Review on Agriculture and Rural Development 2014. vol. 3 (1) ISSN 2063-4803

INFLUENCE OF SOME FOOD ADDITIVE CHEMICALS TO *PHOMOPSIS VITICOLA* SACC.

İSMET YILDIRIM

Çanakkale Onsekiz Mart University Agricultural Faculty, Department of Plan Protection
Çanakkale, Türkiye
yismet96@gmail.com

ABSTRACT

The effects of food additives potassium sorbate, sodium benzoate, sodium bicarbonate, and classical mancozeb on mycelial growth and the yield of pycnidium and pycnidiospore production of *Phomopsis viticola* were tested in vitro. Food additive chemicals were tested at five different dosages, 100; 300; 600; 1000 and 1600µg ml-1, mancozeb was tested only at recommended dose. Food additives showed inhibitory effect at different levels on mycelial growth of fungus isolates. According to the ED_{50} values at the 5th day, potassium sorbate (375 µg ml^{-1} for isolate İzmir 2 and 700 µg ml^{-1} for isolate Salihli 2) showed significant inhibition effect on the radial mycelial growth of fungal isolates. Similar efficacy for İzmir 2 and Salihli 2 was obtained at the highest doses (990 µg ml^{-1} for İzmir 2 and 1150 µg ml^{-1} for Salihli 2) from sodium bicarbonate and (1040 µg ml^{-1} for İzmir 2 and over 1600 µg ml^{-1} for Salihli 2) from sodium benzoate. MIC values of food additives on mycelial growth of *P. viticola* were found higher than 1600 µg ml^{-1}. The food additives at increasing doses were more successful on the pycnidia and pycnidiospores inhibition than on mycelial growth. The strongest inhibition among food additives on the pycnidia and pycnidiospores production was determined in potassium sorbate with dose between <100 µg ml^{1} and 620µg ml^{-1}. However, ED_{50} values of sodium benzoate were found lower than potassium and sodium bicarbonate had. While MIC of potassium sorbate was 1000µg ml^{-1} on pycnidium production; on pycnidiospore it was 600 µg ml^{-1}. Whereas MIC values of sodium benzoate and sodium bicarbonate were determined higher than potassium sorbate. Data obtained from the research demonstrated that food additives may be used against *P. viticola* in organic and traditional viticulture after field experiments.

Keywords: *Phomopsis viticola*, food additives.

INTRODUCTION

Phomopsis viticola is a well-known pathogen of *Vitis vinifera* cultivated in many of the vine-growing areas of the World (HEWITT AND PEARSON, 1998; PEARSON AND GOHEEN, 1994). Phomopsis causes important crop losses due to shoots breaking off near the basis where the lesion formed, reduced growth in vine shoots, loss of vigour, smaller bunches and sometimes as a result of fruit being infected (PINE, 1959; PSCHEIDT AND PEARSON, 1989). The fungus overwinters as mycelium and pycnidia in the bark, and also mycelium occurs in dormant buds. Both forms, especially pycnidia are significant for primer infection of *P. viticola*. Infection generally occurs in spring when shoots begin to grow, and mycelia and pycnidiospores, especially α-spores play important role for this infections. Spores are released in large quantities from the pycnidia on infected canes, spurs and bark, are splashed by rain onto newly developing shoots (HEWITT AND PEARSON, 1998). Infection occurs when free moisture remains on the unprotected green tissue for many hours, and symptoms become visible shortly afterwards. A synthetic fungicide may be used against to Phomopsis cane and leaf spot in the dormant period to help clean up overwintering inoculum and lessen the risk of new shoot infection. In severely affected vineyards, both dormant and spring treatments may be advisable (HEWITT AND PEARSON, 1998). Protective fungicides on new plant growth early in the growing season and eradicant fungicides could be applied during dormancy (CHAIRMAN ET AL., 1982). Some fungicides like captan, maneb, mancozeb, propineb, methiram complex and bordo mixture have been registered against *P. viticola* in Turkey. Phomopsis cane and leaf spot may be controlled by synthetic fungicides; however, most of the fungicides embody the risk of leaving residue on products and have negative effects on human beings, animals and environment (WIGHTWICK AND ALLINSON, 2007; KOMAREK ET AL., 2010). In this context, recent studies have focused on alternative chemicals and biological agents in the control of pathogens (ARCHBOLD ET AL., 1997; SKIRDAL AND EKLUND, 1993; YILDIRIM AND YAPICI, 2007).

In this study the effect of food additives, potassium sorbate, sodium benzoate and sodium bicarbonate on the mycelial growth and yield of pycnidia and pycnidiospore of *P. viticola* were determined *in vitro*.

MATERIAL AND METHOD

Origin of *Phomopsis viticola* isolates

P. viticola isolates (İzmir 2 and Salihli 2) were obtained from infected cane (*Vitis vinifera* L. cv 'Sultana Seedless') obtained from vineyards in İzmir and Manisa, Türkiye. Cultures were grown on PDA in petri plates at 21 °C in the incubator, and then transferred into tubes including PDA medium for obtaining stock culture.

Natural Chemicals

To determination of inhibition effects of the food additives against *Ph. viticola*, potassium-sorbate, sodium benzoate, sodium bicarbonate, and a synthetic fungicide mancozeb were tested *in vitro* essays. The food additives were used in dose series as 0 (control); 100; 300; 600; 1000 and 1600 $\mu g\ ml^{-1}$. Mancozeb was used only in registered dosage (160 g a.i. 100 l^{-1} water). Some characteristics of the test chemicals are shown in *Table 1*.

Table 1. Some properties of the test chemicals

Commercial name	Formulation and ratio of a.i. (%)	Firm
Potasyum sorbate	$C_6H_7O_2K$, 150.22	Selen Kim. Tiç. Ltd.
Sodium benzoate	$C_7H_5NaO_2$, 144.11	Selen Kim. Tiç. Ltd.
Sodium bicarbonate	$NaHCO_3$ 99.00	Carlo Erba
Fumazin 80 WP	Mancozeb 80 WP	Hektaş AŞ.

Effects of Test Chemicals on Mycelial Growth

The stock isolates were grown on PDA medium for 5 days, and agar plugs (Ø4 mm) taken from fungal cultures by a sterile cork-borer were then placed onto surface of PDA medium with or without test chemical. The petri dishes sealed with plastic film after inoculation were incubated at 23 ºC in dark. Colony diameters at the widest point were measured at 24 hours intervals during 5 days. The inhibitory effects of chemicals were determined by comparing with colony diameters in the supplemented and un-supplemented medium. ED_{50} values of chemicals were found by semi-logarithmic graphics. The trials were arranged in a randomized complete block design with three replications.

Effects on Yield of Pycnidia and Pycnidiospores

After 20 days from inoculation, the numbers of Pycnidia were determined on the supplemented and un-supplemented media. Counted pycnidia were taken by means of forceps, and were transferred into tubes including 10 ml sterile deionized water. Pycnidiospores were provided to pass into water by stirring for 10 min by means of vortex, which was then filtered through cheesecloth to remove the pycnidia and other debris. α-spores densities were determined with a haemocytometer (Precicolor HBG, Germany) by adding 20 ml sterile deionized water into the suspension.

The inhibitory effects of chemicals were determined by Abbot Formulation, and ED_{50} values (effective concentrations of the chemicals that reduced the pycnidia and Pycnidiospores numbers by 50%) were found by semi-logarithmic graphics.

RESULTS

The effects of the chemicals on colony growth of *Phomopsis viticola* (isolates: İzmir and Salihli 2) at 5th days after inoculation are presented in *Figure 1*.

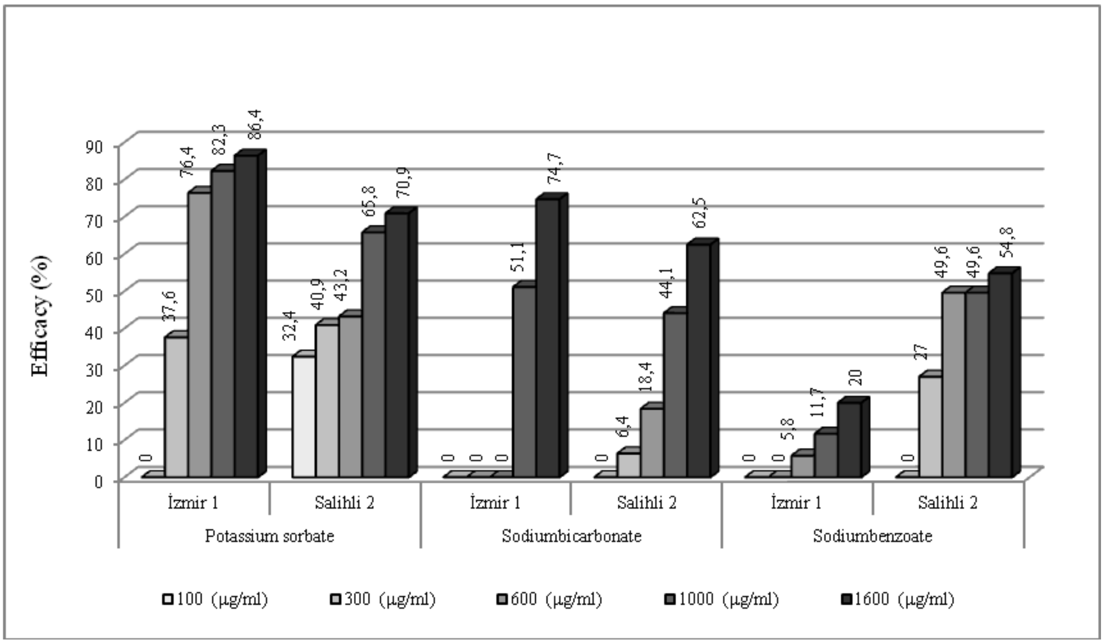

Figure 1. Efficacies of test chemicals on mycelial growth of *Phomopsis viticola* (isolates: İzmir 2 and Salihli 2) at 5th day

Test chemicals exhibited varying degrees of effect on mycelia growth of both isolates of *P. viticola* (*Figure 1*). Potassium sorbate at high dosages shown good antifungal activity on mycelium growth. While potassium sorbate at high dosages (600-1600 µg ml^{-1}) was the most effective against İzmir 2 isolate, it showed moderate effect to Salihli 2 isolate at all dosages. Sodium bicarbonate was found effective only at high dosages (1000-1600 µg ml^{-1}) to both isolates (İzmir 2 and Salihli 2). Sodium benzoate didn't have high inhibition effect to İzmir 1; but was moderately effective to Salihli 2. On the other hand, synthetic fungicide mancozeb at application dosage (%0.16) totally inhibited both isolates.

Minimum Inhibition concentration (MIC) and values of ED_{50} of the test chemicals on radial growth of the isolates of *P. viticola* are given in *Table 2*.

Test chemicals showed low activity with MIC values of >1600 µg ml^{-1}, but according to ED50 values, potassium sorbate was higher effective to the pathogen tested as compared to potassium bicarbonate and sodium benzoate.

Table 2. MIC and ED50 Value of Test Chemical on *Phomopsis viticola*

Test chemicals	İzmir 2				Salihli 2			
	At 4th day		At 5th day		At 4th day		At 5th day	
	MIC	ED_{50}	MIC	ED_{50}	MIC	ED_{50}	MIC	ED_{50}
Potassium sorbate	>1600	270	>1600	375	>1600	680	>1600	700
Sodium bicarbonate	>1600	1400	>1600	990	>1600	1230	>1600	1150
Sodium benzoate	>1600	>1600	>1600	>1600	>1600	1140	>1600	1040

The effects of the chemicals on the yield of pycnidia and pycnidiospores of *P. viticola* (isolates: İzmir and Salihli 2) at 20th days after inoculation are presented in *Figure 2* and *3*.

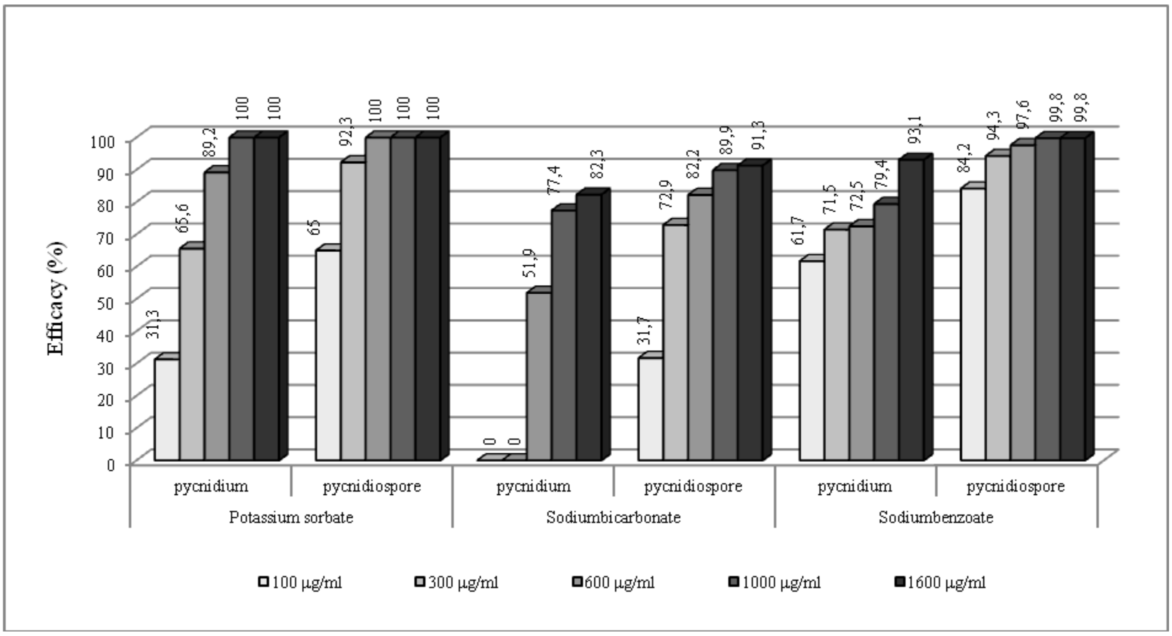

Figure 2. Efficacies of test chemicals on the yield of pycnida and pycnidiospores of *Phomopsis viticola* (isolate İzmir 2)

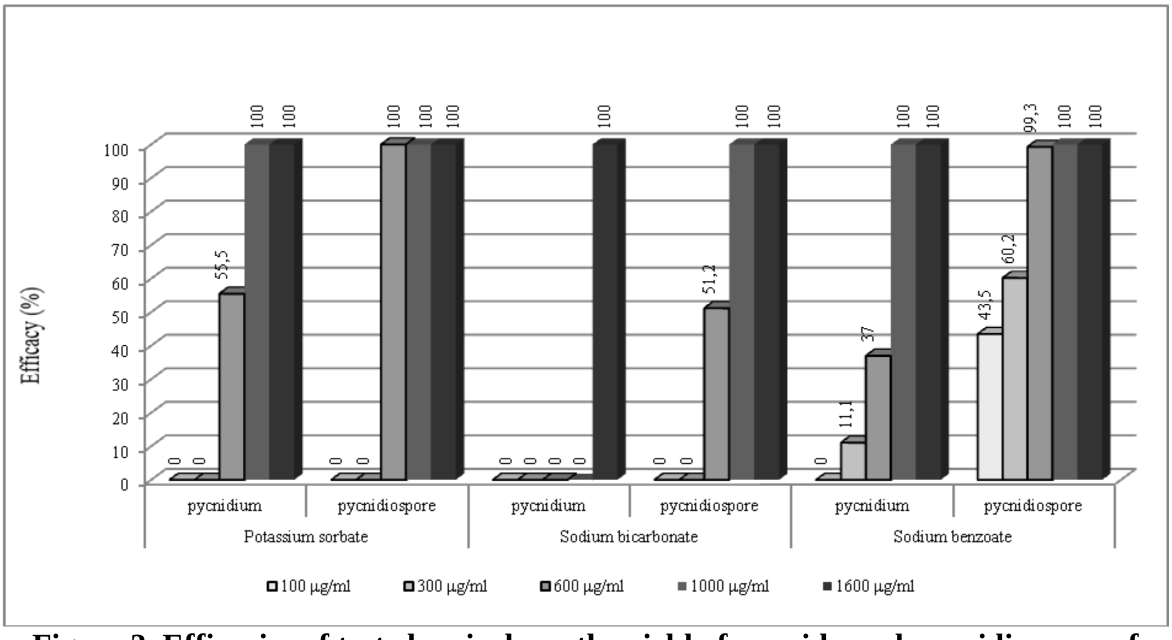

Figure 3. Efficacies of test chemicals on the yield of pycnida and pycnidiospore of *Phomopsis viticola* (isolate Salihli-2)

Test chemicals showed significant effects on the production of pycnidia and pycnidiospores of isolate İzmir 2 (*Figure 2*). Sodium benzoate and potassium sorbate were much effective to both structures of pathogen at all trial dosages. While sodium bicarbonate at the dosages between 600 and 1600 µg ml^{-1} could be effective on the pycnidia formation; it was more effective on the pycnidiospores formation at all dosages (*Figure 2*).

Effects of test chemicals on the pycnidia and pycnidiospores production of Salihli 2 were different to the effects of that isolate İzmir 2 (*Figure 3*). Test chemicals at high doses displayed important effects on pycnidium and pycnidiospore production of *P. viticola* (Salihli 2). On the other hand; potassium sorbate at doses of 100 and 300 µg ml^{-1} against both forms of pathogen, sodium bicarbonate at doses 100, 300 and 600 µg ml^{-1} against pycnidium formation and at 100 and 300 µg ml^{-1} against pycnidiospore formation, and sodium benzoate at 100 µg ml^{-1} dose against pycnidium formation were found not to be effective (*Figure 3*). However, mancozeb at application dose (0.16%) completely inhibited both forms of *P. viticola* (İzmir 2 and Salihli 2).

Minimum Inhibition Concentration (MIC) and values of ED_{50} of the test chemicals on pycnidia forming and pycnidiospore numbers of the isolates of *P. viticola* are introduced in *Table 3*.

Table 3. MIC and ED50 values of test chemicals on pycnidium and pycnidiospore formations of *Phomopsis viticola*

	İzmir 2				Salihli 2			
	Pycnidium		Pycnidospore		Pycnidium		Pycnidospore	
	MIC	ED_{50}	MIC	ED_{50}	MIC	ED_{50}	MIC	ED_{50}
Potassium sorbate	1000	185	600	<100	1000	620	600	370
Sodium bicarbonate	>1600	590	>1600	162	>1600	>1600	1600	980
Sodium benzoate	>1600	<100	>1600	<100	1000	640	1000	150

MIC values of sodium bicarbonate and sodium benzoate on both forms of pathogen were detected at 1000 µg ml^{-1} and higher doses (*Tabel 3*). Whereas MIC values of potassium sorbate on the pycnidium formation was 1000 µg ml^{-1} that was found 600 µg ml^{-1} for pycnidiospore formation. On the other hand, ED_{50} values of sodium benzoate were close to the values of mancozeb. ED_{50} values of other test chemicals were higher than that of mancozeb (*Table 3*). Among the test chemicals, sodium bicarbonate at all doses had lowest ED_{50} values on pycnidium and pycnidiospore formation of both isolates.

DISCUSSION AND CONCLUSIONS

In this study; potassium sorbate, sodium benzoate and sodium bicarbonate, which are food additives that may be alternative to classical fungicides, were analyzed in vitro conditions regarding their effects on mycelium growth and yield of the pycnidium and pycnidiospore of *Phomopsis viticola*.

Among food additives, potassium sorbate showed significant inhibition effect on mycelial growth of *P. viticola* at increasing doses. But, sodium benzoate and sodium bicarbonate couldn't inhibit the mycelial growth enough. Food additive chemicals could not completely inhibit mycelial growth of both isolates; their MIC values were found over 1600 µg/ml. However, ED50 values for potassium sorbate and sodium benzoate were lower than MIC values. Sodium bicarbonate could not show sufficient efficacy on mycelial growth (MIC value for both isolates were higher than 1000 µg ml^{-1}).

On the other hand, all the food additives at higher dosages were more successful on the pycnidia and pycnidiospores inhibition. Food additives are being used widely for a long time in cosmetic and in the field of medicine, as well as in food preservation due to their anti-microbial effects and they are higlyh efficient against yeast and mould (Wade and WELLER 1994; YILDIRIM AND YAPICI 2007). Potassium sorbate and sodium benzoate had antifungal activities against postharvest decaying fungi (ABDEL-KADER ET AL., 2011; AL-ZAEMEY ET AL., 1993; OLİVİER ET AL., 1999). The strongest inhibition among food additives against the pycnidia and pycnidiospores production of both isolates was determined in potassium sorbate with 600 µg ml^{-1} and higher doses. Potassium sorbate exhibited complete inhibition on the pycnidium production of both isolates with 1000 µg ml^{-1} and 1600 µg ml^{-1} and on the pycnidiospore production with doses between 600 and 1600 µg ml^{-1}. But, according to ED_{50} values, sodium benzoate showed better performance on the pycnidium and pycnidiospore production than potassium sorbate did. While ED_{50} values of sodium benzoate on pycnidium and pycnidiospore production of İzmir 2 was <100 µg ml^{-1}; in Salihli 2 were 640 µg ml^{-1} on pycnidium, and 150 µg ml^{-1} on pycnidiospore. MIC values of food additives may show differences not only related to the type and dosages of additives, but also on the species of microorganisms on which they act. STANOJEVICH ET AL. (2009) had reported that while MIC values of potassium sorbate on *Fusarium oxysporum*, *Trichoderma harsianum*, *Penicillium italicum* and *Aspergillus flavus* were respectively 20, 30, 20 and 50 mg ml^{-1}; of sodium benzoate were respectively 7.25, 30, 15 and 50 mg ml^{-1}. In a study with same additives, YILDIRIM AND YAPICI (2007) determined that MIC value of potassium sorbate on mycelial growth of *Botrytis cinerea* was 1000 µg ml^{-1}, of sodium benzoate was higher than 1000 µg ml^{-1}.

The results of this study demonstrate that food additives *in vitro* did not show high affection on the growth of *P. viticola*, but they have strong inhibition on the pycnidium and pycnidiospore of it. This shows that the food additives could be used against *P. viticola* in organic and traditional viticulture after field experiments.

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