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Antifungal and Herbicidal Activity of *Trachystemon orientalis* (L.) G. Don against Some Plant Pathogenic Fungi and *Cuscuta campestris* Yunck*

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ABSTRACT: The antifungal and herbicidal activity of *Trachystemon orientalis* (L.) G. Don aqueous plant extracts were evaluated against important plant pathogenic fungi (*Sclerotinia sclerotiorum* Lib. De Bary, *Alternaria solani* (Ellis & G. Martin) Sorauer, *Phytophthora infestans* (Mont.) de Bary, and *Botrytis cinerea* Pers.,) and *Cuscuta campestris* Yunck. Antifungal activity experiments were conducted under laboratory conditions and herbicidal activity was tested in the greenhouses. The leaves, root and flowers of aqueous extracts of *T. orientalis* at 1, 3, 5, 7, 10 and 20% doses were applied on Potato Dextrose Agar (PDA) and tested to plant diseases, and the dose of 5% of flowers and leaves aqueous extracts of *T. orientalis* were examined on tobacco (*Nicotiana tabacum* L.) and sugar beet (*Beta vulgaris* L.) against *C. campestris*. Antifungal activity displayed differences inhibited effects on mycelium growth of plant pathogenic fungi according to increasing extract dose and plant pathogenic fungi. The results showed that mycelial growth inhibitions were found effective in all extracts (leaf, flower and root) on *S. sclerotiorum* 0 to 100%, on *A. solani* 21 to 100%, on *P. infestans* 25 to 100% and, on *B. cinerea* 0 to 100% depending on the extract dose. According to herbicidal activity result the development of *C. campestris* has been reduced by looking the fresh (in tabocco: 0.3639 g and in sugar beet:0.6749 g) and dry (leaf extracts: in tabacco:0.0675 g and sugar beet: 0.1546 g; flower extracts: in tobacco 0.3246 g and sugar beet: 0.3421g) weight.

Keywords: Antifungal activity, *Cuscuta campestris*, herbicidal activity, plant extract, *Trachystemon orientalis*.

Bazı Bitki Patojeni Funguslar ve *Cuscuta campestris* Yunck.'a Karşı *Trachystemon orientalis* (L.) G. Don'un Antifungal ve Herbisidal Aktivitesi

ÖZET: *Trachystemon orientalis* (L.) G. Don bitkisinin su ekstraktlarının bitki patojeni funguslara (*Sclerotinia sclerotiorum* Lib. De Bary, *Alternaria solani* (Ellis& G. Martin) Sorauer, *Phytophthora infestans* (Mont.) de Bary, ve *Botrytis cinerea* Pers.,) ve *Cuscuta campestris* Yunck. karşı antifungal ve herbisidal aktiviteleri belirlenmiştir. Antifungal aktivite denemeleri laboratuvar koşullarında, herbisit etkinlik denemeleri ise sera koşullarında yürütülmüştür. *T. orientalis*'in %1, 3, 5, 7, 10 ve 20 dozundaki yaprak, kök ve çiçek ekstraklarının bitki patojenlerine karşı Patates Dekstroz Agar (PDA) ortamı üzerinde antifungal aktivite çalışmaları yürütülmüştür. Herbisidal aktivite çalışmalarında ise, *C. campestris*'e karşı *T. orientalis*'in çiçek ve yaprak ekstraktlarının %5 dozları tütün (*Nicotiana tabacum* L.) ve şeker pancarı (*Beta vulgaris* L.) bitkileri üzerinde uygulanmıştır. Bitki patojeni funguslar ve ekstraktların doz artışına göre antifungal aktivitelerde farklılıklar gözlemlenmiştir. Bu sonuçlara göre, ekstraktın doz miktarına bağlı olarak bütün ekstraktlar için (yaprak, çiçek ve kök) *S. sclerotiorum*'da %0-100, *A. solani*'de %21-100, *P. infestans*'da %25-100 ve *B. cinerea*'da %0-100 arasında yüzde miselyum gelişim engellemeleri gözlenmiştir. Herbisidal aktivite çalışmaları sonucunda, *C. campestris*'in kontrol bitkilerine göre taze (tütünde; 0.3639 g ve şeker pancarında; 0.6749 g) ve kuru (yaprak ekstraktı:tütünde 0.0675 g ve şeker pancarında: 0.1546 g; çiçek ekstraktı: tütünde 0.3246 g ve şeker pancarında: 0.3421g) ağırlıklarına bakılarak gelişimini azalttığı ortaya konmuştur.

Anahtar kelimeler: Antifungal aktivite, bitki ekstraktı, *Cuscuta campestris,* herbisidal aktivite, *Trachystemon orientalis*.

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INTRODUCTION

Weeds and plant diseases are the important reasons to yield losses in crop all around the world. Weeds constitute a problem due to increasing costs and hosting diseases and pests in competition with the cultivated plants. Plant diseases cause to be infection and reduce the quality and production in agricultural products (Dellavalle et al., 2011; Işık et al., 2016). Synthetic pesticides are generally used intensively in the control of weeds and plant diseases. The extensive use of synthetic pesticides has brought negative effect on human health and the environment (Işık et al., 2016). In addition, intensive pesticide that is used to control weed and plant diseases bring about resistance problems. For this reason, alternative methods need to be developed for pesticides.

Therefore, researchers focus on developing new bio-pesticide against weed and controling of plant diseases by using new active molecules such as seconder metabolites (Onaran et al., 2014; Yılar and Kadıoğlu, 2016; Onaran, 2016). Secondary metabolites are naturally contained in plants and their effectiveness on diseases and harmful weeds have been studied by isolating the allelochemicals with the potential of biological activity from the plants.

Sclerotinia sclerotiorum (Lib.) De Bary is the causal agent of white mold, which is common in cucumber in Turkey (Yanar and Onaran, 2011). *Alternaria solani* (Ell. and G. Martin) Sorauer and *Phytophthora infestans* (Mont.) de Bary cause intense damage in tomato both in the world and in our country. *A. solani* is an early blight agent, is very common in tomatoes in Turkey (Yazıcı et al., 2011). *P. infestans* is the causal agent of late blight, is an important fungal disease of tomato worldwide (Soylu et al., 2006). *Botrytis cinerea* Pers., causes grey mold disease in strawberries all around the world (Grabke et al., 2014).

Cucuscuta campetris Yunck belongs togenus of Cuscuta (Convolvulaceae) is an obligate parasite (Dawson et al., 1994). This parasite has a wide range of host species. It parasites tobacco, sugar beet, some horticultural crops, legumes and broadleaved weeds (Yuncker, 1932).

Trachystemon orientalis (L.) G. Don (Boraginaceae) is known colloquially as kaldirik, fish poison and borage in Turkey, is a perennial plant at heights of 30-40 cm with rhizome. It can be seen in the Black Sea Region in Turkey, especially under beech forests, in different habitats of the Black Sea Region, in addition to east Bulgaria and the west Caucasus. It has diuretic, blood cleansing, emollient and antipyretic effects. It can be used internally as infusion, and flower buds and leaves are consumed as vegetables. It contains tannins, essential oils, nitrate salts, mucilage, saponins and resin (Karagöz et al., 2002; Akçin et al., 2004). The antioxidant, antifungal and herbicidal properties of this plant were determined in previous studies and found to contain phenolic compounds and flavonoids (Özen, 2010; Onaran and Yılar, 2012; Ayvaz, 2015).

The purpose of this study was to determine the effect of *T. orientalis* on plant pathogenic fungi (*S. sclerotiorum*, *A. solani*, *P. infestans*, and *B. cinerea*), and against *Cuscuta campestris,* which is an important parasite of the plants.

MATERIALS AND METHODS

Plant Material

Plant materials were collected from the Saz Village in the city of Düzce in April 2014. The leaves, flowers, and roots of the collected plants were dried at room temperature. The dried plant materials were passed through the electric grinder. Plant powder was kept room temperatures in the jar until using the experiment.

Fungi Cultures

The plant pathogenic fungi (Table 1) were obtained from the stock cultures in the phytopathology laboratories of the Plant Protection Department at Gaziosmanpasa University, Faculty of Agriculture. Fungus cultures were used in the study after being developed for seven days at 25±2°C in 60 mm petri plates, containing 10 ml Potato Dextrose Agar (PDA).

Table 1. Species of fungi used in study

Plant Pathogens	Isolated Plants
Sclerotinia sclerotiorum (Lib.) de Bary	Cucumber (*Cucumis sativus* L.)
Alternaria solani (Ell. and G. Martin) Sorauer	Tomato (*Solanum lycopersicum* L.)
Phytophthora infestans (Mont.) de Bary	Tomato (*Solanum lycopersicum* L.)
Botrytis cinerea Pers.	Strawberry (*Fragaria* L.)

Preparation of Extracts

Four hundred grams of each grained plant material (leaf, flower and root) were weighed and placed in an Erlenmeyer flask and 1000 ml sterile purified water (pH:6.5) was added. This solution was left for 24 h on a shaker at room temperature for extraction. Then it was filtered through filter paper. The obtained extracts were filtered with the Millipore mechanism by using 0.45μm membrane paper after being centrifuged for 15 minutes at 5000 rpm. The obtained solutions were used in the tests (Onaran and Yılar, 2012).

Weed Cultures

Cuscuta campestris Yunck plants were grown on sugar beet plants in the greenhouses from Gaziosmanpasa University (Tokat-Turkey) in 2014. *C. campestris* transferred to the new sugar beet and tobacco seedlings from sugar beet plants (all experiment pot sizes were 16 (w) x 21 (h) cm). *C. campestris* was infected to plants and waited approximately 3-4 weeks for development (Çakmakcı et al., 2006). The same grown of *C. campestris* intensity were attentive to use for experiment.

Herbicidal Activity

The resulting stock solution was adjusted to 10% dose. The 95 ml stock solution (% 5 dose) was taken, and added 5 ml of acetone (5% acetone v/v) inside the taken solution for preparing the application (Onaran and Yılar, 2012). This solution was applied by spraying methods on sugar beet and tobacco plants, which were infected by *C. campestris*. The changes in the plants were recorded daily. Two weeks after application, *C. campestris* was taken on sugar beet and tobacco plants and then recorded fresh and dry weights (Khaliq et al., 2013). This experiment was set up 3 replicate and repeated twice in the greenhouse conditions.

Antifungal Activity

The specific dose of the obtained extracts was 10%. The extracts of 2.5, 7.5, 12.5, 17.5, 25 and 50 ml respectively were added to 250 ml PDA and final doses were obtained at 1%, 3%, 5%, 7%, 10% and 20% respectively. These PDAs including different doses were cooled to 45-50^{o}C, and poured into the 60 mm petri plates in 10 ml.The mycelium discs (diameter: 5mm) were taken from 7 days old plant fungus cultures were placed in extract with PDA added. As control, fungi were added to PDA plate without contained plant extract. The fungus cultures were left for incubation for seven days in 25±2^{o}C after inoculation. The changes in the mycelium growht of the pathogen were started to record 24 h after inoculation and continued 7 days. This experiment was set up 4 replicate and repeated twice (Onaran and Yılar, 2012). The growing mycelium diameters were measured with a digital calliper and mycelial growth inhibition (MGI) was calculated based on the following formula (1) (Pandey et al., 1982);

$$MGI = 100 \times (dc - dt) \div dc \quad (1)$$

dc: Mycelial growth in control
dt: Mycelial growth in treatment

Statistical Analysis

Data were analyzed statistically using SPSS 16.0 program (SPSS, Chicago, IL). Differences among doses and each pathogen were compared with using DUNCAN Multiple Range Test of $p<0.05$.

RESULTS AND DISCUSSIONS

The effect on leaf, flower, and root extracts of *Trachystemon orientalis* L. on plant pathogenic fungi are summarized in Table 2.

Table 2. The antifungal activity of flower, leaves, and root extracts of *Trachystemon orientalis* on mycelium growth of plant pathogens

Plant pathogens	Doses	Plant Parts		
		Leaf	Flower	Root
		Mycelium Growth (mm)	Mycelium Growth (mm)	Mycelium Growth (mm)
Sclerotinia sclerotiorum	Control	60.00[1]a[2]±0.00	60.00a±0.00	60.00a±0.00
	1%	60.00a±0.00	0.00b±0.00	60.00a±0.00
	3%	36.85ab± 0.80	0.00b±0.00	60.00a±0.00
	5%	27.64b±1.99	0.00b±0.00	60.00a±0.00
	7%	0.00c±0.00	0.00b±0.00	60.00a±0.00
	10%	0.00c±0.00	0.00b±0.00	60.00a±0.00
	20%	0.00c±0.00	0.00b±0.00	44.45b±0.65
Alternaria solani	Control	53.13a±0.36	53.13a±0.36	53.13a±0.36
	1%	32.07b±1.02	28.48b±0.37	43.19b±0.33
	3%	19.05c±0.82	15.33c±0.78	40.67b±1.82
	5%	18.68d±1.16	0.00d±0.00	33.15c±0.29
	7%	0.00e±0.00	0.00d±0.00	32.01c±0.28
	10%	0.00e±0.00	0.00d±0.00	31.23c±0.52
	20%	0.00e±0.00	0.00d±0.00	30.83c±1.58
Phtophthora infestans	Control	46.19a±0.97	46.19a±0.97	46.19a±0.97
	1%	22.85b±0.27	0.00b±0.00	34.58bc±0.78
	3%	19.62c±0.75	0.00b±0.00	26.86c±1.67
	5%	12.45c±1.80	0.00b±0.00	25.69c±0.59
	7%	0.00d±0.00	0.00b±0.00	23.30c±1.56
	10%	0.00d±0.00	0.00b±0.00	22.76c±0.35
	20%	0.00d±0.00	0.00b±0.00	20.35d±0.54
Botrytis cinerea	Control	60a±0.00	60a±0.00	60a±0.00
	1%	56.52ab±0.59	52.45b±2.08	60.00a±0.00
	3%	55.27ab±0.18	0.00c±0.00	60.00a±0.00
	5%	54.97ab±0.58	0.00c±0.00	60.00a±0.00
	7%	53.91ab±0.69	0.00c±0.00	48.84b±0.55
	10%	52.75ab±2.72	0.00c±0.00	45.72b±1.48
	20%	50.45b±0.19	0.00c±0.00	41.60c±0.56

Mycelial growth± Standard deviation.[1]Mycelial growth after 7 days (mm). [2]Different letters represent statistically significant differences among mycelia growths between treatments according to Duncan's test ($P < 0.05$)

T. orientalis extracts were showed a significant effect on the inhibition of plant diseases. This effect has varied depending on the extracts, pathogens and doses. The flower extract has showed highest effect on pathogens, this was followed by leaves, and root extract. Mycelial growth inhibition of *S. sclerotiorum*, *A. solani*, *P. infestans* and *B. cinerea* at a rate of 100% according to the control were affected by the flower extract of *T. orientalis* (Figure 1 and 2). Mycelium growth has negatively affected by the leaves extract of *T. orientalis* depending on increasing applied dose. The 20% dose of *T. orientalis* was inhibited mycelium growth of *S. sclerotiorum*, *A. solani* and *P. infestans* at a rate of 100% but *B. cinerea* mycelium growth was reduced at a rate of 16% compared to the control (Figure 1 and 2). The root extract of *T. orientalis* showed lower effect on plant pathogens compared with flower and leaf extracts, in general. The 20 % dose of *T. orientalis* root extract inhibited the mycelium growth of *S. sclerotiorum* was 26%; (Figure 1) *A. solani* was 44%; (Figure 1) *P. infestans* was 56%, (Figure 2) and *B. cinerea* was 31% (Figure 2) compared with the control.

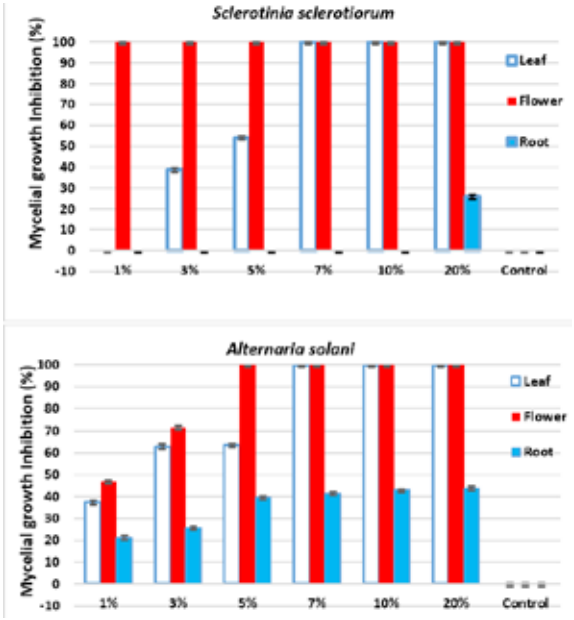

Figure 1. Mycelial growth inhibition values *Trachystemon orientalis* extracts (leaf, flower and root) against *Sclerotinia sclerotiorum* and *Alternaria solani*. Bars show standard deviation

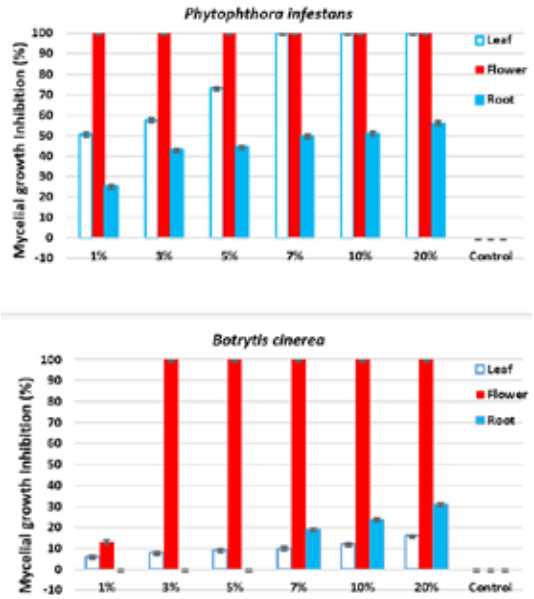

Figure 2. Mycelial growth inhibition values of *Trachystemon orientalis* extracts (leaf, flower and root) against *Phtophthora infestans* and *Botrytis cinerea*. Bars show standard deviation

With this study, herbicidal activity of *T. orientalis* was examined. The leaves and flower extracts were applied against development of *C. campestris*. During the experiment, the control plants grew vigorously and developed normally. The 5 % of leaves and flower extracts of *T. orientalis* was applied by spray on *C. campestris* and were obtained significantly reduced the fresh (control) and dry weight of *C. campestris* (Figure 3). In addition, after application of extract, *C. campestris* was observed brownish colouring. The flower extracts were found more effective than leave extracts. This result is similar with antifungal experiment results. Özkurt et al., (2007) reported that *T. orientalis* plant water extracts inhibited seed germination and seedling development of *Sinapis arvensis* L., *Agrostemma githago* L., *Triticum vulgare* L., *Lepidium sativum* L., and *Lactuca sativa* L. In another study, Hassannejad and Ghafarbi, (2013) reported that the aqueous extracts of medicinal plants (*Lavandula vera* DC., *Rosmarinus officinalis* L., *Salvia officinalis* L., *Thymus vulgaris* L., and *Melisa officinalis* L.) had inhibited effect the germination percentage of *C. campestris* and reduced seedling length of *C. campestris*. These results have shown that *T. orientalis* has herbicidal activity on weeds. Thus, the use of extracts could be potentially an effective way of controlling *C. campestris*.

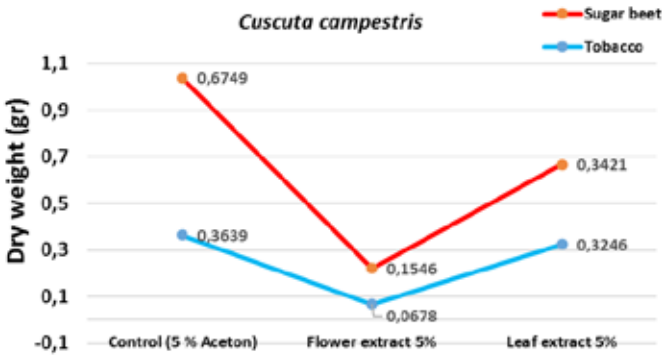

Figure 3. Herbicidal effect of *Trachystemon orientalis* flower and leaf extracts against *Cuscuta campestris* on the tobacco and sugar beet plants

According to the results of this study, the flower extracts of *T. orientalis* was showed the greatest effect, this was followed by leaf and root extracts respectively. This difference came from secondary metabolites, which were possession of different parts of plant contain different level. Similar result has been reported by many researchers (Okigbo and Ogbonnaya, 2006; Kanan and Al-Najar, 2008; Yılar and Kadıoğlu, 2016). Onaran and Yılar, (2012) reported that the aqueous flower extract of *T. orientalis* showed inhibitory effect against the plant pathogens (*Ascochyta rabiei*, *Fusarium oxysporum* f.sp. *melonis*, *Fusarium oxysporum* f.sp. *radicis-lycopersici*, *Verticillium dahliae* and *Rhizoctonia solani*). The flower extract completely inhibited mycelial growth of *A. rabiei*, *V. dahliae* and *R. solani* in all applied doses. In this study, the extracts of *T. orientalis* were observed the different antifungal activity rates against plant pathogens. The most tolerant plant disease was found *B. cinerea*, in contrast *S. sclerotiorum* was observed more susceptible against plant extracts. This difference arises from the structural diversities of pathogens and effect of the chemical substance mechanisms in plant extracts (Türküsoy and Onogur, 1998; Kordali et al., 2009). The previous studies showed that *T. orientalis* plant extracts have antiviral, antioxidant, allelopathic, and antimicrobial activities (Karagöz et al., 2002; Uzun et al., 2004; Özkurt et al., 2007; Özen, 2010). As result of this study, *T. orientalis* has showed herbicidal and antifungal activities, these results are consistent with previous studies.

CONCLUSION

Consequently, *Trachystemon orientalis* aquatic extract has demonstrated antifungal activity on *Sclerotinia sclerotiorum*, *Alternaria solani*, *Botrytis cinerea* and *Phytophthora infestans* plant pathogens, and has showed herbicidal activity on *C. campestris*. From these results, *T. orientalis* has determined that could be an alternative to synthetic pesticides against important plant diseases and weed problems.

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