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Effects of Some Plant Essential Oils Against Botrytis cinerea and Tetranychus urticae on Grapevine

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Abstract

The cultivation of grapes in the world in terms of the first-ranked Turkey as well as the appropriate climatic zone, has potential for rich gene and ancient viticulture culture. Grape growers, starting from production until it reaches the consumer is faced with various problems in the process. Plant protection faced in the vineyards of the manufacturers in terms of the most important causes of losses; Downy Mildew (*Plasmopara viticola*), Powdery Mildew (*Uncinula necator*), Gray mold (*Botrytis cinerea*), European Grapevine Moth (*Lobesia botrana*), Spidermite (*Tetranychus* spp.), Vine weevil (*Otiorhynchus* spp*., Megamecus* spp.). The impact of pests and diseases due to changing climate conditions with increased losses, these factors makes it difficult to control. In order to achieve higher efficiency and quality of synthetic chemicals used in viticulture, many benefits they provide, as well as the nature of the effect is known to be negative. In this sense, the effect of plants compounds on diseases and pests is a prominent work area. In this study, 7 different plant essential oil; grape seed (*Vitis vinifera*), thyme (*Thymus* sp.), Rosemary (*Rosmarinus officinalis*), ozone oil (*Olea europaea*), mint (*Menta piperita oleum*), Basil (*Ocimum basilicum*) and Sage (*Salvia* spp.), were examined on *Botrytis cinerea* and *Tetranychus urticae* which are important in terms of viticulture.

Keywords: Essential oil, *Botrytis cinerea*, *Tetrancyhus urticae*, Grapevine

_____Bazı Bitki Uçucu Yağlarının Asmalarda *Botrytis cinerea ve Tetranychus urticae* Üzerine Etkisi_____

Özet

Üzüm yetiştiriciliği açısından Dünya'da ilk sıralarda yer alan Türkiye uygun iklim kuşağında yer almasının yanı sıra zengin asma gen potansiyeline ve eski bir bağcılık kültürüne sahiptir. Üzüm yetiştiricileri, üretimden başlayan ve tüketiciye ulaşana kadar geçen süreçte çeşitli sorunlarla karşı karşıya kalmaktadır. Bitki koruma açısından üreticilerin bağlarda karşılaştığı en önemli kayıp nedenleri; Mildiyö (*Plasmopara viticola*), Külleme (*Uncinula necator*), Kurşuni küf (*Botrytis cinerea*), Salkım güvesi (*Lobesia botrana*), Kırmızı örümcekler (*Tetranychus* spp.), Bağ maymuncukları (*Otiorhynchus* spp*., Megamecus* spp.) olarak sıralamak mümkündür. Değişen iklim koşulları nedeniyle hastalık ve zararlıların etkileri ile kayıplar artarken, bu etmenlerin kontrolünü de zorlaştırmaktadır. Kurşuni küf, bağlarda çiçeklenmeden itibaren hasat sonuna kadar ve hasat sonrasında görülen önemli hastalıklardan bir tanesidir. Sıcaklık ve nem şartlarına bağlı olarak etkisi değişen bir diğer zararlı ise kırmızı örümceklerdir. Asma yapraklarında renk açılmaları, fotosentez miktarının değişmesine bağlı olarak bitkide stres yaratmakta ve yaprakların dökülmesi neticesinde salkımların güneşten olumsuz etkilendiği belirlenmiştir. Daha yüksek verim ve kaliteye ulaşabilmek için bağcılıkta kullanılan sentetik kimyasalların, sağladıkları faydanın yanı sıra doğaya birçok olumsuz etkisinin olduğu bilinmektedir. Bitkilerin bünyelerinde barındırdıkları bileşikler ile bunların hastalık ve zararlılar üzerine olan etkisinin belirlenmesi bu anlamda öne çıkan bir çalışma alanı oluşturmaktadır. Bu çalışmada 7 farklı bitkisel yağ; üzüm çekirdeği (*Vitis vinifera*), Kekik (*Thymus* sp.), Biberiye (*Rosmarinus officinalis*), Ozon (*Olea europaea*), Nane (*Menta piperita oleum*), Fesleğen (*Ocimum basilicum*) ve Adaçayı (*Salvia* spp.)'nın bağcılık açısından önemli olan; Kurşuni küf (*Botrytis cinerea*) ve İki noktalı kırmızı örümcek (*Tetranychus urticae*) üzerine 3 farklı dozdaki etkisi ve kullanım olanakları incelenerek, değerlendirilmiştir.

Anahtar Kelimeler: Bitki Uçucu Yağları, *Botrytis cinerea*, *Tetrancyhus urticae*, Asma

Introduction

Grapevine, which is one of the oldest plant, is spread over large areas of the world. Our country has appropriate climate for grape cultivation within the world. Cultivation has carried out on Turkey in 3,969.379 acres in the area with 3,556.153 tons products (Anonymous 2014).

The necrotrophic ascomycete *Botrytis cinerea* Pers.:Fr. causes gray mold disease on more than 200 plant species. The gray mold fungus leads important losses in yield and quality of numerous crops in temperate climates under humid conditions as well as pre- and postharvest (Williamson et al.,2007; Staats 2005). B. *cinerea* which can be infect different stage of vine from the flowering period, can cause important disease in favorable climate conditions in vineyards in addition downy mildew and powdery mildew diseases (Delen 2001, Roslenbroich and Stuebler 2000). It has been known for many years that this pathogen is a serious problem in vineyard in Turkey (Delen 2001; Köycü et al. 2005) like all over the world (Roslenbroich and Stuebler 2000). Especially close to the grape harvest season gray mold occurs as a problem againts us in Trakya Region (Köycü and Özer 2005).

The two-spotted spider mite, *Tetranychus urticae* Koch (Acarina: Tetranychidae) is the most polyphagus species of spidermites. The mite often feed on the under surface of the leaf and may cause economic losses in over 150 host plants which list include grapevine (Zhang, 2003). Control of this pest several syntetic pesticides which compound pyretroids or organophates are used. Enviromental pollution is negative effect of these chemicals (Kumral et al., 2010). The mite rapidly developt resistance this pesticides after some application. A number of resistance mechanism in T. *urticae* have been recently characterised at the molecular level (Leeuwen et al, 2010). For this reason enviroment friendly apllication methods and materials study are trend of twenty century.

Plants have individual morphologic and biochemical properties such as leaf structure, oil compounds etc. to protect themselves from several disease and pests. Researchers focus on plant essential oil (EOs) potencial effect and usage for alternative pest management step (Isman, 2000). Many EOs and their major terpenoid constituents are neurotoxic to insects and mites and behaviourally active at sublethal concentrations (Isman et al., 2004). At the same time many EOs have antifungal effect for phytopathogen fungi; *Alternaria*, *Sclerotinia* etc. (Tripathi et al. 2008; Zechini et al., 1998).

In this study, 7 different plant essential oil; Grape Seed (*Vitis vinifera*), Thyme (*Thymus* sp.), Rosemary (*Rosmarinus officinalis*), Ozone Oil (*Olea europaea*), Mint (*Menta piperita oleum*), Basil (*Ocimum basilicum*) and Sage (*Salvia* spp.), were examined on two agent. Grape Seed and Ozone Oil usage on *Botrytis cinerea* and *Tetranychus urticae* which are important in terms of viticulture are first report.

Materials and Methods

1. Biological Material

1.1. Spider mite

T. urticae originated from a research colony maintained on İtalia grapes without any pesticide exposure from greenhouse. The colony was maintained in laboratory at 26±1°C, 60–65% R.H. and a 16:8 h L:D photoperiod.

1.2. *Pathogen*

B. cinerea, was isolated from decayed Italia berries and maintained on potato dextrose agar (PDA). Fresh cultures of the fungus were prepared by subculture of mycelia onto new PDA plate and the incubated at 22 °C for 3-5 days.

1.3. Essential Oil

Commercial preparation of Grape seed (*Vitis vinifera*), Thyme (*Thymus* sp.), Rosemary (*Rosmarinus officinalis*), Ozone oil (*Olea europaea*), Mint (*Menta piperita oleum*), Basil (*Ocimum basilicum*) and Sage (*Salvia* spp.) oils were used in this study.

The effect of four concentrations of oil (1%, 3%, 6% and 12%) were studied in acaricide effects of essential oil. Extracts of oil compounds was prepared with distilled water and Triton X.100 (Sigma T8787).

2. Bioassay

2.1. Acaricidal effects of essential oils

Leaf-Dipping Method

İtalia grape leaf discs (3cm) were prepared and dipping them into extract solutions. Dry leaf discs and 10 adult mites were placed on moistened filter paper in the petri dishes (9 cm) after 30 minutes (Barış ve Çobanoğlu 2009, Erdoğan et all., 2012).

Leaf-Spraying Method

İtalia grape leaf discs and adult mites were placed into petri dishes on moistened filter paper. Different concentrations of extract sprayed (17–20 µL/cm2) on them by using a hand sprayer (Erdoğan et all., 2012) .

Ten replications were made for each concentrations for both method. Untratened control was used and they were kept under controlled conditions at 26±1°C, 60–65% R.H. and a 16:8 h L:D photoperiod in laboratory. The results

were assayed after 1, 3, and 6 days by counting the number of living adults.

2.2. Antifungal effects of essential oils

Antifungal effect of essential oils were tested for assessing its contact and volatile phase effect towards the B. *cinerea*. For the determination of contact effects, essential oils were prepared in solutions (0,01% Tween 20, 1% Ethanol) and different concentrations (0,5-1,5-2,5-5 µl/ml) were poured into autoclaved and cooled (45°C) PDA. Medium PDA with essential oil was immediately poured into sterile 70 mm Petri dishes (nearly 15 ml/plate). Then the fungal discs of 5 mm diameter from the young cultures were placed in the middle of petri dishes and incubated at 22°C. When the fungal vegetative growth was cover in control petri dishes, colony diameters of fungus with essential oils were measured and inhibition percentage of mycelial growth (MGI) by using formula MGI (%) = [(dc – dt) / dc] x 100 (dc: mycelial growth diameter in control, dt: mycelial growth diameter in treatment).

In volatile effects of essential oils, PDA was pour into petri dishes (70 mm). Steril filter papers impregnated with different concentration of essential oil (50-100-150-200 µl/petri dishes) were placed in petri dishes lid. Active growing fungal discs (5 days) were put in the middle of PDA and petri dishes were sealed with parafilm. After incubation at 22° C, colony diameters were measured when fungi covered petri in control.

3. Data analysis

For acaricide effect all mortalities were corrected against control (Abbott, 1925) and the data were analysed with ANOVA followed by Duncan test at $p < 0.05$ using JMP 7.0 (SAS, 2007). Mortality rate was calculated as;

$$\text{Mortality} = \frac{\text{After treatment the number of died mites}}{\text{Before treatment the number of mites}} \times 100$$

In antifungal effect, values obtained were analsed using JMP statistical program and differences between the datas were determined by Duncan test at $p \leq 0.05$.

Results

Essential oils, secondary metabolites derived from various organs of aromatic plants belonging mostly to a few families (e.g., Asteraceae, Apiaceae, Lamiaceae, Myrtaceae, Rutaceae) are important source of natural products showing pesticide activity (Marcic 2012). In several study basil, mint, peppermint, rosemary, oregano, thyme, and other plants have shown a significant acaricidal activity (Choi et al. 2004; Miresmailli et al. 2006; Han et al. 2010, Marcic 2012). In this research we found Thyme (85-88%), Mint (71-74%) and Rosemary (61-67%) were most effective oils on *T. urticae* similar results to previous studies. The acaricidal activity of seven plant essential oils experiments were indicate in Figure 1 and Figure 2. Positive corelations between insect mortality rate and oil concentrations; The increased concentrations led to increased adult mortality according to the istatistical analyzed. Leaf dipping method and Leaf spraying method were showed similar results on *T. urticae.* In addition to basil have minimum effect 20-21% from unlike other plant essential oils.

Phytotoxicity was observed in grape leaves due to Rosemary oil applied among the seven EOs. Grape seed oil (24-27%) and Ozon oil (20-22%) were tested firstly on *T. urticae* and showed promising results.

The antifungal effect of seven essential oils to *Botrytis cinerea* applied three different dosage in *in vitro*, was found to be at various levels. Percentage of inhibited redial growth calculated from measured colony diameter was given in Table 1 and Table 2.

Thyme, Minth and Rosemary oils were found more effective to *Botrytis cinerea*. As well as contact and votalite effects on mycelial growth were reached up to a hundered percent. Sage, Grape seed, Ozone and Basil oils were showed varying effect. Thyme oil which was the most effective in our study, has been studied by many researchers (Vitoratos et al., 2013; Koçak and Boyraz, 2006). It seems fungicidal even at the lowest dosage based on previous studies. This study antifungal data for thyme oil were seems to support also onother studies (Çakır ve Yeğen, 1991; Walter et al., 2001).

MGI (%) in contact effect of grape seed oil were found 19,7 (%), 25 (%), 22,1 (%) and 20,7 (%) from the lowest dosage respectively. That was found 2,1 (%), 17,1 (%),14,2% and 7,1% in votalite effect. In two method, effectiveness of oil was more effective in the second and third dosage. However, a decrease was observed at the highest dosage. In ozone oil similar effect was seen.

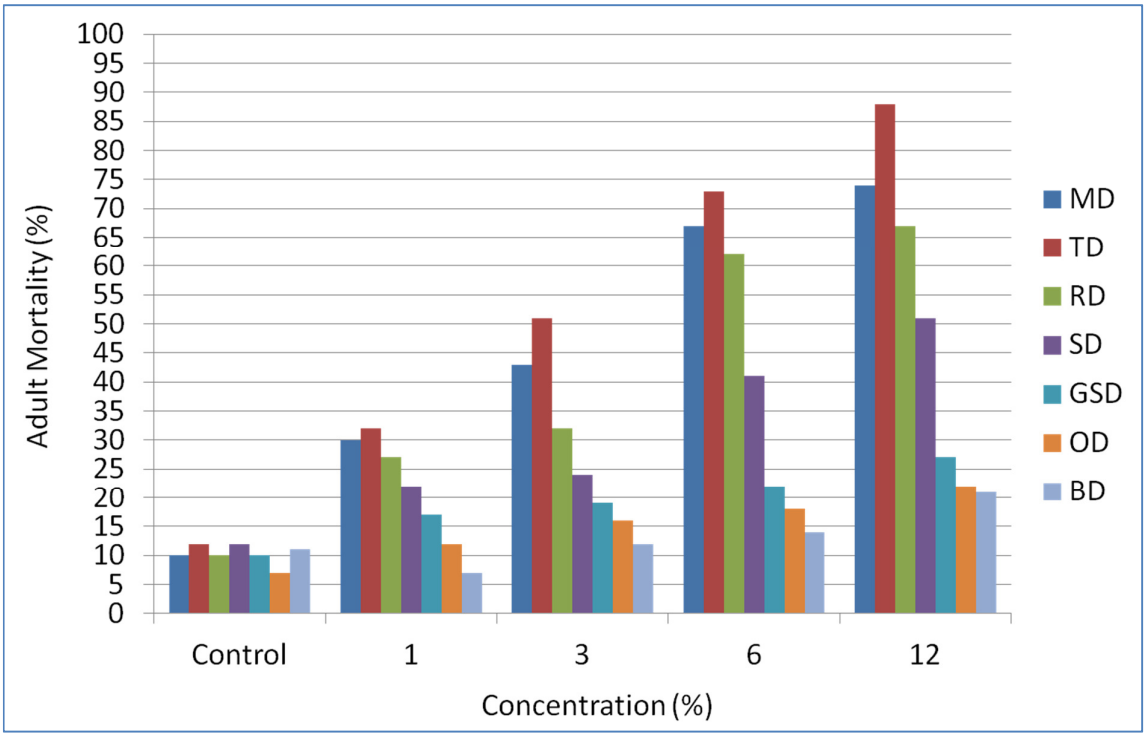

Figure 1. The effects of different concentrations of essential oils on percent mortality of *Tetranychus urticae* adults in leaf dipping method.

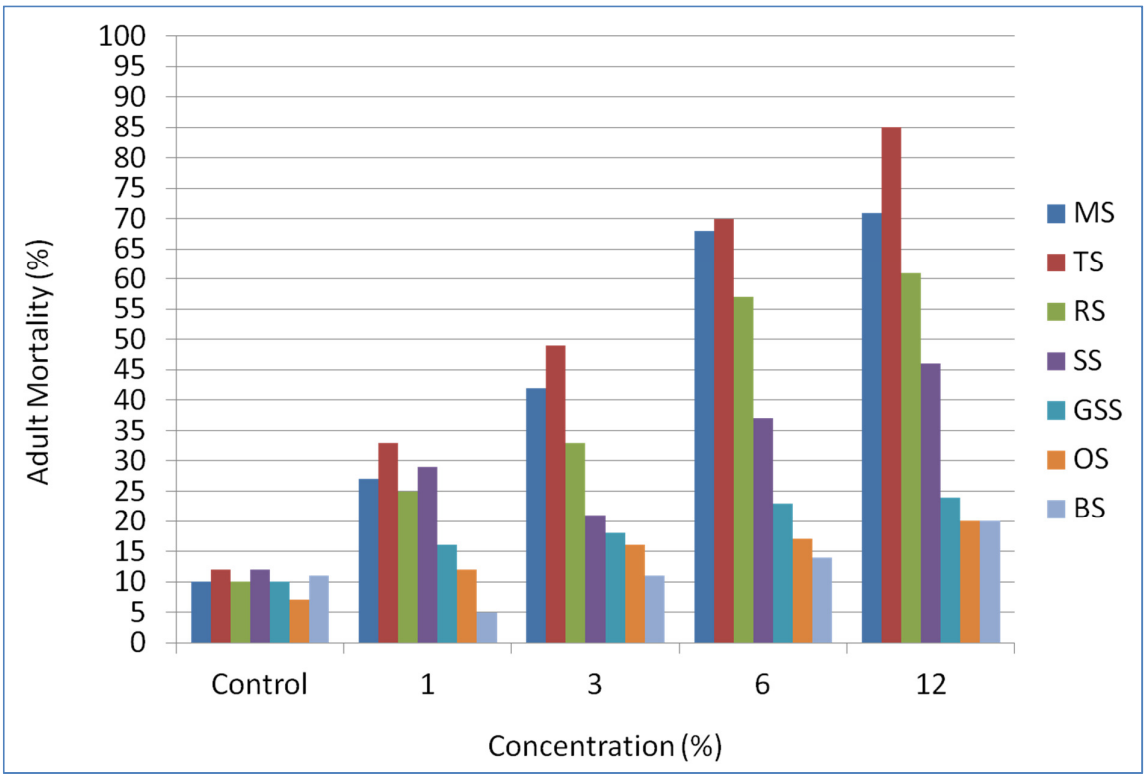

Figure 2. The effects of different concentrations of essential oils on percent mortality of *Tetranychus urticae* adults in leaf spraying method.

Table 1. Antifungal contact effects of essential oils on mycelial growth of *Botrytis cinerea*.

	Minth		Thyme		Rosemary		Sage		Grape Seed		Ozone		Basil	
Dosage	MG*	MGI%	MG	MGI%	MG	MGI%	MG	MGI%	MG	MGI%	MG	MGI%	MG	MGI%
Control	70a		70a		70a		70a		70a		70a		70a	
0,5 µl/ml	17,5b	75	10,2b	85,4	22b	68,5	60b	14	56,2ab	19,7	48,3b	31	54,6b	22
1,5 µl/ml	0c	100	0c	100	13,6c	80,5	57,5b	17,8	52,5b	25	56,5b	19,2	52,8b	24,5
2,5 µl/ml	0c	100	0c	100	0d	100	55,8b	20,2	54,5b	22,1	54,6b	22	46c	34,2
5 µl/ml	0c	100	0c	100	0d	100	45c	35,7	55,5ab	20,7	57,5ab	18,2	40,8c	41,7

* Means with the same letter in a column are not statistically significant different from each other according to the Duncan test at P≤0.05.

Table 2. Antifungal votalite effects of essential oils on mycelial growth of *Botrytis cinerea*

	Minth		Thyme		Rosemary		Sage		Grape Seed		Ozone		Basil	
Dosage	MG	MGI%	MG	MGI%	MG	MGI%	MG	MGI%	MG	MGI%	MG	MGI%	MG	MGI%
Control	70a		70a		70a		70a		70a		70a		70a	
50 µl/petri	0b	100	0b	100	12b	82,8	62,5b	10,7	68,5b	2,1	61,5d	12,1	55b	21,4
100 µl/petri	0b	100	0b	100	0c	100	45c	35,7	58e	17,1	62c	11,4	55b	21,4
150 µl/petri	0b	100	0b	100	0c	100	35d	50	60d	14,2	65b	7,2	30c	57,1
200 µl/petri	0b	100	0b	100	0c	100	31e	55,7	65c	7,1	60e	14,2	13d	81,4

* Means with the same letter in a column are not statistically significant different from each other according to the Duncan test at P≤0.05.

Conclusion

In conclusion of Plant essential oils which we use in our research, these oil can be promising in management of *Botrytis cinerea* important disease agent in vineyard and *Tetrancyhus urticae* which causes chlorotic syptoms on leaves.

The result of the study shows that thyme oil has fungicide and acaricide effects at the same time.

Temperature, sunligth and humidity can be influce phytotoxic effect of essential oil on leaves. Because of this reason concentration of oil can investigate in further study.

Ozone and grape seed oils usage for disease and spider mite have effects but it is not found very efficient. These oils should be studied in more detail.

In conclusion the essential oil from thyme, mint and rosemary may have potential to be used in organic vineyard management of B. *cinerea* and T. *urticae*. However further studies need to be conducted to avaluate usage and efficacy of these essential oils dosage in vineyard.

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