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THE EFFECTS SOME PLANT (*Coridothymus Capitatus* L.) RCHB. F. AND *Mentha Pulegium* L.) EXTRACTS ON THE CONTROL OF AN INVASIVE WEED (*Oxalis Pes-Caprae* L.)

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ABSTRACT

This study was carried out to determine the effect of some plant extracts in the control of weed (*Oxalis pes-caprae* L.) which is an invasive weed species. The phenolic components of *Coridothymus capitatus* (L.) Rchb. F. and the *M. pulegium* L. samples UPLC-ESI-MS / MS. determined with the device. In our study, a total of 10 phenolic components were detected in *C. capitatus* and *M. pulegium* samples. Among the phenolic components obtained from the samples of *C. capitatus* plant, the most effective components are; Vanilic acid (2103,24) (mg/kg), Caffeic acid (444,50) (mg/kg), Protocatechuic acid (240,47)(mg/kg) and those of *M. pulegium* were Caffeic acid 335,67(mg/kg), Protocatechuic acid 144,73 (Mg/ kg), 3-4-Dihydoxy benzaldehyde 93.18 (mg /kg) plant. In the experiment, control effects were investigated by applying 3 different doses of *C. capitatus* and *M. pulegium* plant extracts applied on germination of stolon tubers of *O. pes-caprae*, which were placed in petri dishes of 90 X 14 mm. The control effectiveness of plant extracts on the development of root shoots of *O. pes-caprae* was statistically determined. As a result, it was determined that 20% dose of *M. pulegium* application was the most effective application that prevented the root development of *O. pes-caprae* by 96.53% compared to control petri. The effects of the other application, the dose of 20% *C. capitatus*, on the root development of O. *pes-caprae* were determined that prevented by 98.65%. Control activities of other applications have followed this application.

KEYWORDS:
Invasive weed, Phenolic compound, *Oxalis pes-caprae* L., *Coridothymus capitatus* (L.) Rchb. f. and *Mentha pulegium* L.

INTRODUCTION

The presence of weeds leads to considerable losses in agriculture. Production is damaged both qualitatively and quantitatively, because weeds constantly compete with culture plants for their nutrient, light and humidity needs. Weeds colonize well, reproduce faster, and their ability to stay alive in large numbers in small areas of soil for a long time leads to the continuation of the weed problem [1]. On average, weeds lead to a 34% reduction in production [2]. The increase in the world's population and the simultaneous spending of existing resources have led agriculture towards using synthetic herbicides without reservations for weed control. This broad usage has caused serious problems like herbicide resistance and the emergence of invasive species. Invasive species are those that are not naturally present (including seeds, eggs, spores or other propagules) in an area which may induce economic, environmental and human health-related damages. The term "invasive" is used for the most aggressive species [3]. These species grow and reproduce fast, and they lead to substantial problems in the areas they are found. If invasive plant species are left uncontrolled, they may interrupt the sustainable agriculture system by limiting the present and future land use. Living beings or their seeds, eggs, spores or other biological materials with reproductive capacity which are not found in the natural flora or fauna of a certain limited geographical area and are carried in from the outside for a certain purpose, therefore not being among the native species in this area, may be defined as "Alien Species" or "Exotic Species". Among alien species that enter this geographical area this way using various routes, those threatening human health, inducing economic or environmental damages or having the potential to induce such damages may be defined as "Invasive Alien Species" [4]. Invasive alien plants, which have a substantially high capacity for adaptation, achieve advantages over other plant species (including weeds) in their newly entered area due to reasons such as their genetic diversity and strong development capacities and that there are no negative pressures (herbivore species, disease factors, etc.) over them as there are no natural enemies in their new environment [5-7]. As a result of this, they constitute a significant problem regarding the continuity of the natural areas they have entered [8]. Invasive species may also be harmful by reducing or annihilating the diversity of the species found in the natural vegetation [9-10]. For example, invasive species affect the nutrient cycle in the vegetation due to their different nutrient collection strategies, nutrient intake and output and higher nutrient utilization efficiency than native plants [11, 12]. Therefore, it was determined that invasive species, whose effects on soil processes were investigated, affected the nutrient cycle by increasing the presence of nutrients [10].The increase in CO_2 concentrations, which is one of the most significant factors causing global warming and climate change which are among the most important problems of the era emerging as a consequence of human presence in the world, is also a factor that speeds up the process of invasive species becoming the dominant species in natural and agricultural ecosystems [13]. Thus, in relation to climate changes, it is projected that the spread of invasive alien weed species will probably continue [14]. The damages created by such species include the harm on agricultural products, as well as funds that are spent for control of such species. It is more difficult to measure the ecological effects of invasive species, but, these calculations are critically important for estimation of the damages. Invasive plants may affect natural ecosystems in various ways. They may reduce the natural diversity of plants by competing for resources such as sunlight, water or minerals. Additionally, they may also change the soil conditions around them by secreting chemicals that prevent seeds from germinating or other plants from growing (an effect known as allelopathy). They may change the nutrient cycle and soil properties in the invaded area by changing the composition of their plant-based wastes or their degradation rate. Invasive plants that are closely related to native species may hybridize with their native relatives. Moreover, quarantine precautions may sometimes fall short, and this makes it easier for invasive species to be introduced to the environment. If we left uncontrolled, invasive species may limit the present and future land use, and thus, if we neglect the significance of this problem, its solution and control will be much difficult and expensive. The damages generally related to invasive species are estimated as hundreds of billions of dollars per year. The United States of America spends 1.1-120 billion dollars annually due to economic damages created by exotic and invasive species. In addition to this, approximately 42% of endangered species are under risk due to non-native invasive species [4].

The fact that invasive species have become a highly prevalent problem in the world has been brought about by the increase in the activities of humanity (commerce, tourism, etc.), suitable conditions brought by climate change, intensive use of dangerous chemicals and changes in land use. The evolution of herbicide-resistant alien weed populations and their negative effects on environmental, human and animal health are highly intensive [15]. Therefore, there is a need for discovering environmentally friendly strategies for the control of weeds and invasive species.

The Use of Secondary Metabolites in Weed Control. Plants have developed various defense mechanisms to protect themselves against plant diseases, parasites and weeds. These mechanisms include physical characteristics that play a role in their protection against the harms of these factors and chemical compounds they synthesize. The most significant defense mechanism developed by plants against these harmful factors is based on the secondary metabolites they synthesize. Secondary metabolites are chemical compounds that do not have effects on the growth and development of plants but take a role in the protection mechanisms of plants against harmful factors [16]. These chemical compounds that have behavioral and biological effects on pests are classified in many different categories [17]. Secondary metabolites are generally classified in three main groups as alkaloids, terpenoids and phenols [18]. Chemical substances that are secreted by plants and have allelopathic effects are known as allelochemical substances. Most secondary metabolites released by plants have allelochemical properties [19-24]. Allelochemicals are found in almost all plant parts and tissues including stems, leaves, flowers, buds, bark, pollens, seeds, fruits, roots and rhizomes [25]. Furthermore, the allelochemical concentrations of plants may vary from a part to another [26]. As an alternative method, allelopathic substances (secondary metabolites, allelochemicals) are being used against weeds, pests and plant diseases. The increasingly higher usage area of secondary metabolites with allelopathic effects involves their use in biological struggle against weeds in plant production [25]. The use of allelopathic effects in the field of agriculture requires long-term and highly disciplined work when possible. The objective of this study, in allelopathy studies, the priority issue is to determine whether or not natural compounds have effects on the targeted factors (plant, bugs, nematodes, disease factors, etc.). For this purpose, in allelopathic struggle against *Oxalis pes-caprae* (Bermuda buttercup), extracts obtained from the plants *Coridothymus capitatus* (L.) Rchb. f. and *Mentha pulegium* L. were utilized.

MATERIALS AND METHODS

Material. The material of the study consisted of *Coridothymus capitatus* (L.) Rchb. f. and *Mentha pulegium* L. that grow naturally in Muğla-Ula region and *Oxalis pes-caprae* L.. that grow naturally in İzmir. *Coridothymus capitatus* (L.) Rchb. f.; was collected from in Muğla Akyaka area (6 m), *Mentha pulegium* L. was collected from Karabörtlen area

(81m), *Oxalis pes-caprae* L. stolon tubers were obtained by field study in Karaburun location (İzmir). The leaved shoots and flowers of each plant were collected to be used in extraction analyses. The collected plants were put in bags, and each bag was labeled. The data related to the collection time, place, and elevation were written on the label of each bag. These plants were then dried in a semi-shadowy and airy place at room temperature (at 25°C) to be used in extraction analyses.

Standards and reagents. Methanol, acetonitrile, and hexane for extraction of phenolic compounds and chromatographic separation were HPLC grade, and were purchased from Merck (Darmstadt, Germany). The standards used for identification and quantification of phenolic compounds were: pyrogallol, homogentisic acid, 3,4-dihydroxybenzoic acid, gentisic acid, pyrocatechol, galantamine, 4-hydroxy benzoic acid, 3,4-dihydroxybenzaldehyde, catechin hydrate, vanillic acid, caffeic acid, syringic acid, vanillin, epicatechin, catechin gallate, *p*-coumaric acid, ferulic acid, rutin, trans2-hydroxy cinnamic acid, myricetin, resveratrol, transcinnamic acid, juteolin, quercetin, naringenin, genistein, apigenin, kaempferol, hesperetin, chlorogenic acid chrysin were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). HPLC grade water was 18.2 MΩ

Ultrasonic-assisted extraction of phenolic compounds from plant samples. The ultrasonic-assisted extraction of phenolic compounds from samples of *Coridothymus capitatus* and *Mentha pulegium* was based on liquid-liquid extraction procedure previously described by Kıvrak et al. (2018) with slight modifications. Briefly, 2.0 g of *C. capitatus* and *M. pulegium* samples weighed into a centrifuge tube, and 15 mL of n-hexane and 30 mL of acetonitrile were added [27]. The mixture is mixed for 2 min then, extracted in an ultrasonic bath for 10 min followed by centrifuge at 4000 rpm for 5 min. Then, the acetonitrile layer was separated. Those steps were applied two times more. The acetonitrile extracts were combined and washed with petroleum ether, and then evaporated to dryness using nitrogen evaporator. The residue was dissolved in water: methanol mixture (60:40, v/v), and then filtered through 0.20 µm PTFE syringe filter and 2 µL injected to UPLC-ESI-MS/MS instrument, which stated in a previous method with slight modification [28]. The phenolic compounds analyses of oil samples were provided by C18 column, and the separation of compounds was performed by gradient elution. The mobile phases were composed of (S1) 0.5% acetic acid in water and (S2) 0.5% acetic acid in acetonitrile. Elution was done at 40°C column oven temperature with a flow rate of 0.650 mL/min with eluent (S1) and eluent (S2). Elution sequence was composed of a linear gradient mode 1 min at 99% of (S1), from 99% to 70% of (S1) in 10 min, from 70% to 99% of (S1) in 2 min, and finally a 3-min plateau at 99% of (S1). The re-equilibration of the column was obtained in the last plateau. Tandem mass spectrometry parameters were ion mode (ESI±), nebulizer 7.0 bar, source temperature 150°C, desolvation temperature 500°C. The confirmation/quantification of mass transitions (*m/z*), and their collision energies are explained in Table 1.

UPLC-MS/MS analysis of individual phenolic compounds. Phenolic profiles of samples of *Coridothymus capitatus* and *Mentha pulegium* were determined based on previous procedure [27]. The analysis of individual phenolic compounds in samples of *Coridothymus capitatus* and *Mentha pulegium* were determined by an UPLC–ESI–MS/MS instrument). The mass spectrometry parameters, confirmation, and quantification mass transition (*m/z*), and their collision energies are explained in previous literature [28]. Separation operations were accomplished using a C18 column

Seed Germination. The obtained product was kept in storage to organize laboratory trials for determining allelopathic effects. Extracts obtained from the *Coridothymus capitatus* and *Mentha pulegium* plants were filtered through a Whatman-filtered Büchner funnel, extracts with concentrations of 5%, 10% and 20% were obtained, kept at +8°C for a week and stored. After washing Bermuda buttercup (*O. pes-caprae*) tubers with distilled water, 3 *O. pes-caprae* tubers were put inside 90 × 14 mm sterilized petri containers equipped with 3 layers of filter paper. Afterwards, the petri containers were put into a Plant Growth Cabin adjusted to a temperature of 14°C for 12 hours of dark and a temperature of 20°C for 12 hours of light. Including the 3 doses (5%, 10% and 20%) of the allelopathic extracts of the *C. capitatus* and *M. pulegium* plants and a control (0%), the trials were organized to include 4 treatments and 4 replications (Figure 1). Seven days after the application of plant extracts, to determine the germination of the *O. pes-caprae* stolon tubers, the lengths of the root shoots were measured by the help of a caliper and recorded in units of mm. The root shoot measurements were compared in the SPSS 22 package software with Duncan's multiple comparisons test on the level of P=0.05. (Figure 1).

RESULTS AND DISCUSSION

The contents of the phenolic compounds obtained from *M. pulegium* and *C. capitatus* were analyzed (Table1). Among the total of 12 phenolic compounds, those most abundantly found in *Coridothymus capitatus* were as: 2103.24% Vanillic acid, 444.50% Caffeic acid, 240.47% Protocatechuic acid.

For *Mentha pulegium,* these were as: 335.67% Caffeic acid, 144.73% Protocatechuic acid, 93.18% 3-4-Dihydoxy benzaldehyde.

Effects of phenolic compounds on germination rates. The effects of the plant extract of *M. pulegium* used for weed control on the root development of the invasive weed species of *O. pes-caprae* in its tubers' germination were as shown in Table 2. In the statistical analysis of the root developments of the weed, it was determined that the differences in length were significant on the level of P=0.05. Among the treatments, the most effective treatment was found to be the 20% *M. pulegium* (0.4489 mm) extract application. This was followed by the other extract doses of *M. pulegium* respectively as 10% and 5% (Table 2).

The 20% *M. pulegium* extract application prevented the root development of *O. pes-caprae* by 96.53% in comparison to the control group. Moreover, the 10% extract prevented root development by 64.00%, and the 5% extract prevented it by 29.21%. Another study also reported that the root extracts of *M. pulegium* prevented the germination of the seeds of *Plagiobthrys hirtus* and *Perideridia erythrorhiza* to a large extent [29]. In similarity to the results of our study, 10% and 15% extracts of another *Mentha* species, *Mentha piperita* L., had effects of up to 100% in preventing the seed germination of plants such as *Beta vulgari*, *Cucumis sativus*, *Lactuca sativa*, *Lupinus luteus*, *Phaseouls vulgaris*, *Raphanus sativus* v. *radicula*, *Sinapis alba*, *Triticum aestivum*, *Lycopersicon esculentum* and *Zea mays* [30].

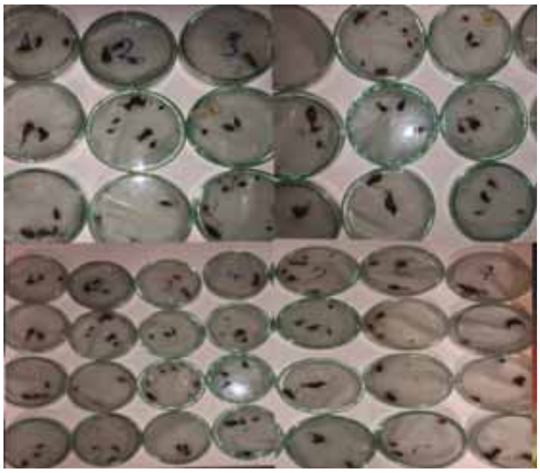

FIGURE 1
View from petri experiment

TABLE 1
Phenolic compounds of M. pulegium and C. capitatus(mg/kg)

Compounds	*Coridothymus capitatus* L.	*Mentha pulegium* L.
4-Hydroxy benzoic acid	88,11	38,31
3-4-Dihydoxy benzaldehyde	201,48	93,18
***t*-Cinnamic acid**	ND	ND
Vanillin	10,33	ND
Gentisic acid	34,55	21,41
Protocatechuic acid	240,47	144,73
***p*-Coumaric acid**	7,74	2,01
Vanilic acid	2103,24	90,65
Caffeic acid	444,50	335,67
Ferulic acid	16,90	28,52
Kaempferol	21,54	ND
Myricetin	ND	ND

ND: not determined.

TABLE 2
Effects of M. pulegium applications on the root length of O. pes-caprae

M. pulegium Extract Concentration (%)	Root Length (mm)	Effect (%)
5	9,1657	29,21
10	4,6608	64,00
20	0,4489	96,53
Control	12,9479	-
Sig. (P=0,05)	0,000	

Means for groups in homogenous subsets are displayed Based on observed means. The error term is Means Square (Error)=43,760 a.Uses Harmonic Mean Sample Size=100,000. b.Alpha=05.

TABLE 3
The Effects of the plant extract of *C. capitatus*

C. capitatus Extract Concentration (%)	Root Length (mm)	Effect (%)
5	4,5066	62,16
10	2,2958	80,72
20	0,1608	98,65
Control	11,9123	-
Sig. (P=0,05)	0,000	

Means for groups in homogeneous subsets are displayed Based on observed means. The error term is Mean Square(Error) = 25,467. a. Uses Harmonic Mean Sample Size = 100,000. b. Alpha = 0,05

The effects of the plant extract of *C. capitatus* used for weed control on the root development of the invasive weed species of *O. pes-caprae* in its tubers' germination were as shown in Table 2. In the statistical analysis of the root developments of the weed, it was determined that the differences in length were significant on the level of P=0.05. Among the treatments, the most effective treatment was found to be the 20% *C. capitatus* (0.1608 mm) extract application. This was followed by the other extract doses of *C. capitatus* respectively as 10% and 5% (Table 3). The 20% *C. capitatus* extract application prevented the root development of *O. pes-caprae* by 98.65% in comparison to the control group. Moreover, the 10% extract prevented root development by 80.72%, and the 5% extract prevented it by 62.16%. Many previous studies have also found that extracts obtained from allelopathic plants could be used to fight against weeds [31-33]. Furthermore, studies in similar with the effect results of our study [34-35] stated that *C. capitatus* may have a potential of use as a bioherbicide. In another petri study in laboratory conditions, the same plant extract was found to prevent the seed germination of *Erucaria hispanica* by up to 69%, *Plantago psyllium* by up to 65% and *Lactuca sativa* and *Anastatica hierochuntica* by up to 96% [36].

CONCLUSIONS

The findings showed that these plant extract could be used in the struggle against invasive species as they contain several chemical compounds with allelopathic effects. It is a necessity to develop alternative control methods to eliminate the effects of the increasingly prevalent use of pesticides on environmental and human health. The intensive use of pesticides especially for this purpose may lead to the emergence of new invasive species. In this study, it was determined that the plant extracts of *Coridothymus capitatus* (L.) Rchb. f. and *Mentha pulegium* L. in the control of *O. pes-caprae* among invasive species constituted and effective method. However, to improve the doses used and their effectiveness, it is thought that studies need to continue.

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