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The use of *Marrubium vulgare* L. plant extracts in the control of fungal plant pathogens

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Abstract

In this study, we investigated anti-fungal properties of *Marrubium vulgare* L. plant extracts dissolved in four different solvents against five different plant pathogenic fungi, namely, *Fusarium oxysporum* f. sp. *lycopersici, Macrophamina phaseolina, Phytopthora infestans, Sclerotinia sclerotiorum* and *Rhizoctania solani*. We used 1.0%, 2.5% and 5.0% plant extract concentrations against these fungi species which cause a significant decrease in the production yield of certain plants. In all pathogenic fungi species analyzed, we observed an increase in the anti-fungal effect of *Marrubium vulgare* L. plant extract preparations in a dose-dependent manner. *Marrubium vulgare* L. extracts dissolved in chloroform and dichloromethane at 2.5% concentration showed higher inhibition against *Fusarium oxysporum* compared to other solvents at this concentration. At the same concentration, extracts dissolved in methanol resulted in higher anti-fungal activity against *Phytopthora infestans* and intermediate activity against *Macrophamina phaseolina* and *Sclerotinia sclerotiorum*. As a result, it can be stated that *Marrubium vulgare* L. plant extracts dissolved in different solvents display anti-fungal properties against certain plant pathogens.

Keywords: *Marrubium Vulgare* L., Anti-fungal activity, Plant extracts, Plant pathogens

Introduction

Marrubium vulgare L. which is a perennial plant from *Labiatae* family, is a very common plant in North Africa, Central and West Asia, and Southern Europe (Weel et al., 1999). *Marrubium vulgare* L. was reported to be used in the treatment of certain diseases including bronchitis, asthma, tuberculosis, respiratory system infections and diarrhea (Gruenwald et al., 1998). Many studies showed that *Marrubium vulgare* L. has various biological properties such as cytotoxicity (Argyropoulou et al., 2012), anti-cancer (Yamaguchi et al., 2006), anti-hypertensive (Bardai et al., 2001), analgesic (Meyre-Silva et al., 2005), antibacterial (Gonza'lez and Marioli, 2010; Bouterfas et al., 2018), antioxidant (Amri et al., 2017), antidiabetic (Boudjelal et al., 2012) anti-inflammatory (Schlemper et al., 1996), hypoglycemic (Roman Ramos et al., 1992) effects and wound healing (Bokaeian et al., 2014). Considering the biological importance of this plant, scientists have shown an increasing interest in the research of *Marrubium vulgare* L.

Plant fungal diseases cause serious problems in agricultural production and decrease annual yield significantly. Plant pathogenic fungus *Fusarium oxysporum* f. Sp. *Lycopersici* causes wilting in tomato (Rai et al., 2011), *Phytophthora infestans* induces late blight in tomato and potato (Marcin et al., 2012), *Rhizoctonia solani* and *Macrophamina phaseolina* give rise to root rot (Gautam et al., 2003) and *Sclerotinia sclerotiorum* infection results in white mold in plants (Visser, 2007). Several synthetic drugs are widely used for the control

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of diseases caused by these fungal pathogens; however, these chemicals lead to toxic residues in food and to environmental pollution. Recently, many researchers have focused on the use of environmentally-friendly biological methods for the protection of plants from damages and the loss of yield associated with plant pathogenic fungi (Koul and Dhaliwal, 2001; Copping and Menn, 2000). Plant extracts used in these methods are quite beneficial since they easily decompose, have no harm on the environment and have low phytotoxicity (Benner 1996). A review on the use of plants against plant pathogenic fungi species can be found elsewhere (Shuping and Eloff, 2017).

In the current study, we determined anti-fungal properties of *Marrubium vulgare* L. plant extracts dissolved in methanol, ethyl acetate, chloroform and dichloromethane against five plant pathogenic fungi species (*Fusarium oxysporum* f. sp. *lycopersici*, *Macrophamina phaseolina, Phytopthora infestans*, *Rhizoctania solani* and *Sclerotinia sclerotiorum*).

Materials and Methods

Preparation of plant extracts

100 g dried *Marrubium vulgare* L. plant was mixed with 250 mL methanol, chloroform, dichloromethane and ethyl acetate solutions separately after grinding, and stirred for 48h with a magnetic stirrer. Then, plant particles in solvents were filtered, solvents were evaporated and extracts were obtained in a viscous form. These extracts were diluted with 10 % aceton and kept at 4 ºC as 50% stocks for further use.

Microorganisms used in the study

Microorganisms used in this study (*Fusarium oxysporum* f. sp. *lycopersici*, *Macrophamina phaseolina*, *Phytopthora infestans*, *Rhizoctania solani* and *Sclerotinia sclerotiorum*) were obtained from stock cultures in Phytopathology Laboratory at Faculty of Agriculture, Tokat Gaziosmanpasa University.

Anti-fungal studies

Marrubium vulgare L. extracts prepared with different solvents were used in *in vitro* anti-fungal tests. *Marrubium vulgare* L. extracts dissolved in methanol, dichloromethane, ethyl acetate and chloroform were added to autoclaved Potato Dextrose Agar (PDA) as final concentrations of 1.0%, 2.5% and 5.0%. 5 mm mycelium disks for *Fusarium oxysporum* f. sp. *lycopersici*, *Macrophamina phaseolina, Phytopthora infestans, Rhizoctania solani* and *Sclerotinia sclerotiorum* pathogens were inoculated on solidified PDA growth media and placed in a 25 ºC incubator. Experiments were performed in triplicates. After 7-day incubation period, fungi mycelium radius measurements were carried out. PDA growth media without any plant extract was used as a control.

Evaluation of antifungal properties

In these anti-fungal tests, percent inhibition values of plant extracts against pathogenic fungi species were determined by comparing mycelium radial growth measurements of pathogens with that of controls. Inhibition rates were determined using the following formula by Deans and Soboda (1990):

$$MGI\ (\%) = [(dc - dt) / dc] \times 100$$

MGI = Inhibition (%)

dc = Radial growth in control petri dish (mm)

dt = Radial growth in petri dish with plant extract (mm)

Data visualization

Graphs were drawn in R statistical programming environment (R Core Team, 2018). Following R packages were used in data analysis and visualization: tidyverse (a collection of R packages including ggplot2 designed for common data science tasks such as data cleaning and visualization) (Wickham et al., 2019), readxl (data import into R from excel) (Wickham and Bryan, 2019). The following convention for star symbols indicating statistical significance was used in the plots: ns: p > 0.05, *: p <= 0.05, **: p <= 0.01, ***: p <= 0.001, ****: p <= 0.0001 (ggpubr package, Kassambara, 2020).

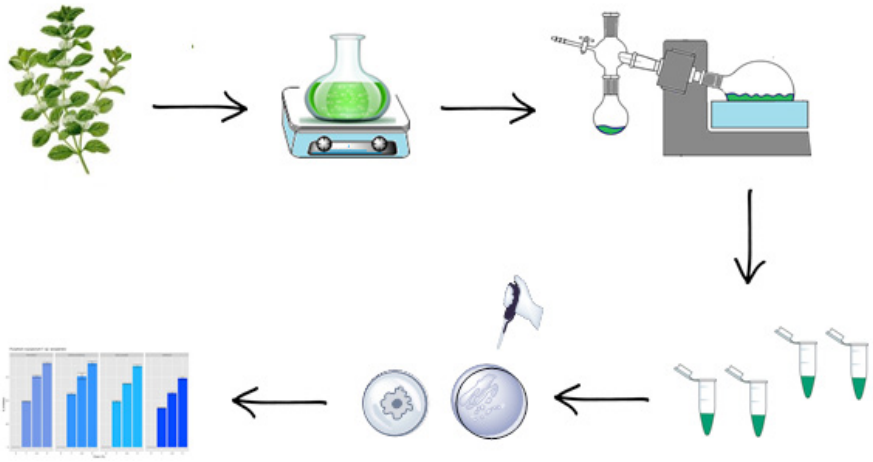

Figure 1. Stages of plant extract and fungus study

Results

The effect of *Marrubium vulgare* L. extracts dissolved in chloroform, dichloromethane, ethyl acetate and methanol on *Fusarium oxysporum* f. sp. *Lycopersici* mycelium growth was shown in Figure 2. Extracts dissolved in all solvents showed an increased anti-fungal activity in a concentration-dependent manner. The inhibitory roles of extracts on mycelium growth in terms of solvents were mostly parallel; however, extracts dissolved in methanol had lower inhibitory effect on mycelium growth compared to other solvents. This difference on inhibition of fungal growth might be due to the fact that different solvents can dissolve different organic metabolites in varying amounts. The highest anti-fungal activity on *Fusarium oxysporum* f. sp. *Lycopersici* relative to other solvents at the

same concentrations was observed with 2.5% plant extract in dichloromethane and 5.0% plant extract in chloroform, resulting in growth inhibition of 76% and 90%, respectively. 5.0% plant extract dissolved in methanol resulted in the lowest inhibitory effect (74%) compared to other solvents at this concentration.

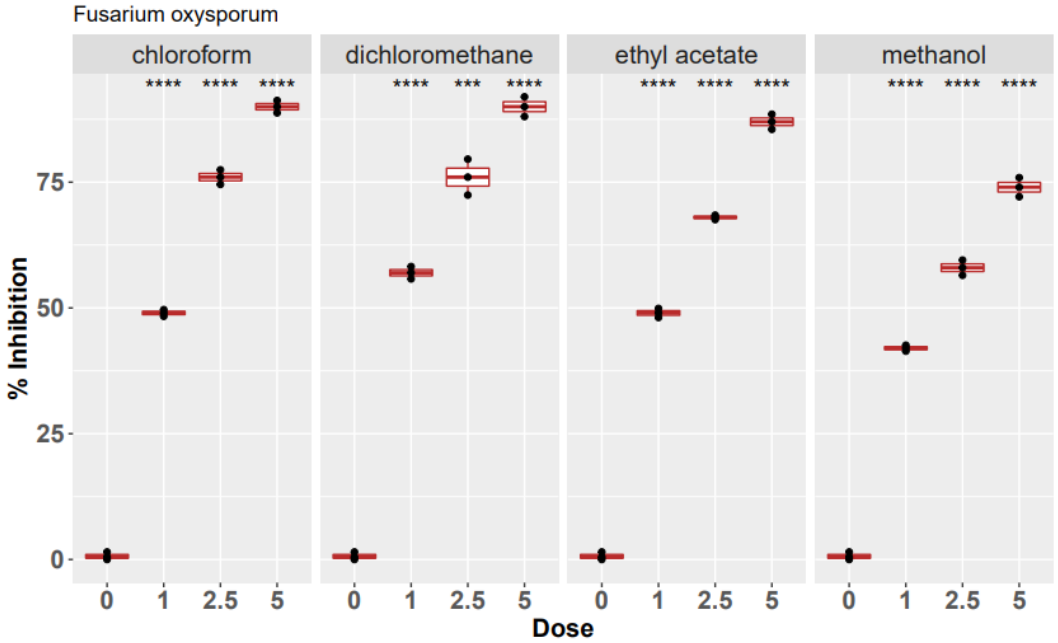

Figure 2. Percent inhibition of *Fusarium oxysporum* f. Sp. *Lycopersici* mycelium growth with increasing doses of *Marrubium vulgare L.* extracts dissolved in chloroform, dichloromethane, ethyl acetate and methanol. ns: p > 0.05, *: p <= 0.05, **: p <= 0.01, ***: p <= 0.001, ****: p <= 0.0001.

The percent inhibition values for *Marrubium vulgare* L. extracts on *Macrophamina phaseolina* mycelium growth were given in Figure 3. The inhibitory effects of these extracts on *Macrophamina phaseolina* were seen to be parallel with that on *Fusarium oxysporum* f. sp. *Lycopersici,* similiar dose-dependent increase in inhibition was observed. For this fungi species, at the same concentration of *Marrubium vulgare* L. plant extract, we did not observe any significant difference between different solvents. At the highest dose (5.0%), inhibition rates were calculated to be between 88 – 90%.

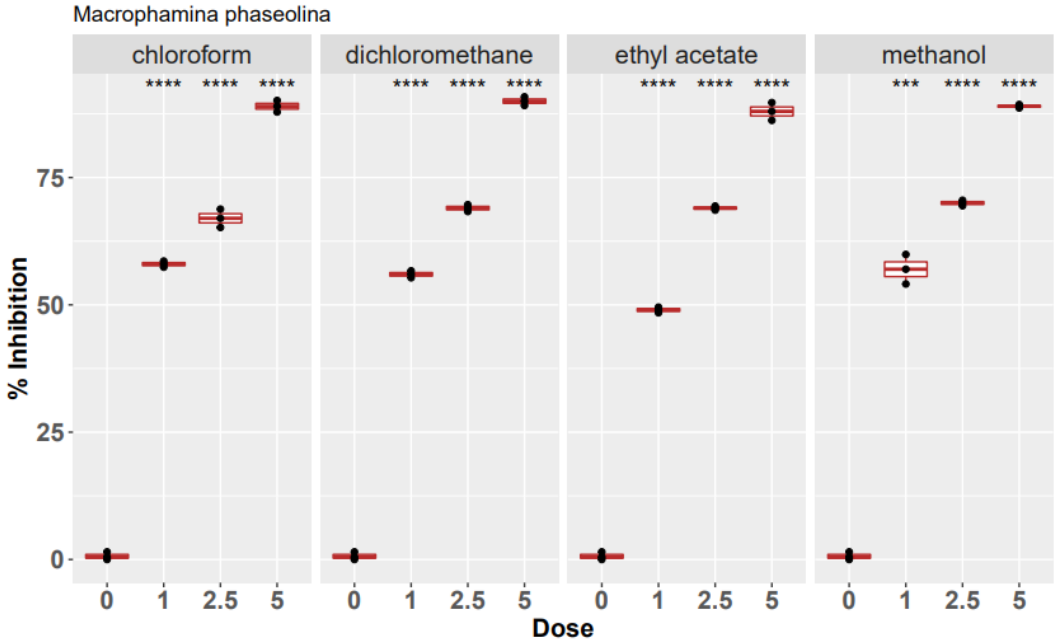

Figure 3. Percent inhibition of *Macrophamina phaseolina* mycelium growth with increasing doses of *Marrubium vulgare L.* extracts dissolved in chloroform, dichloromethane, ethyl acetate and methanol. ns: p > 0.05, *: p <= 0.05, **: p <= 0.01, ***: p <= 0.001, ****: p <= 0.0001.

The highest inhibitory effect on *Phytopthora infestans* mycelium growth was seen when methanol was used as a solvent at all concentrations of plant extract tested (58% inhibition at 1.0% concentration, 76% at 2.5%, 89% at 5.0%). Figure 4 shows dose-dependent increase in the inhibiton of *Phytopthora infestans* with *Marrubium vulgare* L. extracts dissolved in four solvents. At the highest concentration used, plant extract in dichloromethane resulted in the second highest inhibition against *Phytopthora infestans.*

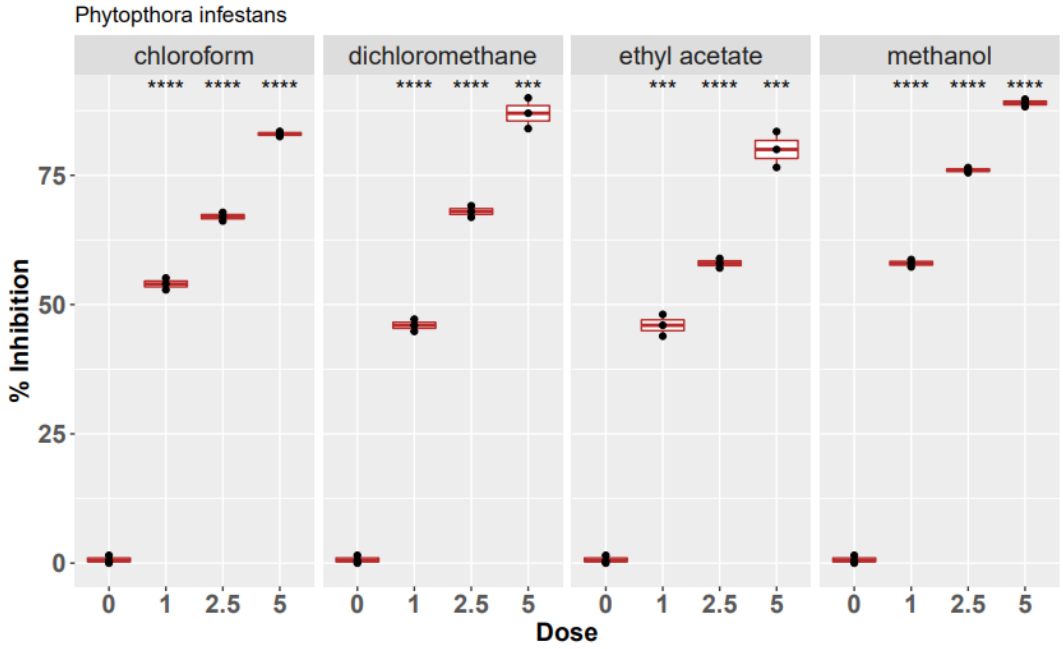

Figure 4. Percent inhibition of *Phytopthora infestans* mycelium growth with increasing doses of *Marrubium vulgare L.* extracts dissolved in chloroform, dichloromethane, ethyl acetate and methanol. ns: p > 0.05, *: p <= 0.05, **: p <= 0.01, ***: p <= 0.001, ****: p <= 0.0001.

The anti-fungal effect of *Marrubium vulgare* L. extract against *Sclerotinia sclerotiorum* mycelium growth was the highest with plant extract dissolved in dichloromethane at 1.0% dose; mean inhibition for all solvents was 67.25% at 2.5% dose. However, at 5.0% dose, extract in dichloromethane showed the highest inhibitory effect against *Sclerotinia sclerotiorum* relative to other solvents at this concentration (91% growth inhibition). For other solvents, mean growth inhibition was 84% at the highest dose used. Results were summarized in Figure 5.

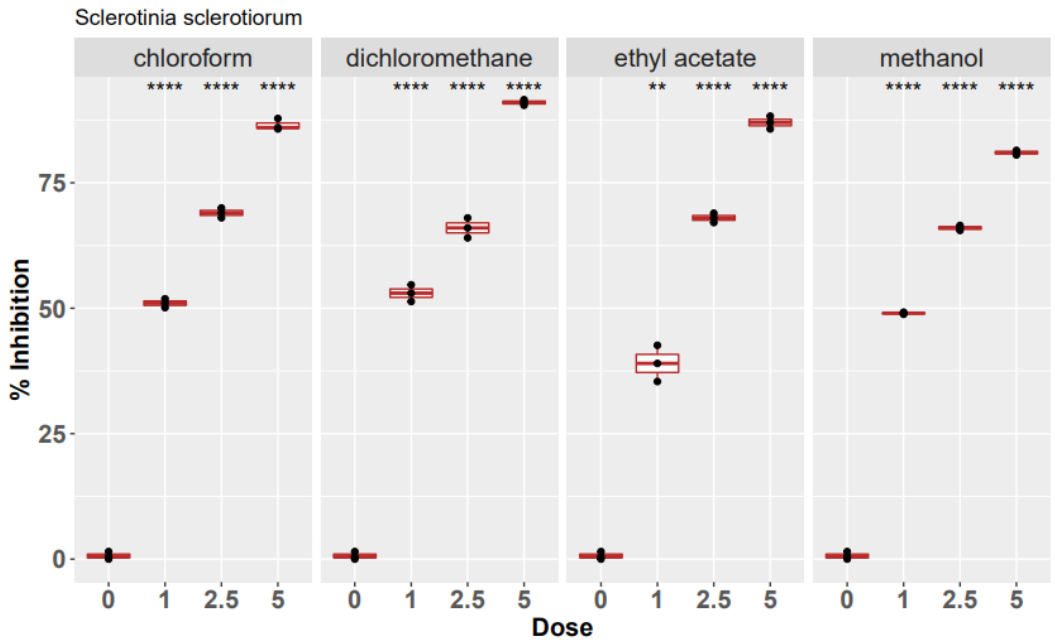

Figure 5. Percent inhibition of *Sclerotinia sclerotiorum* mycelium growth with increasing doses of *Marrubium vulgare L.* extracts dissolved in chloroform, dichloromethane, ethyl acetate and methanol. ns: p > 0.05, *: p <= 0.05, **: p <= 0.01, ***: p <= 0.001, ****: p <= 0.0001.

Rhizoctania solani was shown to be least effected fungi species by the treatment with *Marrubium vulgare* L. extract in all solvents used in this study. Data on this fungus species were given in Figure 6. Highest growth inhibition (53%) against this pathogen was obtained with 5.0% *Marrubium vulgare* L. extract dissolved in methanol. Plant extract in dichloromethane followed methanol with 43% growth inhibition rate. At lower concentrations of plant extracts in solvents, significant inhibition percentages were not observed against *Rhizoctania solani.*

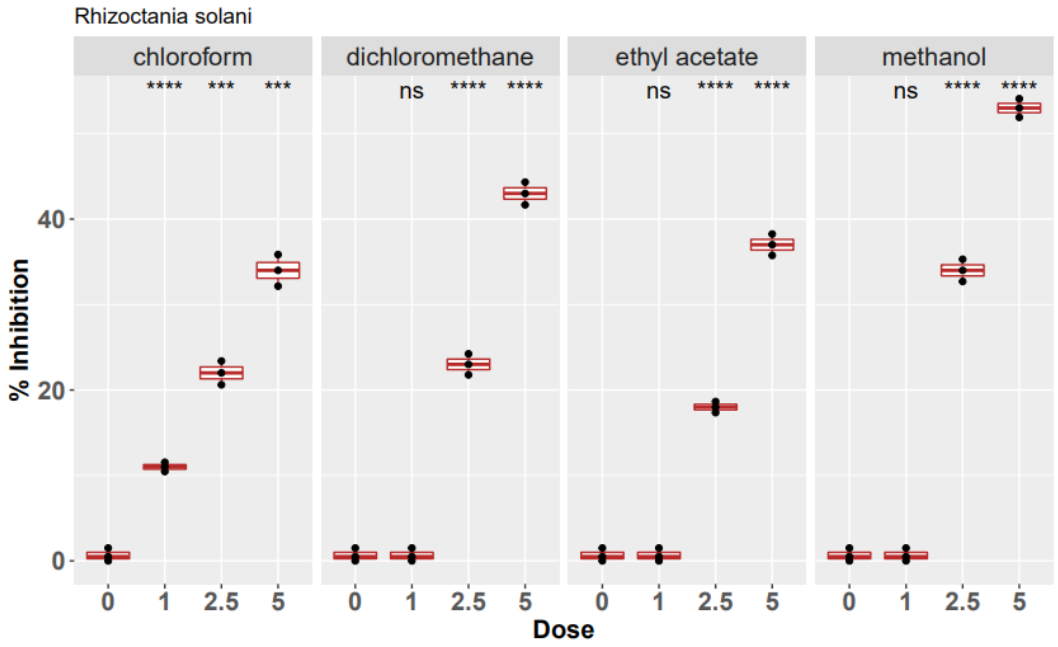

Figure 6. Percent inhibition of *Rhizoctani solani* mycelium growth with increasing doses of *Marrubium vulgare L.* extracts dissolved in chloroform, dichloromethane, ethyl acetate and methanol. ns: p > 0.05, *: p <= 0.05, **: p <= 0.01, ***: p <= 0.001, ****: p <= 0.0001.

Conclusion

In the current study, anti-fungal properties of *Marrubium vulgare* L. extracts in different solvents against certain plant pathogenic fungi were determined. Today, diverse set of organic and inorganic molecules were synthesized in the laboratory conditions using different methodologies, and their multiple biological functions including anti-fungal properties were studied (Özbek et al., 2017; Gürdere et al., 2020; Özbek and Gürdere, 2020). However, in the present study, natural plant-based extracts were analyzed in terms of their anti-fungal activities. In all the analysis, we showed that increased dose resulted in higher inhibition on fungal mycelium growth. At 2.5% dose, higher anti-fungal activity against *Fusarium oxysporum* was observed when *Marrubium vulgare* L. extracts dissolved in chloroform and dichloromethane. At the same concentration, plant extract dissolved in methanol showed increased inhibitory activity on *Phytopthora infestans* growth; however, intermediate effects were observed against *Macrophamina phaseolina and Sclerotinia sclerotiorum* pathogens. Significant inhibitory effect against *Rhizoctani solani* was observed only with plant extract dissolved in methanol, though this anti-fungal effect is intermediate. Other doses and solvents did not show any considerable inhibition on mycelium growth of *Rhizoctani solani.* Finally, it can be stated that *Marrubium vulgare* L. extracts dissolved in methanol, dichloromethane, ethyl acetate or chloroform have anti-fungal properties against certain plant pathogens and can be used as biofungicides in the future to prevent agricultural losses associated with fungal infections.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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