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Research Article

The antifungal effect of propolis extract against cotton wild disease (*Verticillium dahliae* Kleb.)

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Abstract: The aim of this study is to investigate the antifungal activity of propolis against *V. dahliae* Kleb. under both *in vitro* and *in vivo* conditions. Firstly, the inhibitory effect of the propolis on mycelial growth in Potato Dextrose Agar (PDA) media containing its ethanol extract (PE) at various concentrations (0.003, 0.06, 0.125, 0.25, 0.5, and 1 ppm/mL) was investigated under *in vitro* conditions. Then to assess the effect of PE on non-defoliating (PHCVd3 isolate) and defoliating (PHCVd47 isolate) pathotypes of *V. dahliae*, the varieties Giza 45 (resistant), Carmen (tolerant), and Acala SJ2 (susceptible) treated with PEE (1 ppm/mL) were observed in the plant growth chamber up to the 4-6 leaf stage. The whole *in vitro* experiments were carried out with three replicates, and the studies *in vivo* experiment were with five replicates depending on a completely randomized parcels design. The most effective dose of PEE with1 ppm/mL dose resulted in 75.2% suppression against the PHCVd3 isolate, while the effect of the same dose against the PHCVd47 isolate was 74.4%. The lowest disease severity index (DSI) values against PHCVd3 and PHCVd47 isolates in cotton cultivars treated with PE were 1.34 and 1.64 in the Giza 45, respectively, and the highest DSI values were 3.80 and 3.90 in the Acala SJ2 cultivar *in vivo* experiment, respectively. The findings indicate that PE treatment has a promising effect against cotton wilt disease that could be combined with known plant protection strategies.

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1. INTRODUCTION

Cotton (*Gossypium* spp.) is an industrial crop grown worldwide in tropical and subtropical warm-climate regions. Cotton fiber is used as a raw material for the textile industry, and cotton pulp and seed husks are used as animal feed. In addition, the seed's residual linter is utilized by the cellulose and chemical industries, the military industry, and the filling business (Gokdogan *et al.,* 2016). Cotton is grown on 35 million hectares in around 90 countries worldwide, and an average of 26.7 million tons of lint cotton is produced in these areas. Türkiye is the sixth largest cotton producer in the world after India, China, the United States, Brazil, and Pakistan (USDA, 2021). In Türkiye, cotton is grown in 477.000 hectares in 4 main regions (Southeastern

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Anatolia, Aegean, Çukurova, and Antalya), yielding 2.2 million tons of seed cotton yield. Some nations, including Türkiye, produce around 80% of the cotton used worldwide (TSI, 2021).

Wilt disease caused by *Verticillium dahliae* Kleb is one of the stress factors affecting yield and fiber quality traits in cotton cultivation (El-Zik, 1985). *V. dahliae* Kleb. causes wilting of more than 400 plant species (vegetables, legumes, ornamental plants, industrial plants, fruit trees and weeds, and so on), especially cotton (Berlanger & Powelson, 2000). In regions where cotton is grown, the pathogen can remain in the soil as microsclerotia for about 15 years and cause a wilt disease that can lead to significant yield losses (Chen *et al.,* 2016).

Nowadays, places where cotton is grown have both defoliating and non-defoliating pathotypes of the disease. The non-defoliating pathotype results in less leaf shedding by inducing wilting, while the defoliating pathotype causes the cotton plants to shed their leaves completely and die (Bejarano-Alcazar *et al.,* 1995). Of two pathotypes detected in our country, 93% of the defoliating pathotype is in the Aegean region, and 77% of the non-defoliating pathotype has been reported in the Çukurova and Southeastern Anatolia regions (Göre, 2007).

First, the fungus blocks the movement of water and other minerals from the root to the leaves and tissues. Then it causes wilting, desiccation, reduced photosynthesis, shedding of small bolls, and changes in yield and fiber quality characteristics, starting with the lower leaves (Agrios, 2005). The disease reported causes a yield loss of 480 million bales in the US between 1990 and 2014 (Lawrence *et al.,* 2016).

Currently, there is no effective and economical chemical control against Verticillium wilt. Alternative control methods are necessary for the control of the disease. Propolis and bee products with antimicrobial properties are considered to be one of the alternative measurements against plant pathogens. Understanding of the interaction between disease agent and the host plant is important in view of the disease control (Koral & Türktaş, 2018).

Apitherapy is one of the ways of using bee products to treat or prevent diseases from ancient to modern times. Beeswax, honey, honey milk, pollen, bee larvae, bee venom, and propolis are some bee products used in apitherapy. The propolis contains a large number of active chemicals as it exhibits a variety of biological and pharmacological activities, including antibacterial, antifungal, antiviral, antitumor, and anti-inflammatory effects (Kujumgiev *et al.,* 1999; Basim *et al.,* 2006; Pereira *et al.,* 2008). In general, raw propolis consists of 50% resin and vegetable balsam, 30% wax, 10% essential and aromatic oils, 5% pollen, and 5% various other substances including organic residues (Cirasino *et al.,* 1987). The ethanolic extract of propolis (EEP) is the most widely used preparation and over 200 compounds have been identified (Burdock, 1998). Galangin, caffeic, gallic acid, and quercitin are just a few examples of the flavonoids and aromatic acids found in propolis and its preparations that are beneficial in their biological action. Lisa *et al.* (1989) reported that propolis ethanol extract inhibited the growth of 60 yeast isolates and 38 fungal isolates. Some researchers have reported in their studies that propolis has antifungal effects against various plant-pathogenic fungi (Yanar *et al.,* 2005; Özdemir *et al.,* 2010; Curifuta *et al.,* 2012; Manty *et al.,* 2014; Manty, 2015; Araujo *et al.,* 2016; Er, 2021; Çakar *et al.,* 2022). Hegazi *et al.* (2014) reported that geography, plant species, and harvest timing all impact the propolis's biological activity.

This study aims to investigate the antifungal activity of propolis collected from Muğla region against defoliating and non-defoliating pathotypes of Verticillium wilt (*V. dahliae* Kleb.) under both *in vitro* and *in vivo* conditions.

2. MATERIAL and METHODS

2.1. Plant Material, Fungal Pathogen and Propolis Sample

Cotton varieties resistant to Verticillium wilt Giza 45 (*Gossypium barbadense* L.), tolerant Carmen (*Gossypium hirsutum* L.), and susceptible Acala SJ2 (*G. hirsutum*) were used as plant material (Bolek *et al.,* 2005; Erdoğan *et al.,* 2014). The raw propolis of the study was sourced from Muğla province in 2021. Isolates of PHCVd3 (non-defoliating pathotype) and PHCVd47 (defoliating pathotype) were obtained from the Plant Protection Laboratory, Department of Plant Protection, Hatay Mustafa Kemal University.

2.2. Preparation of Propolis Ethanol Extract (PEE)

Raw propolis was purchased from Muğla, frozen at -18°C in the laboratory condition, and then chopped into tiny pieces while still frozen. It was first made from a 1:3 mixture of propolis and ethyl alcohol, which was ground up in a blender for 2 minutes before being homogenized in an ultrasonic bath for two days. Then it was prepared from 80% ethyl alcohol (80 mL ethyl alcohol + 20 mL clean water (Rios) = 100 mL). Inverting and mixing at least twice daily, the homogenized mixture was kept in a dark area for five days. At the end of this time, the extract was filtered using Whatman No. 1 filter paper, and PEE was prepared by isolating the propolis components from the wax. The alcohol in each combined filtrate was concentrated by rotary evaporation using an IKA RV10-Germany rotary evaporator and then cooled to +4°C.

2.3. Calculation of Total Phenolic Substance, Total Antioxidant Activity, and Total Flavonoid Content of PEE

According to Oruç *et al.* (2021) separation of phenolic compounds in propolis samples and high-performance liquid chromatography (HPLC) analyses were performed. A C18 column (Inertsil ODS-3.5 mm, 4.6 x 150 mm) was used to separate the propolis samples.

By reducing the ferric ion in the presence of antioxidants, the FRAP technique (Ferric Reduction/Antioxidant Power) becomes (Fe(III)-TPTZ-2,4,6-Tris(2-Pyridyl)-S-Triazine)-based on TPTZ synthesis. To do this, 100 mL sample and 3 mL of the FRAP reagent (300 mM pH 3.6 acetate buffer, 10 mM TPTZ and 20 mM $FeCl_3$ (10:1:1) were combined, and after 4 minutes this combination formed at a maximum absorbance of 593 nm (Benzie & Strain, 1999). The standard graph was created with different concentrations of $FeSO_4.7H_2O$ (31, 25, 62.5, 125, 250, 500 and 1000 M). Results are presented as antioxidant potency equivalent to $FeSO_4.7H_2O$.

The 4-keto and C-3 or C-5 hydroxyl groups (or both) of the flavonoids combine to form a stable acidic complex that forms the basis of the Fukumoto and Mazza method, also known as the aluminum chloride colorimetric method (Fukumoto & Mazza, 2000). The standard graphic was created using quercetin at different concentrations (1, 0.5, 0.25, 0.125, 0.0625, 0.03125 and 0.015625 mg/mL). The amount of flavonoid substance corresponding to quercetin was detected, as shown by the graphic constructed using absorbance values at 415 nm versus concentration.

2.4. Determination of the Antifungal Effect of PEE *In Vitro* on Pathotypes of *Verticillium dahliae*

To evaluate the mycelial growth in PDA (Difco) media containing propolis at various concentrations (0.003, 0.06, 0.125, 0.25, 0.5 and 1 ppm/mL) under *in vitro* conditions, the effect of PEE on both *V. dahliae* pathotypes was noted. A variable concentration of propolis was added to the sterilized PDA medium before being dispensed in 20 mL portions into sterilized Petri plates (100 mm). PDA medium with PEE was kept at room temperature for 24 hours. After that, propolis was used to inoculate the 7-day-old cultures of both pathotypes of *V. dahliae* grown in PDA medium with 5 mm mycelial discs cut with a mushroom drill. Petri dishes were

cultured at 24 ± 1ºC for 7-10 days. Only the pathogen was inoculated into the control Petri plates. Growth inhibition rates were separated using calipers after the pathogen had grown in the control plates and the treated petri plates, *in vitro* experiment was performed using a fully randomized parcels design with three replicates, and was replicated twice. The following formula was used to calculate the level of antifungal activity of propolis (Deans & Svoboda, 1990).

Percentage of mycelial growth inhibition (%) = [(dc-dt/dc)] x 100

where dc is the average mycelial growth diameter in the control petri plate (mm) and dt is the average mycelial growth diameter in the treatment petri plate (mm).

2.5. Determination of the Antimicrobial Effect of PEE *In Vivo* against *Verticillium dahliae*

The *in vivo* pot experiment included drilled, fungicide-free seeds of cotton varieties resistant Giza 45, tolerant Carmen, and susceptible Acala SJ2. First, the autoclave-sterilized (1 hour at 121°C) soil mixture (1/3 soil + 1/3 sand + 1/3 peat) was filled into sterile plastic pots (10 cm diameter). Then 4 cotton seeds were planted in each pot (2 mL/seed) coated with the effective dose of propolis extract (1 ppm/mL). Pots were cultivated in a plant development plant growth chamber with 12 hours light and 12 hours dark at 24 ± 1ºC. Then, when the cotton seedlings reached the cotyledon stage, thinning was performed and one seedling was left in each pot. The plant maintenance procedures were completed on schedule and the cotton seedlings were grown until they had 4-6 leaves.

To determine the susceptibility of PEE-coated cotton cultivars to *V. dahliae* (Erdoğan *et al.,* 2014) two-week-old spores cultured in broth medium (0.01 g $FeSO_4.7H_2O$, 0.5 g $MgSO_4.7H_2O$, 2 g $NaNO_3$, 1 g K_2HPO_4, 0.5 g KCl, and 7.5 g sucrose, 1 L sterile distilled water) cultured isolates of PHCVd3 and PHCVd47 were filtered through 2 layers of cheesecloth and mycelium and pieces of agar were removed from the suspension and then the spore concentration was adjusted to 4×10^6 spores/mL using a Thoma slide in the light microscope (Leica) and used for the inoculation of cotton plants. The plants were transplanted into new plastic pots (10 mL) with spore solution when they reached the 4-6 leaf stage. Only sterile distilled water was added to the plastic pots bottoms as a control. The pot experiment was performed with five replicates in a fully randomized parcels design in the plant growth room (under a 12-hour light/12-hour dark cycle at 24 ± 1ºC). The severity of the disease on cotton plants was evaluated by the wilt index according to the percentage of affected leaves using a scale of 0 to 5 (0 = no symptoms, 1 = chlorosis in the lower leaves, 2 = moderate (30-50% of the leaves) wilt with severe chlorosis, 3 = moderate wilting and necrosis, 4 = severe (more than 50% of leaves) wilting and necrosis, 5 = dead plant) after about 3-5 weeks (Tsror *et al.,* 2001). The following formula was used to calculate the Disease Severity Index (DSI) value caused by *V. dahliae* and the data obtained from the pot experiment were transformed using Arcsin (Karman, 1971).

$$DSI= (a \times 0) + (b \times 1) + (c \times 2) + (d \times 3) + (e \times 4) + (f \times 5) / M$$

where a, b, c, d, e, and f are the plant numbers with degrees 0, 1, 2, 3, 4, and 5, respectively, and M is the overall plant number.

2.6. Statistical Analysis

The data were analyzed by performing the ANOVA (one-way analysis of variance). Statistically significant differences between mean values were determined using Least Significant Differences (LSD) Test ($p \leq 0.01$). All statistical analyses were performed using JMP software version 13 (SAS Institute Inc., Cary, NC, USA).

3. RESULTS

3.1. Antifungal Effect of PEE on Mycelial Growth of Pathotypes of *Verticillium dahliae*

The effects of PEE concentrations on mycelial growth and inhibition rates of non-defoliating (PHCVd3 isolate) and defoliating (PHCVd47 isolate) pathotypes under *in vitro* conditions are given in Table 1. The PEE doses were found to be significant according to the statistical analysis results ($p \leq 0.01$) of the *in vitro* experiment. At various concentrations, PEE reduced mycelial growth in both non-defoliating (PHCVd3 isolation) and defoliating pathotypes (PHCVd47 isolate). PEE doses showed percentage inhibition rates of the non-defoliating pathotype (PHCVd3 isolate) ranging from 18.30 to 75.20%. The 1 ppm/mL dose produced the most potential antifungal effect (75.20%), followed by the 0.5 ppm/mL dose (61.80%). The defoliating pathotype (PHCVd47 isolate) showed inhibition rates from PEE dosages between 13.00 and 74.40%. The highest antifungal effect was determined at 1 ppm/mL dose (74.40%), followed by 0.5 ppm/mL dose (61.00%). Compared to the other treated petri dishes, the mycelium diameter in the control petri dish was statistically different (Table 1).

Table 1. Antifungal effect of PEE on mycelial growth of pathotypes of *Verticillium dahliae*.

Concentration (ppm/mL)	PHCVd3 isolate		PHCVd47 isolate	
	Mycelial growth (mm)*	MGI	Mycelial growth (mm)*	MGI
0.003	16.17 B	18.30	17.25 B	13.00
0.06	15.08 C	23.90	15.17 C	23.70
0.125	11.50 D	42.00	11.67 D	41.30
0.25	9.00 E	54.60	9.17 E	53.90
0.5	7.58 F	61.80	7.75 F	61.00
1	4.92 G	75.20	5.08 G	74.40
Control	19.83 A	0.00	19.88 A	0.00
CV $_{(0.01)}$	2.43		2.32	
LSD	0.52		0.50	

Each observation is based on three replicate plates. Arcsine transformation was performed prior to statistical analysis. *Mean values followed by different letters within the column are significantly different according to LSD Test ($p \leq 0.01$). MGI: Mycelial growth inhibition rate (%)

3.2. Determination of The Antimicrobial Effect of PEE *In Vivo* against *Verticillium dahliae*

The effects of the effective dose of PEE (1 ppm/mL) on PHCVd3 and PHCVd47 isolates of *V. dahliae* Kleb. in disease-resistant Giza 45, tolerant Carmen and susceptible Acala SJ2 cultivars under pot conditions are given in Table 2. The statistical analysis of the pot experiment's data revealed that cotton variety differences were significant ($p \leq 0.01$). The range of 1.34 to 3.80 was discovered for the mean disease severity index values in cotton cultivars treated with PEE for the PHCVd3 isolate. The lowest disease severity index value was found in the resistant Giza 45 variety (1.34) and the tolerant Carmen variety (1.82), and these varieties were statistically in the same group. The mean disease severity index values for the PHCVd47 isolate ranged from 1.64 to 3.90. The resistant Giza 45 variety came in first with a disease severity index value of 1.64, followed by the tolerant Carmen variety (2.32). When both disease pathotypes were present, the Acala SJ2 cultivar had the highest Disease Severity Index value (3.80-3.90) (Table 2).

Table 2. Disease severity index values in cotton plants treated with PEE after PHCVd3 and PHVd47 inoculation.

Variety	PHCVd3 isolate DSI*	PHCVd47 isolate DSI*
Acala SJ2 (Susceptible)	3.80 A	3.90 A
Carmen (Tolerant)	1.82 B	2.32 AB
Giza 45 (Resistant)	1.34 B	1.64 B
CV $_{(0.01)}$	3.25	4.34
LSD	1.10	1.66

Each observation is based on five replicate plates. Arcsine transformation was performed prior to statistical analysis. *Mean values followed by different letters within the column are significantly different according to LSD Test ($p \leq 0.01$). DSI: Diseases severity index value.

3.3. Total Phenolic Analysis, Total Antioxidant Analysis and Total Flavonoid Substance Amount Values of Propolis

The propolis samples used in the HPLC-DAD analyses of the studies are given in Table 3. It was determined that propolis samples contained high levels of phenolic chemicals. According to the HPLC-DAD results of propolis, phenological chemicals such as galangin, pinosembrine, quercetin, chrysin and naringenin were found in significant amounts (Table 3).

Table 3. HPLC-DAD analysis results of propolis.

Identified phenolic compounds	Amounts found (µg/mL) *
Gallic acid	30.28
Epigallocatechin gallate	24.34
Caffeic acid	292.55
p-Coumaric acid	116.68
trans-Ferulic acid	86.00
trans-Isopherulic acid	225.25
3-4-Dimethoxycinnamic acid	142.16
Quercetin	468.02
trans- Cinnamic acid	44.29
Naringenin	367.28
Apigenin	287.01
Kaempferol	172.73
Krisin	419.76
Pinosembrine	958.08
Galangin	959.83
Caffeic acid phenethyl ester	2102.26
trans- Chalcone	443.85

*Analysis results include µg/g amounts of liquid propolis in 1 mL.

The total antioxidant capacity of propolis used in the study is given in Table 4. Table 4 shows that propolis has a high level of overall antioxidant ability.

Table 4. Total antioxidant capacity of propolis.

Sample	Total antioxidant capacity FRAP
liquid ethanolic propolis	222.85±1.67 Mmol $FeSO_4.7H_2O$/mL
Raw propolis	197.79±2.593 (mmol $FeSO_4.7H_2O$/g)

FRAP: Ferric reducing/antioxidant power.

The results of the total content of flavonoids in propolis are given in Table 5. Flavonoids, which are the main source of antioxidants, have analytical results suggesting that the propolis used in the study has an effective content (Table 5).

Table 5. Total amount of flavonoid substance of propolis.

Sample	Total amount of flavonoid substance mg QE/g
liquid ethanolic propolis	10.18±0.06 mgGAE/mL
Raw propolis	4.779±0.140 mgQE/g

4. DISCUSSION and CONCLUSION

In vitro studies conducted in petri dishes the various doses of PEE inhibited both pathotypes to varying degrees. The highest antifungal effect was obtained at 1 ppm/mL dose between 75.20% and 74.20% in non-defoliating (PHCVd3 isolate) and defoliating (PHCVd47 isolate) pathotypes, respectively. In a similar study Kurt and Şahinler (2003) reported that increasing concentrations of PEE reduced mycelial growth of the pathogens tested and the effect of PEE on *V. dahliae* was 84.8% and 83.3% at 1.0 and 0.5 ppm concentrations, respectively. Gallez *et al.* (2014) have shown the inhibitory effect of the propolis ethanol extract (PEE) which inhibited mycelial growth by 70-78% of *Didymella bryoniae* and *Rhizotocnia solani in vitro*. They have suggested to have its fungistatic effect. Abd-El-Kareem *et al*. (2017) have reported that the antifungal effect of PEE increased with increasing doses, and 10% EEP dose inhibited sclerotid germination by 91% of *Sclerotinia sclerotiorum*. In another study, 3% propolis ethanol extract strongly inhibited the mycelial growth of the green mold disease agent *Penicillium digitatum* in lemon, and an inhibition zone was formed (Abo-Elyousr *et al*., 2021). Türk *et al*. (2022) showed that mycelial growth of *F. oxysporum* decreased depending on PEE concentrations increased, PEE collected from Muğla at the highest concentration (50 ppm) was 77.81% against *F. oxysporum*, and PEE collected from Denizli had the lowest antifungal effect (64.52%). The reason for the varying antifungal effects of propolis extract against different or same pathogens is due to its chemical content, which is significantly depending on the plant flora where the propolis content has been supplied (Ali & Kunugi, 2020).

As known in previous studies, PEE was used against many phytopathogenic fungi *in vivo*, whereas fewer studies were conducted against *V. dahliae*. Soylu *et al*. (2008) have reported, all doses of PEE prepared in 70% ethanol prevented disease emergence in citrus fruits artificially infected with *Penicillium digitatum*, PEE at 100 mg/mL concentration reduced natural disease emergence by 100% *in vivo* conditions. In a previous study, the results showed that 3% dose of Iraqi origin PEE prevented the rot caused by *P. digitatum* on oranges for three weeks at room temperature (Matny, 2015). In another study, the effectiveness of PEE increased as the concentration increased. The studies carried out *in vivo* conditions indicated that 5% concentration of PEEs completely prevented fruit infections, 3% and 5% concentrations of PEE were also effective against quince brown rot disease (*M. fructigena*) that can be used as an edible coating (Özyiğit *et al.,* 2018). Er (2021) tested PEE under *in vivo* conditions. An alcohol-based propolis extract at a concentration of 60 mg/mL applied to seeds seed + foliar cabbage showed up to 97.9% protection against *A. brassicola*. Spraying seeds + leaves with the same concentration of water-based propolis extract gave the highest antimicrobial effect with of

91.6% inhibition. The raw propolis used in the study was rich in view of phenolic compounds, high in total antioxidant capacity and, effective in total flavonoid substance content. In the studies carried out, propolis has been reported as inhibitory compound against selected plant pathogenic fungi due to the antifungal properties of phenolics, flavonoid aromatic acids in its chemical composition (Bancova *et al.,* 2000). Kordali *et al.* (2009) stated that terpenes have an antifungal effect against plant pathogenic fungi and this effect changes depending on the type and structure of the molecule. In the studies carried out on the chemical composition of propolis, researchers reported that propolis contains chemical compounds such as myristic acid, benzoic acid, benzyl alcohol, vanillin, cinnamic acid, pinosembrin, pinobanksin, quercetin, galangin, apigenin, chrysin, caffeic acid, acacetin, campheride, and isovaniline (Burdock, 1998; Salomão *et al.,*2004; Uzel *et al.,* 2005). Keskin & Kolaylı (2018) reported that the total phenolic content of Anatolian propolis ranges between 16.13-178.34 mg GAE/g for raw propolis. In a similar study conducted, the total phenolic content of propolis obtained from different regions of Anatolia was found to be between 2748 mg GAE/100 g and 19969 mg GAE/100 g (Ozdal *et al.,* 2019). Our findings revealed that samples with high total phenolic content had also high antioxidant effects. In accordance with our findings, Aygun (2017) reported that although the chemical composition of propolis is complex, its antimicrobial effect is due to flavonoids from phenolic acids, phenolic acid esters and terpenes.

In the pot experiment, seed application of PEE suppressed the non-defoliating and defoliating pathotypes of Verticillium wilt disease agent. PEE was a promising treatment against both pathotypes of *V. dahliae* on the resistant Giza 45 cultivar and then in the tolerant Carmen cultivar, which was determined according to the disease severity index values. In this context, the combination of resistant variety + propolis can be suggested against Verticillium wilt disease as biological control, which is the best alternatives within the scope of integrated control. However, we need detailed studies related to assessing the effective dose of PEE in cotton varieties and Verticillium wilt under field conditions. The results of the propolis analysis showed that it is rich in phenolic compounds with a high total antioxidant capacity, and that the total amount of flavonoid substances is the reason for being effective against the pathogen. However, no studies have been conducted on the propolis extract's mechanism of action. For this purpose, detailed studies should be carried out to determine the mechanism of action and plant growth promoting effects of PEE, which is found to be effective. The study is pioneer to determine the effects of PEE to cotton seed against Verticillium wilt under pot conditions. Seed coating studies carried out with simple laboratory facilities are both effective and its application commercially uncomplicated. Therefore, the results obtained from the study will shed light on biological control studies for the future.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the author(s).

Authorship Contribution Statement

Melike Mutlu Yilmaz: Literature survey, Data collection, Edit data. **Yesim Kara:** Methodology, Supervision, Resources, Reading and Editing of article. **Oktay Erdogan:**

Methodology, Supervision, Data analyses, Writing, Reading and Editing the original draft of the article.

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