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Antifungal Activity of Turkish Propolis Against *Phytophthora* Species

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Abstract: The antifungal effects of 10 different concentrations (10, 7, 5, 3, 1, 0.1, 0.07, 0.05, 0.03 and 0.01 μg mL^{-1}) of propolis methanol extract (PME) on *Phytophthora infestans, P. capsici* and *P. parasitica* were evaluated *in vitro*. PME was mixed with steril Corn Meal Agar medium (CMA) at various concentrations. Aceton of 4% and recommended dose of Metalaxyl (1.8 mg a.i. mL^{-1}) were used as control. Mycelial discs (5 mm in diameter), taken from actively growing margin of five-day-old culture of each species, were placed in each extract medium and incubated for 4 days at 24°C in the dark. Significant differences occurred among the extract concentrations. Four of PME concentrations (10, 7, 5 and 3 μg mL^{-1}) tested completely inhibited the mycelial growth of the three *Phytophthora* species and even killed the mycelium on PDA disc, while rest of the concentrations tested had fungistatic effects on these pathogens. The results indicated that PME had compound with fungicidal effects on *Phytophthora* species.

Key words: Propolis, *Phytophthora*, antifungal, extract

INTRODUCTION

Phytophthora species (*Phytophthora infestans, Phytophthora capsici* and *Phytophthora parasitica*), causal agents of late blight, root and crown rots and fruit rot, one of the most destructive pathogen of tomato, potato, pepper and many cucurbit species worldwide due to rapid asexual reproduction under conducive weather and soil conditions[1,2]. Control of these pathogens mainly relies on modification in cultural practices, crop rotation and proper and effective use of selective fungicides, especially the phenylamid fungicides[3]. The phenylamid fungicide metalaxyl provides systemic protection against oomycete pathogens[1,3,4]. Metalaxyl has been used extensively for control of many different oomycete pathogens, including *P. infestans, P. capsici* and *P. parasitica*. The intensive use of metalaxyl led to rapid selection for metalaxyl-resistant strains of these pathogens in many European countries[5-7]. Besides metalaxyl-resistant strains development in the pathogens populations, there is a growing global concern, over the intensive use of synthetic fungicides on agricultural crops because of their potential effects on human health and on the environment. These concerns have resulted in a renewed interest in the search for alternative control measures.

Propolis is one of the several non-synthetic compounds control options. The anti microbial effects of propolis have long been recognized. Propolis is a sticky, gummy and resinous substance collected by honeybees (*Apis mellifera*) from various plant sources around their hives. Bees collect the propolis to seal holes in the hives, to reinforce the combs, smooth out the internal walls and protect the entrance against intruders. Propolis also is used to keep the hive environment aseptic[8]. Raw propolis composed of approximately 50% of resin (polyphenolic fraction), 35% of wax, 10% of essential oil, 5% of polen and 5% of various organic and inorganic compounds[8,9]. More than 200 compounds have been identified[8]. Its biological activity depends on compounds polyphenolic fraction, mainly flavonoids, followed by aromatic acids, phenolic acid esters, triterpenes, lignans etc.[8,10]. It is known that biological activity of propolis is effected by flavonoids concentration[11]. Flavonoid concentration of propolis depends on the geographical origin and plant sources. Thus the anti-microbial properties of the propolis may vary as well.

The aim of this study was to evaluate the anti-fungal activity of Turkish propolis against *Phytophthora* species.

MATERIALS AND METHODS

One isolate from each of *Phytophthora infestans, P. parasitica* and *P. capsici* was used in this study. The

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isolate of *P. capsici* was recovered from infected bellpepper plant in a commercial field in North East Turkey whereas isolates of *P. infestans* and *P. parasitica* originated from infected tomato plants in the same places.

Approximately 100 g of propolis collected by *Apis mellifera*, were obtained directly from the bee hives in North East of Turkey. The samples of propolis were dried and ground to a fine powder. Methanolic extracts of propolis (PME) were prepared by adding 100 g of the specimens of propolis to 200 mL absolute methanol in 500 mL flask and shaking on an orbital shaker (at 120 rpm) for one day. After extraction, the mixture was passed through four-layer of cheese clothes to separate solid materials. The methanol was evaporated at 32°C using rotary evaporator. The solid phase was dissolved in 10% acetone to obtain 20% concentration of propolis extract as an stock solution[12].

Each species of *Phytophthora* was grown on Potato dekstrose agar (PDA) plates at 24°C for 4 days. Individual agar disks (5 mm in diameter) were removed from the edge of an actively growing culture of each pathogen and placed at the center of plastic petri dishes (90 mm in diameter) obtaining Corn Meal Agar (CMA), amended with 10, 7, 5, 3, 1, 0.1, 0.7, 0.5, 0.3 and 0.1 μg mL^{-1} concentrations of propolis extract. All tests were performed in duplicate, using a 10% acetone solution without propolis as a control to test the inhibitory effect of the solvent. Metalaxyl+Mancozeb (Ridomil MZ 72 WP, 1,8 mg a.i. mL^{-1}) was also used to compare the efficiency of propolis with fungicide. Propolis and fungicide were added to CMA after autoclaving when the agar had cooled to approximately 45°C. There were three replicates per species at each propolis concentration. After a four day of incubation period at 24°C in darkness, the radial growth of mycelia was measured. The minimum inhibitory concentration was assumed as the lowest concentration of the propolis extract which inhibited the visible growth of *Phytophthora* species on the plates. The percentage mycelial growth inhibition with respect to the control was calculated as:

$$P = (C-T/C)x100$$

where, P is Inhibition (%), C is Average mycelial diameter of the control (mm) and T is average mycelial diameter of the treated ones[13].

Statistical analysis: The data were analysed using Analysis of Variance (ANOVA) test. The means of treatments were grouped on the basis of Least Significant Difference (LSD) at the 0.05 probability level. The software SAS was used to conduct all the statistical analysis.

RESULTS AND DISCUSSION

The antifungal activity of the propolis methanol extract (PME) against *Phytophthora* species (*P. infestans, P. capsici* and *P. parasitica*) at 10, 7, 5, 3, 1, 0.1, 0.07, 0.05, 0.03 and 0.01 μg mL^{-1} was expressed as mycelial growth inhibition percentages. Significant differences occurred among the extract concentrations. PME, 10-3 μg mL^{-1} showed 100% inhibiting effects on mycelial growth of the pathogens. The minimum inhibitory concentration (3 μg mL^{-1}) of propolis extract was fungicidal to the *Phytophthora* species. At 1 μg mL^{-1}, PME showed 96.86, 96.42 and 95.15% growth inhibition on *P. capsici, P. infestans* and *P. parasitica*, respectively (Table 1). At 0.01 μg mL^{-1}, PME showed the lowest antifungal activity with mycelial growth inhibitions of 6.84, 6.13 and 5.98% on *P. capsici, P. infestans* and *P. parasitica*, respectively. The growth inhibition declined significantly as PME concentration was lowered. Many others reported the inhibitory effect of propolis on fungi[14-19]. Ozcan *et al.*[19] reported that methanolic extract of propolis significantly inhibited the mycelial growth of *Alternaria alternata* and *Fusarium oxysporum* at different concentrations. Kurt and Sahinler[18] showed that ethanolic extracts of propolis were inhibitory against *Verticillium dahliae, Fulvia fulva* and *Penicillium digitatum*. Propolis extracts were also shown to reduce mycelial growth of *Aspergillus parasiticus*[20]. Present results exhibited the inhibitory effect of PME on fungi. However, further research is needed in order to characterize the functional groups, or combinations of groups, responsible for the antifungal of the PME used in this study.

Table 1: Antifungal activity of propolis extract on *Phytophthora* species

Propolis concentration (μg mL^{-1})	Inhibition rate of mycelial growth (%)		
	P. capsici	*P. infestans*	*P. parasitica*
10.00	100.00a[1]	100.00a	100.00a
7.00	100.00a	100.00a	100.00a
5.00	100.00a	100.00a	100.00a
3.00	100.00a	100.00a	100.00a
1.00	96.86b	96.42b	95.15b
0.10	80.97c	79.70c	79.49c
0.07	64.31d	63.60d	63.29d
0.05	45.96e	45.44e	44.42e
0.03	34.52f	33.80f	33.56f
0.01	6.84g	6.13g	5.98g
Ridomil MZ 72 WP[2]	100.00a	100.00a	100.00a
Kontrol (4% Aseton)	0.00h	0.00h	0.00h
LSD	1.99	2.12	1.95

[1]Values within a column followed by the same letter are not significantly different according to Fisher's protected least significance test
[2]For fungicide, the rate of active ingredient is 1.8 mg mL^{-1}

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