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ORIGINAL PAPER

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ANTIFUNGAL EFFECT OF ESSENTIAL OIL AND DIFFERENT EXTRACTS OBTAINED FROM *Nepeta meyeri* ON *Botrytis cinerea*

Sinem Karakuş[1] ✉, Ökkeş Atici[2], Cafer Köse[3], Deniz Tiryaki[2]

[1] Çölemerik Vocational School, Hakkari University, Hakkari 30000, Turkey

[2] Department of Biology, Faculty of Science, Atatürk University, Erzurum 25240, Turkey

[3] Department of Horticulture, Faculty of Agriculture, Atatürk University, Erzurum 25240, Turkey

ABSTRACT

The study concerns the antifungal effect of the aqueous and methanolic extracts, and the essential oil obtained from the aerial parts of *Nepeta meyeri* Benth. on *Botrytis cinerea* Pers. The fungus has been isolated from the infected plants of common grape Karaerik (*Vitis vinifera* L.) cultivating in vineyards in Üzümlü district, Erzincan (Turkey), and was cultured on potato dextrose agar medium in Petri dishes after the identification by 18S rRNA gene-based PCR assay. The concentrations of extracts from *N. meyeri* in Petri dishes were 2%, 5% and 10% (w/v) for aqueous extract (AE); 500, 1000 and 1500 ppm (v/v) for methanolic extract (ME), and 0.6, 0.8 and 1 µL for essential oil (EO). After the treatments, mycelial growth, spore germination, and germ tube elongation were determined. Sterile distilled water at the same ratios was used for the control treatment. Thirty-six different compounds were identified in the EO of *N. meyeri* by GC/MS. The highest antifungal activity has been registered for EO of *N. meyeri*. The inhibition rates in 1 µL/Petri of the EO were 80.72%, 18%, 38.15% on mycelial growth, spore germination and germ tube elongation, respectively. However, AE and ME of *N. meyeri* showed diverse effects on the studied parameters of *B. cinerea*. It is suggested that the favourable concentration of EO from *N. meyeri* can contribute to the prevention of *B. cinerea* infection (grey mould) which causes disease in vineyards.

Key words: *Nepeta meyeri*, *Botrytis cinerea*, grey mould, vine

INTRODUCTION

Considering the increasing world population and diversity in the product range, the studies to prevent product losses due to diseases have been gaining momentum. Along with the development of modern agriculture, global food production has increased substantially. On the other hand, many biotic factors such as insects, fungi, bacteria, viruses, and weeds cause significant losses in agricultural production [Singh et al. 2006]. Nowadays fungicide and pesticide-purpose synthetic chemicals are continuously produced and used extensively in agricultural areas against these factors that cause diseases. However, the widespread and intensive use of such synthetic chemicals in modern agriculture has become a major problem in terms of environmental pollution and human health [Vyvyan 2002]. All these adversities have led researchers to different approaches including biological control [Weston and Duke 2003]. For example, most studies have focused on secondary metabolites synthesized by plants [Moret et al. 2003]. Such compounds are easy to biodegrade in nature and are more reliable for the environment and human health compared to their synthetics [Weston and Duke 2003]. Since the majority of plant secondary compounds are very potent antioxidant

✉ sinemkarakus@hakkari.edu.tr

compounds, they have been used in the natural food and pharmaceutical industry for many years. In addition, using these compounds as natural herbicide, pesticide, and fungicide is gradually increasing [Kordali et al. 2013]. In Morocco, for example, the essential oils of seven plants of *Labiatae* family have been evaluated in terms of their antifungal activity against *Botrytis cinerea*. Among these, *Origanum compactum* and *Thymus glandulosus* have been determined to significantly inhibit its mycelial growth [Bouchra et al. 2003]. Another study pointed out that the methanolic extract from *Satureja icarica* P.H. Davis in *Lamiaceae* family exhibited a powerful antifungal effect against 14 plant pathogens including *B. cinerea* and *Fusarium oxysporum* [Dulger and Hacıoglu 2008].

The grey mold (*Botrytis cinerea* Pers.) is a pathogen which is difficult to fight and causes significant losses in many crops, especially in greenhouse conditions in the world. Because temperature and humidity conditions in greenhouses are very suitable for the development of this pathogen, it can cause severe infections in vegetables and fruits such as peppers, strawberries, tomatoes, cucumbers, lettuce, and eggplants [Delen et al. 1984]. This pathogen may also pose an important problem in open agricultural areas depending on the seasons. For example, the loss caused by this pathogen in grapevine (*Vitis vinifera* L.), an important agricultural crop, has been known for many years. Despite intense chemical struggle against this pathogen, the yield in grapevine that was infected by this pathogen can be decreased significantly by 70–90% [Delen et al. 1984]. More effective approaches that do not cause troubles on human health and the environment in controlling this pathogen are gaining importance every day. This is because the pathogen is becoming increasingly resistant to the synthetic chemicals used, and sometimes producers are helpless in this fight.

Nepeta species (family *Lamiaceae*) are characterized by their high content of nepetalactone, a kind of essential oil compound. *Nepeta* genus contains about 280 species spreading to Central and Southern Europe, South-West, Central and South Asia [Mojaba et al. 2009]. It has been determined that there are 44 taxa including 22 endemic species belonging to this genus in Turkey. Endemic and non-endemic species are generally found in the Eastern Anatolia and Taurus Mountains [Dirmenci 2005]. *Nepeta* species show great differences in the amount of nepetalactone. The nepetalactone ratio in some species of this genus reaches 80% of the total essential oil [Bisht et al. 2012]. In addition, different *Nepeta* oils such as (*Z*) – sabinene hydrate acetate and caryophyllene oxide have been also determined in the total essential oil composition. *Nepeta* species containing high levels of nepetalactone have strong biological activities. For example, species containing 4aα,7α,7aβ-nepetalactone and 4aα,7α,7aα-nepetalactone have antibacterial, antifungal and antiviral properties [Çalmasur et al. 2006, Mothana 2012]. They are also known to be very potent insect repellents and cat and dog attractants [Bisht et al. 2012]. On the other hand, *N. meyeri* (catmint) plants secrete some natural chemical compounds (allelochemicals) into their growing environments and therefore adversely affect the growth and development of many wild plants in their near vicinity [Mutlu and Atici 2009]. Moreover, this plant is able to isolate itself from other plants by creating a large inhibition zone around it (Fig. 1). The laboratory studies supporting this information have shown that aqueous extract from *N. meyeri* leaves has an allelopathic effect on germination, growth and development of certain crop plants (barley, wheat, etc.) [Mutlu and Atici 2009]. In addition, its essential oil was found to inhibit germination and growth of many weeds (*Amaranthus retroflexus* L., *Portulaca olerace* L., *Bromus danthoniae* Trin., *Agropyron cristatum* L., *Lactuca serriola* L., *Bromus tectorum* L., *Bromus intermedius* Guss., *Chenopodium album* L., *Cynodon dactylon* L., *Convolvulus arvensis* L.) [Mutlu et al. 2010]. According to studies, EO of *N. meyeri* contained more than 80% 4aα,7α,7aβ-nepetalactone and 4aα,7α,7aα-nepetalactone, and it was suggested that its biological activity could be due to the compounds [Mutlu et al. 2011].

Although certain biological activities of *Nepeta meyeri* extracts, a potent allelopathic plant, have been identified, there is little information about its antifungal effect on plant pathogenic fungi. In this study, we hypothesized that aqueous and methanolic extracts, and essential oil from *Nepeta meyeri* stem and flower can have an antifungal effect on *Botrytis cinerea* infecting grapevine plants. For this aim, it was evaluated the *in vitro* antifungal effect of *N. meyeri* extracts and essential oil on mycelial growth, spore germination,

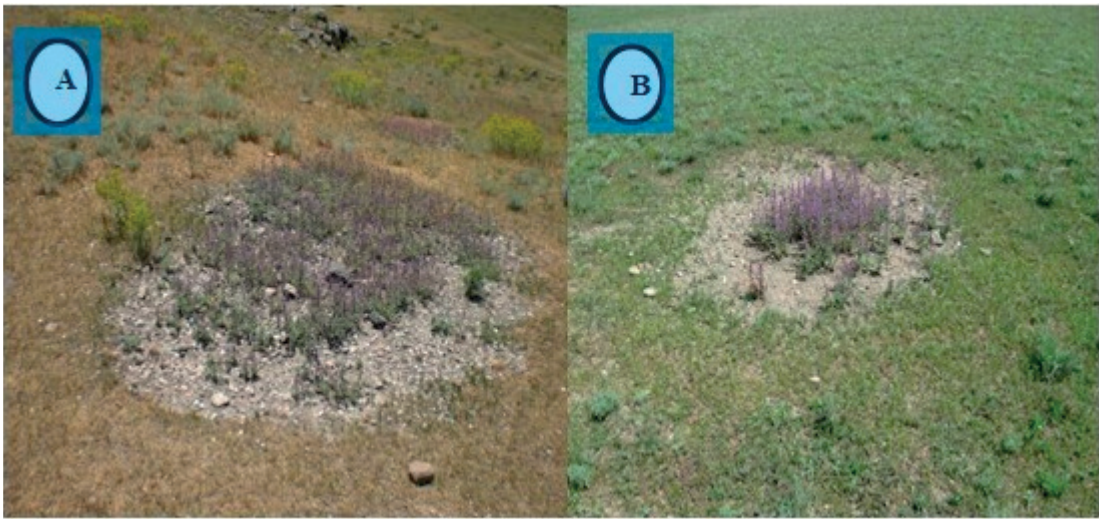

Fig. 1. The inhibition zone of *Nepeta meyeri* (A, B) [Mutlu and Atici 2009, Mutlu et al. 2011]

and germ tube elongation of *B. cinerea* isolated from Erzincan Cimin grape Karaerik (*Vitis vinifera* L.).

MATERIAL AND METHODS

Isolation and identification of *Botrytis cinerea*. *B. cinerea* was isolated from the diseased grapevine plants in the vineyards of Erzincan's Üzümlü district (Turkey). Potato dextrose agar (PDA) medium was used in the isolation and purification of the fungus. Microbial diagnosis of the fungus was realised by RefGen Biotechnology Company, Ankara (Turkey) by using 18S rRNA gene-based PCR assay. *B. cinerea* isolate was stored at +4°C in agar and –20°C in silica gel to use in studies.

Plant material and essential oil extraction. The plant material of *N. meyeri* was collected from the vicinity of Erzurum's Horasan district (Turkey) (GPS coordinates 40°2' 13.2792 2 and 42°11' 5.7984) during its flowering period in June. The collected plants were thoroughly dried in the shade. Their aerial parts were separated and then made into powder using a Waring blender. Essential oils were extracted using hydrodistillation method [Mutlu and Atici 2009, Mutlu et al. 2011]. The analyse of the essential oil was performed using a Thermofinnigan Trace GC/A1300 (E.I. Quadrapole) equipped with a SGE/BPX5 MS capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm). Diluted samples (1/100, v/v, in methylene chloride) of 1.0 µL were injected in the splitless mode. Helium was the carrier gas, at a flow rate of 1 mL/min. Injector temperature were set at 220 and 290°C, respectively. The programme used was 50–150°C at a rate of 3°C/min, held isothermal for 10 min and finally raised to 250°C at 10°C/min.

To use in the experiments, a stock solution of EO extracted from *N. meyeri* was prepared. For this, 10 µL of EO was dissolved in 10 mL of sterile distilled water including 0.1% (v/v) Tween-20. Then, a 600, 800, and 1000 µL volumes from the stock solution (10 µL/10 mL) were added to Petri dishes containing 20 mL PDA. The Petri dishes including sterile distilled water with Tewen-20 (0.1%) was used as controls. The final concentrations for EO in each Petri dishes were 0.6, 0.8 and 1 µL/Petri dish.

Preparation of plant extracts. For aqueous extract (AE), a 20 g of dry plant material which was thoroughly ground was added to 80 mL water and mixed in the shaker at room temperature for 8 h [Schnee et al. 2013]. The mixture was first filtered through a 4-layer cheesecloth and then centrifuged at 3000 rpm. The resulting supernatant was further filtered through Whatman filter paper and then sterilized by Millipore. From this prepared 20% (w/v) stock solution, 2, 5 and 10 mL was added to sterile empty Petri dishes and then poured onto the medium. Since each Petri dish was

adjusted to contain 20 mL of medium, the final concentration was adjusted to be 2%, 5% and 10% (w/v), respectively.

For methanolic extract (ME), 200 g of the ground plant samples were extracted in 1000 mL of absolute methanol for 12 h. This process was repeated 3 times over the same sample and the extracts obtained were combined each time. The methanol was removed from the extract under vacuum at 45°C by means of a rotary evaporator. To remove the lipids in the resulting precipitate, 100 mL of absolute hexane was added in the precipitate and mixed well. The hexane fraction was taken from the medium under the same conditions. This was done twice with the same precipitate and the hexane was evaporated [Satyajit et al. 2006]. One g of ME was dissolved in 100 mL of sterile purified water and then was filtered through Whatman and Milipore filters (0.2 µm), respectively. From this stock solution, 1, 2 and 3 mL was added to Petri dishes and 20 ml sterile PDA medium was poured onto them [Tehranifar et al. 2011]. The final concentrations in the Petri dishes were 500, 1000 and 1500 ppm (v/v).

Determination of antifungal effect on mycelial growth. After the Petri dishes including the concentrations of EO, AE and ME were kept at room temperature for 1 day, 4 mm diameter discs were taken from 7–10 days old fungus cultures grown previously in PDA medium and then were inoculated into the Petri dishes with and without EO, AE and ME. The Petri dishes were sealed with parafilm and allowed to incubate at 25°C for 7 days. The evaluations in control Petri dishes were performed by measuring the mycelium diameters of the fungus on the surface without waiting for the fungi to completely cover the Petri dishes. From the second day of incubation, the Petri dishes were checked daily and the diameters of fungal mycelium were measured and recorded daily. The measurement of the diameter of the mycelium was made by measuring the diameter of the fungus mycelium perpendicular to each other in different directions [Benjılalı et al. 1984]. The experiments were conducted with five replications and control samples.

Percentage of inhibition rates of plant extracts on the fungal growth over the control were calculated according to the formula [Deans and Svoboda 1990]:

$$E = [(K - M)] / K] \times 100$$

where: E – inhibition (%), K – mycelium diameter in the control Petri dish (mm), M – mycelium diameter in the experimental Petri dish (mm).

Determination of antifungal effects on spore germination and germ tube elongation. To collect the spores from 7–10 days old fungal cultures growing in PDA medium, 5 mL sterilized pure water was added in the culture and then the surface of the culture was scraped with a sterile glass rod. The suspension was passed through 3-layer cheesecloth and the mycelial particles were removed spine concentration was adjusted in 1×10^5 conidial/mL. The prepared spores (10 µL) were added to the EO, ME and AE. Samples were allowed to incubate at 25°C under dark conditions. After 24 h, evaluations were carried out under a light microscope through the determination of germinated and ungerminated spores from 100 spores counted by a micrometre. Spores that generate germ tube up to 1/2 of their own length were accepted as germinated. In the evaluation of the length of germ tube, 40 × magnifying lens used and the germ tube elongation (µm) of 30 spores was measured and their averages were calculated. Spores seen in 3 different areas by 40 × magnifying lens were counted and the total number of spores and the number of germinated spores were compared to determine the germination rate (%)

$$\text{Germination (\%)} = [a/(a + b)] \times 100$$

where: a – number of germinated spores; b – number of ungerminated spores.

Statistical analysis. Results, after three independent samples from each application and 2 replicates from each sample, are average of 6 values obtained. For the variables showing normal distribution, one-way analysis of variation (one-way ANOVA) was used for the comparison of the groups. $P < 0.05$ was considered as the level of significance in the analyses. All the analyses were made using SPSS (20.0) software.

RESULTS

The composition of essential oil from *Nepeta meyeri*. The essential oil from *N. meyeri* analysed by GC/MS contained thirty-six kinds essential oil (EO)

Table 1. The essential oil analysis of *N. meyeri*

Compound name	RT	Rate (%)
Methane, thiobis-	7.69	1.63
β-pinen	17.32	3.73
Limonene	23.09	0.13
(z)-β-ocimene	27.1	2.31
1,8-cineole	29.68	3.61
1,3,5,7-cyclooctatetraene	32.39	0.12
2e-hexenal	34.54	0.18
(R)-(+)-3-methylcyclopentanone	36.59	0.05
1-octen-3-Ol	43.79	0.22
1,3-cyclohexadiene, 2-methyl-5-(1-methylethenyl)-	46.4	0.18
β-bourbonene	47.87	1.81
γ-amorphene	52.8	1.1
(E)-caryophyllene	54.25	4.36
Camphor	56.15	0.33
(Z)-α-bisabolene	57.21	0.44
Germacrene D	58.46	7.11
1-isopropenyl-3-propenyl-cyclopent	59.21	0.26
Bicyclogermacrene	59.41	0.48
Benzene acetaldehyde	60.14	0.1
Methyl salicylate	61.4	0.09
cis-calamenene	61.73	0.11
2,6-dimethyl-1,3,5,7-octatetraene,E,E-	62.07	0.14
(E)-anethole	63.02	0.07
Benzenepropanoic acid, methyl ester	64.29	0.1
3-buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-Yl)-, (E)-	66.7	0.12
Caryophyllene oxide	68.21	0.43
(–)-spathulenol	68.74	0.61
4a-α,7-α,7a-β-nepetalactone	70.17	27.87
4a-α,7- β,7a-α-nepetalactone	71.34	28.26
4a-α,7-α,7a-α-nepetalactone	71.68	1.42
1,2-Benzenedicarboxylic acid, dimethyl ester	74.21	0.02
Dıhydroısonepetalactone	74.66	0.02
2-cyclohexen-1-one, 3-methyl-6-(1-methylethyl)-	74.85	0.04
Phenol, 2-(1,1-dimethylethyl)-5-methyl-	77.24	0.19
3-octanone	37.21	0.12

components (Tab. 1). Main components were 4aα, 7α, 7aα-nepetalactone (1.42%), 4aα, 7β, 7aα-nepetalactone (28.26%), and 4aα, 7α, 7aβ-nepetalactone (27.87%). The ratio of nepetalactones in EO was 57.55%. Other high ratios belonged to germacrene (7.11%), caryophyllene (4.36%), and β-pinene 3.73%, respectively.

Findings from EO application. When the effects of EO doses on mycelial growth of *B. cinerea* are evaluated, its inhibition effect on the mycelial growth was induced with the increasing dose rate (Fig. 2). The determined inhibition rates at doses of 0.6, 0.8 and 1 µL/Petri dishes of EO were 25.3%, 49.39% and 80.72% respectively (Tab. 2). In addition, the EO applications negatively affected the morphology of germ tube and spore germination rate. For instance, it was determined an abnormal thickening in germ tubes of the spores exposed to the EO doses while the spores in control showed usually a normal germination (Figs 3 and 4). These spores showing abnormal germination were considered as the ungerminated samples. When the findings of spore germination rate and germ tube elongation were evaluated, the two parameters were inhibited significantly by the EO doses (Tab. 3). For example, the spores treated by 1 µL EO germinated by 18% while the control group by 100% (Tab. 3). In addition, germ tube elongations were decreased by the EO applications in parallel with dose rate. For instance, germ tube elongation average was 134.26 µm in the control group while 38.15 µm at 1 µL of EO (Tab. 3).

Findings from aqueous extract application. The aqueous extracts (AE; 2, 5 and 10%) showed a statistically significant effect on inhibition of mycelial growth but their antifungal effect did not exhibit an increase in parallel with the dose rate of AE (Fig. 5). The highest antifungal effect for this pathogen was achieved at a dose of 10 mL (10% AE) with a rate of 23.52% (Tab. 4). On the other hand, spore germination and germ tube elongation of *B. cinerea* were also negatively affected by AE treatments (Tab. 5). For instance, the spores of *B. cinerea* were germinated 100% in the control group, but the germination

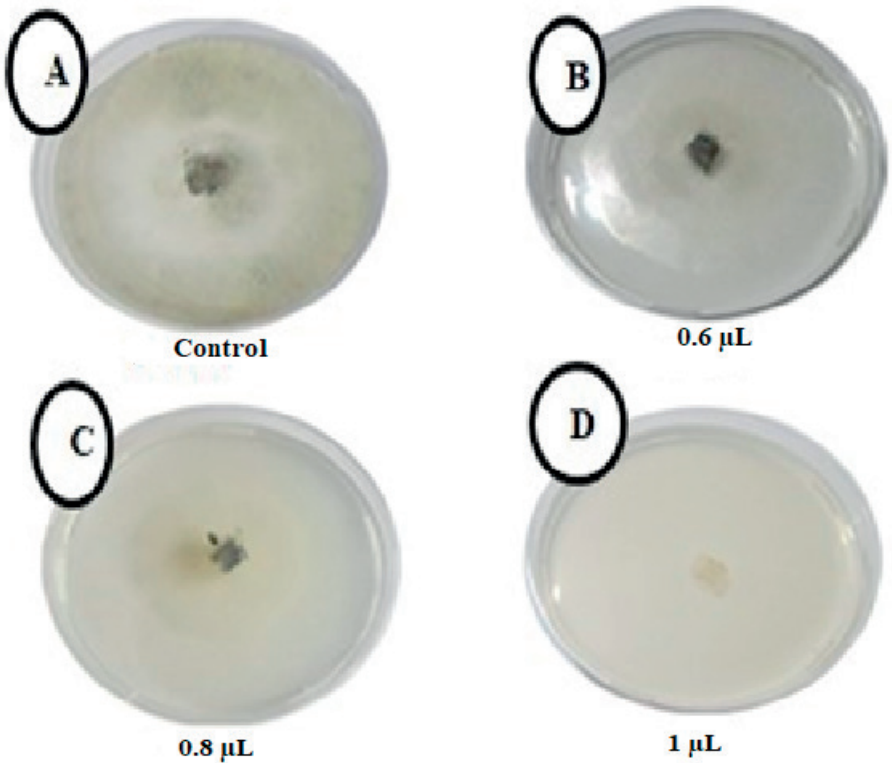

Fig. 2. Control (A) and effects of essential oil on the mycelial growth of *B. cinerea* (B, C, D)

Table 2. Effects of essential oil (EO) doses on mycelium inhibition rate (MIR)

EO doses (µL/Petri dish)	MIR (%)
0.6	25.3 ±0.55[a]
0.8	49.39 ±0.81[b]
1	80.72 ±1.2[c]

The difference between the group averages with different letters is statistically significant, $p < 0.05$. Data represent the mean ±SD of groups

Table 3. Effects of EO doses (µL/Petri dish) on the germination and the germ tube elongation of spore

EO doses (µL/Petri dish)	Spore germination (%)	Germ tube elongation (µm)
Control	100 ±0.0 [d]	134.26 ±1.5[d]
0.6	66 ±1.58[c]	81.04 ±1.06[c]
0.8	55 ±1.37[b]	59.67 ±0.89[b]
1	18 ±0.7[a]	38.15 ±0.41[a]

The difference between the group averages with different letters is statistically significant, $p < 0.05$. Data represent the mean ±SD of groups

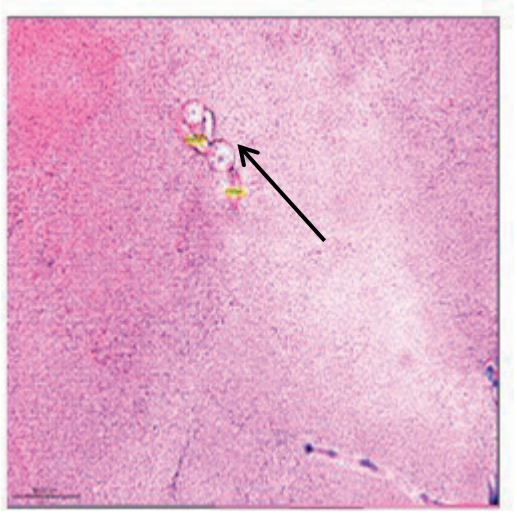

Fig. 3. Abnormally germinated spore

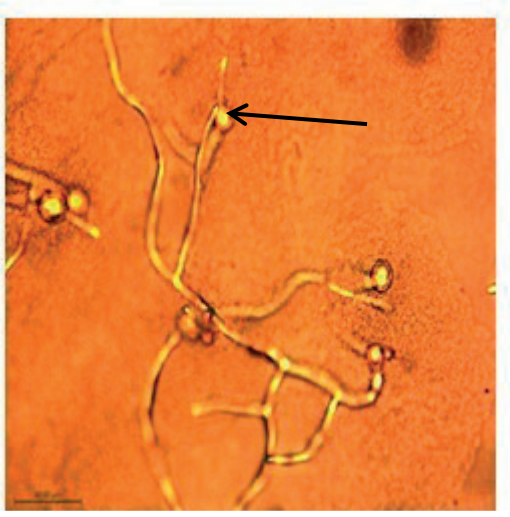

Fig. 4. Normally germinated spore

rates in AE treatments were 79% at 2 mL, 88% at 5 mL, and 72% at 10 mL (Tab. 5). The tube elongation was 122.12 µm in the control group, but 113.4 µm at 2 mL, 118.2 µm at 5 mL, and 110.8 µm at 10 mL in AE treatments (Tab. 5).

Findings from methanolic extract application. None of the methanolic extracts (ME) had a significant effect on mycelial growth of *B. cinerea* (Fig. 6). Since no inhibition was detected, the mycelium inhibition rate was not presented in a figure. In parallel with this finding, an expressive antifungal effect in ME treatments could be not determined on spore germination and germ tube elongation (Tab. 6). For example, spores in ME application groups germinated almost at the control group rate (Tab. 6). The average of germ tube elongation was also

Fig. 5. Effects of 2% (A), 5% (B) and 10% (C) volume of aqueous extract on mycelial growth of *B. cinerea*

Table 4. Effects of aqueous extract doses on mycelium inhibition rate (MIR)

Aqueous extract (%)	MIR (%)
2	15.29 ±0.16^{b}
5	10.58 ±0.07^{a}
10	23.52 ±0.31^{c}

The difference between the group averages with different letters is statistically significant, $p < 0.05$. Data represent the mean ±SD of groups

Table 5. Effect of aqueous extract doses (%) on the germination and the germ tube elongation of spore

Aqueous extract doses (%)	Spore germination (%)	Germ tube elongation (µm)
Control	100 ±0.0^{d}	122.12 ±1.53^{c}
2	79 ±1.09^{c}	113.4 ±0.89^{a}
5	88 ±1.09^{b}	118.2 ±0.35^{b}
10	72 ±1.3^{a}	110.8 ±0.98^{a}

The difference between the group averages with different letters is statistically significant, $p < 0.05$. Data represent the mean ±SD of groups

Table 6. The effect of methanolic extract doses (ppm) on the germination and the germ tube elongation of spore

Methanolic extract doses (ppm)	Spore germination (%)	Germ tube elongation (µm)
Control	100 ±0.0	110.3 ±0.99
500	100 ±0.0	110.0 ±1.3
1000	100 ±0.0	109.0 ±1.31
1500	100 ±0.0	110.8 ±1.02

The difference between the group averages with different letters is statistically significant, $p < 0.05$. Data represent the mean ±SD of groups

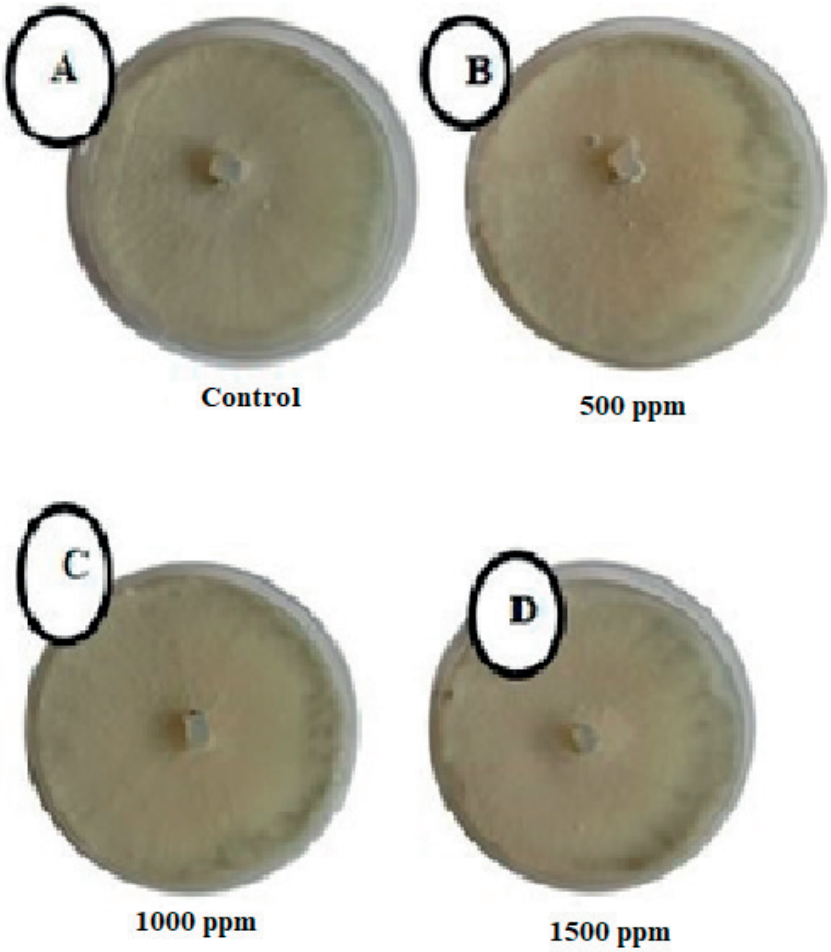

Fig. 6. Control (A) and 500 ppm (B), 1000 ppm (B), 1500 ppm (D) volume of methanolic extract on mycelial growth

110.3 µm in the control group whereas 110.0 µm at 1 mL, 109.0 µm at 2 mL, and 110.2 µm at 3 mL of ME treatments (Tab. 6).

DISCUSSION

The disease caused by *Botrytis cinerea* leads to great losses in both grape yield and quality in vineyards and then under storage conditions. For this reason, a large amount of synthetic fungicide has been used every year in vineyards and grape storages. Today, the struggle by synthetic fungicides against this fungus is often inadequate and generates complicated problems on both environment and human health. Allelopathic approaches to control *B. cinerea* infection, however, may provide a great benefit in vineyard productivity and on the problems. Because some *Nepeta* species have been reported to have significant effects on fungi [Kan 2005], bacteria [Rigano et al. 2011], virus, and tick species [Bourrel et al. 1993, Çalmasur et al. 2006, Rigano et al. 2011] as well as powerful antioxidant properties [Dapkevicius et al. 1999]. In addition, *N. meyeri* does not allow the development of other wild plant species in its natural environment [Mutlu and Atici 2009] and has a strong herbicidal effect on the weeds that can inhibit growth and development of certain cultivated plants [Mutlu and Atici 2009, Mutlu et al. 2010, 2011]. Many researchers reported the high levels of nepetalactone compounds in *N. meyeri* [Sefidkon and Shaabani 2004, Mutlu et al. 2011, Kordali et al. 2013] and these are among the most important causes of the allelopathic effect of the plant [Mothana 2012]. In parallel with the findings, in our study, the EO of *N. meyeri* containing about 55% nepetalactone was able to inhibit the development of *Botrytis cinerea* even at a very low concentration (1 µL/Petri dish) – Table 1 – compared to similar studies in the literature. In such a mixture that is effective at very low concentrations, the determined effect can be attributed to the compounds with the greatest amount in its content. Therefore, we think that the antifungal effect of *N. meyeri* can be caused by nepetalactones in EO. However, in order to determine whether nepetalactones are responsible for these effects, this compound should be obtained and tested purely. Some studies have also propounded that the allelopathic effect can be originated by several compounds, not by a single compound, contained in essential oils [Sefidkon and Shaabani 2004, Kordali et al. 2013]. Moreover, the EO concentrations (0.6, 0.8 and 1 µL/Petri dish) inhibited mycelial growth of *B. cinerea* (Tabs 2 and 3). This effect on the mycelial growth increased in parallel with the increase in EO doses and even 100% inhibition was obtained from 1.2 µL/Petri dish of EO (not presented in the study). As compared similar studies, the concentrations of EO from *N. meyeri* are much lower than the concentrations of EOs obtained from other plants. This finding shows that EO obtained from *N. meyeri* has a strong inhibition on the mycelial growth of *B. cinerea*. Aminifard and Mohammadi [2013], for example, used 400 and 600 µL/Petri concentrations of black cumin, fennel and peppermint oils to achieve similar inhibitions in the growth of *B. cinerea*. We also determined that EO was effective on the inhibition of spore germination of *B. cinera*. The control group spores in EO treatments germinated by 100%, while germinated 66% at 0.6 µL, 55% at 0.8 µL and 18% at 1 µL/Petri dish. At 1.2 µL dose of EO, none of the 100 spores germinated. This effect of EO treatments had also an impact the germ tube elongation of the spores (Tab. 3). In the study, germ tube elongation was decreased significantly and the most powerful impact was obtained from 1 µL of EO. These findings indicate that EO obtained from *N. meyeri* significantly mitigates the disease by inhibiting mycelial growth, spore germination, and germ tube elongation of *B. cinerea*. Also, this EO was able to perform the high antifungal effect at a very low concentration like 1 µL/Petri dish. Kordali et al. [2013] who evaluated the antifungal effect of EO from *N. meyeri* on the growth of 16 phytopathogenic fungi, found that the EO inhibited the development of certain plant pathogenic fungi at different concentrations (0.25, 0.50 and 1.0 mg/mL). Our study additionally revealed EO of *N. meyeri* to inhibit both micelle and spore development of *B. cinerea*. Studies conducted with other species of *Nepeta* have shown that species other than *N. meyeri* also have antifungal activity. For example, containing 4aα, 7α, 7aβ-nepetalactone as the main component, the essential oil isolated from *N. rtanjensis* was found to have a strong antifungal activity against all micromycetes examined [Grbıć et

al. 2008, 2011]. Our study also suggest that EO obtained from *N. meyeri* has the forceful potential to be used as biofungicide against *B. cinerea* infection.

This study also investigated the antifungal effect of AE (2, 5 and 10%) obtained from *N. meyeri* on the mycelial growth, spore germination and germ tube elongation of *B. cinerea*. According to our knowledge, there is no study on the antifungal effects of AE of *Nepeta* species. Of the AE, only 10% extract could weakly (23.52%) inhibit the mycelial growth (Fig. 5), compared to control while the other did not exhibit any antifungal effect on the parameters studied of *B. cinerea.* Certain studies have shown that AE has an antifungal effect [Lee et al. 2007], while some have not determined any antifungal effect [Türküsay and Onoğur 1998]. In addition, a research reported that some plant extracts inhibit the development of certain microorganisms while they have no effect on other microorganisms, even promote their development [Singh et al. 1980]. Studies on AE of different plants reported different types of inhibition of fungi development compared to our study. This difference can be attributed to different plant species used. But, previous studies on *N. meyeri* revealed the allelopathic effects of AE obtained from its root and stem on some cultivated plants [Mutlu and Atici 2009]. These results indicate that the effects of the extracts can be different depending on the type of their active ingredient and the target organism. Sometimes the antifungal effect of the extract obtained from a certain plant is lower than the volatile oil obtained from the same plant. This is believed to be due to the amount, stability and effect level of the active ingredient that is contained in the extract. The ME from *N. meyeri* did not carry out a positive or negative effect on the mycelial development, spore germination, and germ tube elongation of *B. cinerea* (Tab. 6). This can be due to the fact that there are no chemical compounds which will enable the antifungal effect in the ME.

In conclusion, the EO obtained from *N. meyeri* could ameliorate the infection caused by *Botrytis cinerea*, isolated from *Vitis vinifera* L., by inhibiting mycelial growth, spore germination, germ tube elongation. Based on the results, it is suggested that the pure use of nepetalactones, which plays the most important role in the effectiveness of the EO, can further increase its lethal effect on fungi including *B. cinerea*.

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