constituent was carvacrol but it has also p-mentendiol (%10), which is not found in the oil (Başer, 2014). This may be the reason of lower phytotoxic effect of oregano water on seed germination and plant growth. As expected, highest disease severity rates were found in the pathogen inoculated pots, both for root rot and hypocotyl rot (Figure 2). Negative control group yielded the highest emergence rates and plants showed healthy growth (Figure 3).

Table 2. *Pythium deliense* population densities in the soil samples taken every three days after applications (propagules/g soil)

Applications	Days After Applications							
	3 Days	6 Days	9 Days	12 Days	15 Days	18 days	21 Days	24 Days
5% OW[x]	353.33 a[y]	375.00 a	412.67 ab	437.67 ab	504.33 ab	575.33 ab	667.00 ab	742.00 ab
10% OW	315.00 b	330.00 b	366.67 bc	392.00 bc	458.33 bc	517.00 bc	608.67 abc	700.33 abc
20% OW	275.00 c	285.00 c	329.33 cd	367.00 bcd	396.00 c	479.33 c	554.33 bc	637.67 bcd
40% OW	255.00 cd	260.00 c	304.33 cd	337.67 cd	379.33 cd	446.00 cd	496.00 cd	583.67 cde
60% OW	216.67 ef	208.33 d	215.00 ef	275.00 def	308.33 de	362.67 de	413.00 de	504.33 def
80% OW	200.00 f	195.00 de	205.00 ef	245.00 ef	285.00 e	333.33 e	383.67 de	437.67 ef
100% OW	170.00 g	161.67 e	166.67 f	195.00 f	236.67 e	279.33 e	321.33 e	367.00 f
1% NaOCl	240.00 de	253.33 c	279.33 de	321.00 cde	379.33 cd	429.33 cd	492.00 cd	562.67 cde
Control	370.00 a	400.33 a	454.33 a	504.33 a	567.00 a	646.00 a	717.00 a	808.67 a

[x]OW: Oregano water
[y]Means in the columns shown by the same letter were not statistically different from each other according to Tukey test (P=0.05)

Table 3. Effect of oregano water applications on emergence rate, root and hypocotyl rot severity of bean plants

Applications	Emergence rate (%)	Root rot severity (%)	Hypocotyl rot severity (%)
5% OW[x]	6.67 d[y]	98.00 a	98.00 ab
10% OW	16.67 cd	97.33 a	95.33 abc
20% OW	36.67 bcd	95.33 ab	92.00 abcd
40% OW	43.33 bc	92.67 abc	92.67 abcd
60% OW	56.67 abc	80.67 bcd	84.00 bcd
80% OW	56.67 abc	72.67 cd	69.33 de
100% OW	76.67 ab	52.67 d	46.67 e
1% NaOCl	36.67 bc	87.33 abc	82.00 cd
Positive control	20.00 cd	98.00 a	99.33 a
Negative control	90.00 a	12.67 e	11.33 f

[x]OW: Oregano water
[y]Means in the columns shown by the same letter were not statistically different from each other according to Tukey test (P=0.05)

Figure 2. Pre-emergence damping-off symptoms on pathogen inoculated soil samples (Positive control)

Figure 3. Growth of bean plants in the soil samples without pathogen inoculation (Negative control)

Oregano water applications between 5-40% failed to decrease disease severity, while severity rates decreased with higher doses. However, none of the applications totally inhibited the disease. In addition, plants applied with lower doses of oregano water showed chlorosis on the leaves, depending on the unhealthy root development. Emergence rates increased with the increasing oregano water concentrations and plants showed better development with larger leaf areas. In the pots with NaOCl applied soil samples, emergence rates were also lower and plants had smaller leaves. Most of the plants totally dried before the end of the experiment.

Diluted oregano water applications failed to decrease the negative effects of pathogen inoculation on the shoot development of bean plants, while undiluted oregano water application significantly supported shoot development (Table 4, Figure 4). Similar results were obtained with the root development of bean plants. Only the highest two concentrations of oregano water application supported root development against pathogen and mean root lengths of the plants statistically arranged in the same group with negative control. But oregano water applications were not successful to decrease the effect of pathogen in terms of root fresh and dry weights.

Table 4. Effects of oregano water applications on shoot and root development of bean plants

Applications	Shoot development			Root development		
	Shoot length (mm)	Shoot fresh weight (mg)	Shoot dry weight (mg)	Root length (mm)	Root fresh weight (mg)	Root dry weight (mg)
5% OW[x]	8.00 c[y]	89.27 c	8.57 d	4.17 c	5.13 c	1.53 c
10% OW	15.17 c	168.90 c	23.93 cd	6.00 c	11.73 c	3.13 c
20% OW	17.00 c	221.30 c	33.10 cd	9.67 bc	11.07 c	6.07 c
40% OW	22.33 c	316.10 bc	50.63 cd	10.33 bc	15.70 c	11.80 bc
60% OW	53.50 bc	536.10 bc	101.97 cd	22.50 bc	49.57 bc	14.63 bc
80% OW	50.83 c	893.80 b	115.93 bc	31.50 ab	58.77 bc	16.47 bc
100% OW	104.33 a	1596.90 a	209.67 ab	52.83 a	104.23 b	27.87 b
1% NaOCl	27.67 c	247.77 c	54.20 cd	15.50 bc	25.13 c	7.77 c
Positive control	8.67 c	106.83 c	21.53 cd	6.00 c	10.25 c	2.47 c
Negative control	102.17 ab	2038.67 a	259.13 a	53.67 a	216.23 a	53.27 a

[x]OW: Oregano water

[y]Means in the columns shown by the same letter were not statistically different from each other according to Tukey test (P=0.05)

Higher doses of oregano water were found to be more effective on disease severity and root and shoot development of bean plants. In the study, the use of high inoculum density of the pathogen may cause the lower efficiency of oregano water. Lower doses may be more effective in agricultural soils where pathogen density is lower because of complex soil conditions.

Conclusion

Intensive usage of pesticides in agriculture caused problems, such as development of resistance both in pests and disease agents, environmental pollution and residues in crops. Thus development of alternative method and pesticides has gained significance. Pesticides of plant origin are known to decompose easily in the nature and do not cause environmental pollution or residues on crops. Various secondary compounds of plants have commonly been used in agriculture in the last 20 years, especially with the development of sustainable agriculture concept (Aydın and Mammadov, 2017). Antimicrobial effects of plant extracts and essential oils on harmful agents of plants have known for many years (Arslan and Karabulut, 2005; Altundağ and Aslım, 2005; Çelen, 2006).

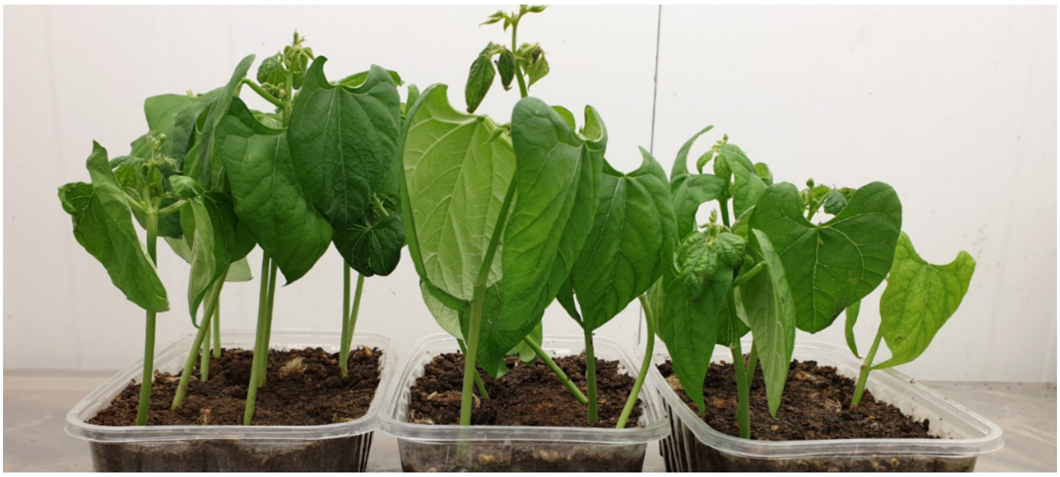

Figure 4. Effect of undiluted oregano water on bean growth

With its geographical location, diverse climate and vegetation and high agricultural potential, Turkey is one of the leading countries in the production and export of medicinal plants. Oregano is one of the medicinal plants mostly been studied for its antimicrobial effects. Its antifungal effects against different plant pathogens were generally studied *in vitro* by using oregano essential oil and found to have higher and wide spectrum effect, in comparison with other plant oils (Sokovic et al., 2002; Daferera et al., 2003). Antifungal effect of oregano oil is mainly attributed to its high carvacrol and thymol content (Kordali et al., 2008). Despite its efficiency on fungal plant pathogens, usage of oregano oil in the control is not common, because it is expensive and impractical. However, oregano water obtained during distillation process as a by-product of oregano oil has similar constituents (Boydağ et al., 2002), while it is cheaper and easier to use. In the present study, it was found that oregano water applications decreased *P. deliense* inoculum density in soil and supported emergence and growth of bean seedlings by decreasing the negative effect of the pathogen. These results showed that oregano water can be used against soil borne plant pathogens in small areas like nurseries and greenhouses. But it will be better to investigate its effect in other host-pathogen combinations.

Compliance with Ethical Standards

Conflict of interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. The authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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