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ALLELOPATIC EFFECTS OF SOME ESSENTIAL OIL COMPONENTS OF THYME AND ROSE ON GERMINATION: CASE STUDY OF BARLEY

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Abstract
The study was carried out to determine whether different concentrations of essential oil components (geraniol, carvacrol, thymol, and alpha terpinene) and essential oil of thyme (Thymus vulgare) have any allelopathic effects on the germination of barley seeds. The study was carried out in the laboratory environment according to the divided plots experimental design in random plots. At the end of the experiment, the effects of allelochemical types of carvacrol, thymol and geraniol doses on germination rate and seedling dry weight were found to be statistically significant (p<0.01). It was determined that geraniol (0.00245 g), carvacrol (0.01790 g) and thymol (0.02590 g) had the most negative effects on seedling dry weight, respectively. As a result, it was determined that some of the herbal allelochemicals used were effective on the germination of barley seed and seedling growth, and the effect rate increased as the dose increased. Thymol, geraniol and carvacrol showed the most negative effects on the germination rate of barley seed at all doses. On the other hand, adverse effects were determined at high doses of alpha terpinene. All doses of thyme essential oil and low doses of alpha terpinene showed similar effects with the control. As a result; It is thought that essential oil components, which have a negative effect on the germination of barley seed, can be used in the production of plant-based bio-herbicides.

Keywords: Carvacrol, Thymol, Geraniol, alpha terpinene, allelopathy, germination

1. INTRODUCTION

The increase in the factors that cause damage to the environment threatens not only the flora and fauna, but also human health. It has been observed that synthetic pesticides, diseases, pests and weeds used especially in agricultural areas cause resistance in themselves, deterioration of the natural balance, and residues in agricultural products. It is desired to reduce this damage due to its negative impact on human health, and studies are being carried out to find alternative methods (Yazlık and Üremiş, 2017).
Allelopathy is one of the alternative solutions in the fight against natural methods (Macias et al., 2007; Özen et al., 2017). Although allelopathy observations started many years ago, it was first scientifically put forward by Molisch (1937) (Gülsoy et al., 2008). The word allelopathy is

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etymologically derived from the combination of the words "allelo" and "pathos" and the interaction of secondary chemicals released through various organs in the plant (Singh et al., 2001; Colquhoun 2006) directly or indirectly affects the growth and development of other plants positively or negatively. (Rice 1984; Oueslati, 2003; Gürsoy et al. 2013). Its general definition is allelopathy when a plant emits a chemical substance to the environment and exerts a positive or negative effect on another plant, and chemicals with allelopathic effects are called "Allelochemicals" (Bhadoria, 2011).

Allelopathy is mostly seen in terrestrial plants. Allelochemicals secreted from terrestrial plants show a stronger allelopathic effect as they enter the soil structure directly or indirectly. Allelochemicals secreted from aquatic plants, on the other hand, are diluted because they mix with water and therefore their effects are weaker than terrestrial plants. These allelochemicals within the body of plants are released from the plant through evaporation, washing, root secretions and decomposition of plant residues. While the positive effects of allelochemicals are on the increase in yield, the negative effect is that they can be used as a natural control method in the control of weeds, diseases and pests (Yılmaz and Köse, 2021). Chemicals with allelopathic potential can be found in the aboveground and underground parts of the plant. Allelopathic agents defined as chemical compounds are simple water-soluble organic acids, straight chain alcohols, aliphatic aldehydes and ketones; simple unsaturated lactones; long chain fatty acids and polyacetylenes; naphthoquinones, anthraquinones and complex quinones; simple phenols, benzoic acid and derivatives; cinnamic acid and its derivatives; coumarins; flavonoids; tannins; terpenoids and steroids; amino acids and polypeptides; alkaloids and cyanohydrins; sulphides and mustard oil glycosides; purines and nucleosides can be divided into groups (Rice, 1984). Plant-based essential oils are shown as an alternative to herbicides due to their allelopathic effect on weeds that inhibit germination, growth and development, and their ability to biodegrade more easily than synthetic herbicides in nature (Özen et al., 2017).

Dudai et al. (1999) examined the allelopathic effects of essential oils obtained from 32 aromatic plants. In this study; They noted that 20-80 ppm applications significantly inhibited the germination of various species, including wheat. Qasem and Hassan (2003) investigated the herbicidal effects of 20 medicinal plant species from 15 families on Malva sylvestris (hibiscus) and Portulaca oleracea (purslane). *Alhagi maurorum, Capparis spinosa, Citrullus colocynthis, Lavandula officinalis [L. angustifolia], Origanum syriacum, Rhus coriaria, Ricinus communis, Rosmarinus officinalis,* and *Teucrium polium* show negative effects against both weed species, root, leaf and stem extracts each of them determined that both species prevented germination and seedling formation. The effect increased as the extract concentration increased. Gulsoy et al. (2008) juniper (*Juniperus excelsa* Bieb.) fruits and thyme (*Origanum minutiflorum* O. Schwarz et. P. H. Davis) leaves were obtained by water distillation. In their study examining the effect of these essential oils on the germination of *Pinus nigra* subsp. *pallasiana* seeds, they found that the germination rate decreased in parallel with the increase in the concentrations of both essential oils. Zahed et al. (2010), in their study investigating the allelopathic effect of *Schinus molle* essential oil on wheat, revealed that these oils have phytotoxic effects on germination and root elongation of the wheat plant. Badmus and Afalayan (2012) determined the allelopathic effects of *Arctotis arctotoides* aqueous extract on germination and seedling growth of cabbage, tomato, spinach and carrot seeds, shortening of plumula and root length. Sharifi-Rad et al. (2014) tested the essential oils obtained from the *Sinapis arvensis* plant on *Hyssopus officinalis* L., *Cardaria draba* (*Lepidium draba* L.), *Amaranthus retroflexus,* and *Taraxicum officinale,* which show medicinal properties, and found that the Sinapis

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arvensis essential oil was effective on the germination and seedling growth of these 4 plants. They concluded that it has a negative allelopathic effect. Yazlık and Üremiş (2015) investigated the allelopathic effects of essential oils of Istanbul thyme (*Origanum vulgare* L.), lavender (*Lavandula angustifolia* L.) and rosemary (*Rosmarinus officinalis* L.) plants on cannabis plant. They revealed that the highest dose of essential oil of rosemary plant gave the best results and reduced the dry weight of cognac by 41% in the pre-emergence period. They found that rosemary oil had the highest effect in the post-emergence essential oils, and that the post-emergence applications of all three essential oils provided a higher effect than the pre-emergence applications. Said et al. (2016) conducted a study in which they revealed the allelopathic effect of the component of *Thymus capitatus* essential oil on the seed germination of *Lactuca sativa* L. They also tested the antibacterial activities of the essential oils used in this study against *Salmonella gallinarum* and *E. coli* bacteria. Zambak et al. (2016) investigated the effect of *Rosmarinus officinalis* L., *Origanum syriacum* L. essential oils on the germination of some weed seeds. As a result, they determined that thyme essential oil was more effective than rosemary essential oil in seed germination. Efil and Üremiş (2019), essential oils obtained from thyme (*Origanum syriacum*) and marjoram (*Origanum majorana*) in different doses, *Amaranthus retroflexus* L., *Portulaca oleracea* L., *Physalis angulata* L., *Echinochloa colonum* (L.) Link., and *Solanum nigrum* L. seeds on germination and plant growth. They determined that the applications of the two essential oils showed a negative allelopathic effect by inhibiting the germination of all weeds and plant growth by 50%. Kong et al. (2021) investigated the chemical compositions of essential oils obtained from the fruit of *Litsea pungens*. They determined the allelopathic effect of these oils on seed germination rate and seedling growth of *Lolium perenne* and *Bidens pilosa*. Zheljazkov et al. (2021), using the essential oil of 6 different plants (*Lavandula angustifolia*, *Hyssopus officinalis*, *Thymus vulgaris*, *Levisticum officinale*, *Chrysanthemum balsamita*, *Cuminum cyminum*) showed an allelopathic effect suppressing seed germination and seedling growth in barley and wheat.

In this study, it was investigated whether essential oil components (geraniol, carvacrol, thymol and alpha terpinene) isolated from the essential oil of thyme and rose plant and thyme essential oil have any allelopathic effects on the germination of barley seeds.

2. MATERIALS AND METHODS

Laboratory experiments were carried out in the laboratory of Çanakkale Onsekiz Mart University, Faculty of Agriculture, Department of Agricultural Structures and Irrigation. Germination experiments were carried out according to 3-replication randomized plots split plots trial design. Barley seeds to be used in germination experiments were disinfected by keeping them in 2.5% sodium hypochlorite for 10 minutes. After the disinfection process, the seeds were taken into sterile distilled water and washed 2 times for 5 minutes. Then, it was placed in sterile distilled water and left for 2 hours for the seeds to swell and become active. 10 barley seeds were sown in petri dishes (9 cm in diameter) containing two layers of Whatman No.1 filter paper. 20 ml of distilled water was added to each petri dish. Different doses (0, 2, 5, 10 and 20 µl) of active substances (thyme essential oil, geraniol, carvacrol, thymol, and alpha terpinene) were impregnated with the blotting paper attached to the inside of the lid of each petri dish. Petri dishes were covered with parafilm to prevent evaporation.

The seeds, which were left to germinate in the petri dish in the laboratory environment, were followed for 7 days. In line with the recommendations of Wang et al., (2009) and Kusvuran (2015), it was accepted that the seed germinated after the rootlets appeared. Then, the rooting seeds for each

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petri dish were placed in a paper bag and dried in an oven at 60°C for 48 hours, weighed with precision scales, and their dry weights were measured and the findings were noted. The germinated seeds in each petri dish were converted to percentages as suggested by Atak et al. (2016). Analysis of variance and Tukey multiple comparison test were applied to the obtained data with the help of JMP 16 statistical package program.

3. RESULTS AND DISCUSSIONS

3.1 Seedling dry weight

As a result of the analysis of variance on the data obtained from the trials, the effect of the applied essential oil component type and essential oil component dose on the dry weight of barley seedlings was statistically significant, while the effect of "essential oil component type x dose" interactions was insignificant.

The lowest average values of seedling dry weight for allelochemical doses were obtained from the 2 µL dose with 0.09371 g, while the highest 0.19833 g was obtained from the control application. All application doses were in different groups than the control, and the seedlings dry weight decreased in parallel with the dose increase (Table 1).

Table 1. Effects of essential oil component doses on seedling dry weight

Dose	Seedling dry weight (g)
Control (0)	0.19833 a
0.8	0.10829 b
1.2	0.10817 b
0.4	0.09724 c
1.6	0.09717 c
2	0.09371 d*
LSD	0.001679

*: The difference between the means indicated with the same letter is not statistically significant.

When we look at the effects of essential oil component type on the seedling dry weight of barley; Thyme essential oil (0.18975 g) was in the same group as the control (0.19800 g), alpha terpinene (0.17125 g) was different from the control but in the same group with thyme essential oil (EO), thymol, carvacrol and geraniol were in different groups. Geraniol (0.00245 g), carvacrol (0.01790 g) and thymol (0.02590 g) had the most negative effects on seedling dry weight, respectively (Table 2). As a result of the analysis of variance applied to the data, the effect of the interaction of essential oil component type, essential oil component dose and "essential oil component type x dose" on the germination rate of barley seeds was statistically significant.

When we look at the effects of essential oil component type on the germination rate of barley barley seed; thyme essential oil (86.78%) was in the same group as the control (90.00%), while alpha terpinene, thymol, carvacrol and geraniol were in different groups. Geraniol (6.82%), carvacrol (14.08%) and thymol (17.09%) had the most negative effects on germination rate, respectively (Table 3).

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Table 2. Effect of essential oil component type on seedling dry weight

Allelochemicals	**Seedling dry weight (g)**
Control	0.19800 a
Thyme EO	0.18975 ab
Alpha terpinene	0.17125 b
Thymol	0.02590 c
Carvacrol	0.01790 cd
Geraniol	0.00245 d*
LSD	0.02259

*: The difference between the means indicated with the same letter is not statistically significant.

Table 3. The effect of allelochemicals on the germination rate of barley seeds

Allelochemicals	Germination rate (%)
Control	90.00 a
Thyme EO	86.78 a
Alpha terpinene	75.87 b
Thymol	17.09 c
Carvacrol	14.08 cd
Geraniol	6.82 d*
LSD	7.2851

*: The difference between the means indicated with the same letter is not statistically significant.

According to the allelochemical application doses, the average germination rate values were obtained with the lowest 43.43% from the 2 µL dose, while the highest 90% were obtained from the control application. All application doses were in different groups than the control, and the germination rate decreased in parallel with the dose increase (Table 4).

Table 4. Effect of essential oil doses on barley seed germination rate

Dose	Germination rate (%)
Control (0)	90.00 a
0.8	53.44 b
1.2	50.83 c
0.4	48.59 d
1.6	45.90 e
2	43.43 f*
LSD	0.07406

*: The difference between the means indicated with the same letter is not statistically significant.

When we look at the interaction table, all doses of thyme essential oil and low doses of alpha terpinene component (0.4, 0.8 and 1.2 µL) are in the same group as the control, while high doses of alpha terpinene (1.6 and 2.0 µL) and other essential oil All doses of the components (thymol, carvacrol and geraniol) were in different groups from the control (Table 5). From this, it was observed that no dose of thyme essential oil and low doses of alpha terpinene component (0.4, 0.8 and 1.2 µL) did not have any allelopathic effect on the germination of barley seeds. It appears that high doses of alpha terpinene (1.6 and 2.0 µL) and all doses of other essential oil components

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(thymol, carvacrol and geraniol) have an allelopathic effect on the germination of barley seeds. Looking at the data in the table, it can be concluded that the allelopathic potential of thymol, geraniol and carvacrol, which are seen to cause the greatest decrease in germination rate, is higher than alpha terpinene.

Table 5. Effect of "essential oil component type X dose" interactions on barley seed germination rate

Allelochemical	x	Dose	Germination rate (%)
Thyme EO	x	2	90.00 a
Alpha Terpinene	x	1.2	90.00 a
Thyme	x	1.6	90.00 a
Thyme	x	0.4	90.00 a
Control	x	0.4	90.00 a
Control	x	0.8	90.00 a
Control	x	1.2	90.00 a
Control	x	1.6	90.00 a
Control	x	2	90.00 a
Alpha Terpinene	x	0.4	90.00 a
Alpha Terpinene	x	0.8	83.54 ab
Thyme	x	1.2	83.54 ab
Thyme	x	0.8	83.54 ab
Alpha Terpinene	x	1.6	80.34 bc
Alpha Terpinene	x	2	67.42 c
Thymol	x	0.4	54.87 d
Thymol	x	0.8	37.13 de
Carvacrol	x	0.8	25.73 ef
Carvacrol	x	0.4	19.64 ef
Carvacrol	x	2	14.26 ef
Geraniol	x	1.6	14.26 ef
Carvacrol	x	1.6	11.12 ef
Carvacrol	x	1.2	11.12 ef
Thymol	x	1.2	11.12 ef
Geraniol	x	0.8	11.12 ef
Geraniol	x	1.2	5.74 f
Geraniol	x	2	5.74 f
Geraniol	x	0.4	5.74 f
Thymol	x	2	5.74 f
Thymol	x	1.6	5.74 f*
LSD			16.2899

*: The difference between the means indicated with the same letter is not statistically significant.

According to the results of Qasem and Hassan's (2003) study, *Alhagi maurorum*, *Capparis spinosa*, *Citrullus colocynthis*, *Lavandula officinalis* [*L. angustifolia*], *Origanum syriacum*, *Rhus coriaria*, *Ricinus communis*, *Rosmarinus officinalis*, and *Teucrium polium* plants on *Malva sylvestris* and *Portulaca oleracea* showed allelopathic features. The results are in harmony with our study in terms of preventing germination and seedling formation and increasing the effect as the dose increases. Gulsoy et al. (2008) determined that the germination rate of larch seeds decreased in parallel with the concentration increases of essential oils obtained from juniper and thyme. Üremis et al. (2009) reported that thyme essential oil has high inhibitory effects on the germination of wild oat seeds.

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Zahed et al. (2010) found that *Schinus molle* L. oils applied on wheat seeds reduced the germination rate. Said et al. (2016) revealed that the component of *Thymus capitatus* essential oil has an allelopathic effect on seed germination of *Lactuca sativa* L. Essential oils of *Origanum vulgare* L., *Lavandula angustifolia* L. and *Rosmarinus officinalis* L. plants of Yazlık and Üremiş (2017); determined allelopathic effects on the development of tomato, peanut and corn plants in the pre-emergence period except rosemary application. In this regard, Özen et al. (2017) has a study in which they revealed the effects of the use of essential oils in the fight against weeds. Coskun et al. (2018) reported that carvacrol, thymol and geraniol had negative effects on the germination and seedling dry weight of spelt wheat seeds at 0.5 μL cm-2 level, but thymol had no effect. Efil and Üremiş (2019) used essential oils obtained from thyme (*Origanum syriacum*) and marjoram (*Origanum majorana*). These oils are *Amaranthus retroflexus* L., *Portulaca oleracea* L., *Physalis angulata* L., *Echinochloa colonum* (L.) Link., and *Solanum nigrum* L. seeds showed a negative allelopathic effect by inhibiting germination and plant growth by 50%. Also, Zheljazkov et al. (2021) determined that essential oils of *Lavandula angustifolia*, *Hyssopus officinalis*, *Thymus vulgaris*, *Levisticum officinale*, *Chrysanthemum balsamita*, *Cuminum cyminum* showed allelopathic effects suppressing seed germination and seedling growth in barley and wheat. When the results of these studies were examined, it was seen that in different studies using different plant species containing citronellol, geraniol, carvacrol, thymol, and alpha terpinene, and essential oils of thyme similar results were reported in terms of the allelopathic effects of essential oils on seedling growth and germination.

4. CONCLUSIONS

As a result; Rose (*Rosa damescana*) essential oil component (geraniol) and thyme (*Thymus vulgare*) essential oil components carvacrol, thymol and alpha terpinene have an allelopathic effect on seedling growth and germination of barley seed, and this effect increases as the dose increases. It is thought that these essential oil components can be used as bioherbicides, together with similar studies on other cultivated plants and weeds and examining their effects on the ecosystem.

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