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Original article

Determination of the Antifungal Effect of Boron, Sodium and Potassium Salts against Pomegranate Fruit and Crown Rot Disease Agent *Coniella granati*

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Abstract

Pomegranate (*Punica granatum* L.) is one of the most important fruit species grown in Turkey. Fungal pathogens cause significant losses in quality and yield of pomegranate fruit in orchards and warehouses. Pomegranate fruit and crown rot, caused by *Coniella granati*, is one of the most serious diseases of pomegranate. No fungicides have been registered in Turkey to control this disease. In this study, the antifungal effect of etidote-67, borax, boric acid, sodium benzoate, sodium nitrite, sodium carbonate and potassium sorbate on mycelial growth of *C. granati* was investigated in vitro. The antifungal effect of the salts on mycelial growth of the fungus was determined at concentrations of 0.01, 0.02, 0.03, 0.04, 0.05, 0.06 and 0.07 (w/v). Increased concentrations of etidote-67, borax, boric acid, sodium benzoate, sodium nitrite, sodium carbonate, and potassium sorbate significantly inhibited mycelial growth of the fungus compared to the control. Significant differences were found between the efficacy of the treatments ($P<0.05$). The concentrations of 0.04% and 0.03% of etidot-67 and borax salts, respectively, were determined to be the doses that completely inhibited mycelial growth of the fungus. Boric acid, sodium nitrite, sodium carbonate and potassium sorbate completely inhibited the fungal mycelial growth at a concentration of 0.05%, while sodium benzoate inhibited fungal mycelial growth at a relatively high concentration (0.07%). When comparing the effective concentrations (EC_{50}) of the salts inhibiting mycelial growth by 50%, sodium benzoate showed a stronger inhibitory effect against the fungus. In conclusion, the results of this study show that boron, sodium, and potassium salts can be used as an alternative to synthetic fungicides to control fruit and crown rot disease caused by *C. granati* in pomegranate.

Keywords: Pomegranate, *Coniella granati*, Sodium and Potassium Salts, Alternative control.

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INTRODUCTION

Pomegranate cultivation has been increasing rapidly in our country and in the world in recent years. Pomegranate (*Punica granatum* L.) is a fruit that is grown in many tropical and subtropical regions of the world, especially in the Mediterranean countries, and can be consumed fresh as well as being used as a colorant and flavoring for fruit juice, jam, various foods (Nanda et al., 2001; Altan & Maskan, 2005).

Pomegranate production and quality in our country are affected by many fungal disease factors both during gardening and storage. Of these, *Botrytis cinerea, Aspergillus niger*, *Penicillium* spp., *Alternaria* spp., *Nematospora* spp., *Coniella granati, Colletotrichum gloeosporioides, Pestalotiopsis versicolor* and *Syncephalastrum racemosum* are important fungal disease agents causing postharvest losses in pomegranate fruits worldwide (Wilson & Ogawa, 1979; Snowdon 1990; Jamadar et al., 2011; Mirabolfathy et al., 2012; Palou et al., 2013; Kanetis et al., 2015; Munhuweyi et al., 2016; Alvarez et al., 2016). Fruit and crown rot disease caused by *C. granati*, one of these disease agents, causes problems in pomegranate fruits during gardening and storage (Uysal et al., 2018). Symptoms in the form of many irregular spots formed on the fruit by the disease agent cause significant quality and yield losses by covering the entire peel surface in time, depending on climate and variety sensitivity, and in conditions where aeration is low and humidity is high (Uysal et al., 2018). It is very important for producers and consumers who consume the products to offer alternative environmentally friendly innovative control opportunities to chemicals for disease agents. These innovative methods are also important in terms of preventing the use of wrong methods in the fight against the disease.

There are no studies on innovative eco-friendly control methods alternative to chemicals for the control of *Coniella granati*, which is one of the disease agents that are a problem in pomegranate growing areas. In this study, the antifungal activity of boron, sodium and potassium salts on the inhibition of mycelial growth of the fruit and crown rot fungal disease agent *C. granati*, which is an important problem in pomegranate cultivation areas, was investigated *in vitro* conditions.

MATERIALS and METHODS

Fungal Culture

In order to obtain the fungal disease agent, isolation was performed from pomegranate fruits that were brought to the laboratory and thought to be diseased (Figure 1A, B). For this purpose, small pieces were cut from the semi-infected and semi-solid fruit tissues with the help of a scalpel. The cut pieces were surface sterilized in 2% sodium hypochlorite (NaOCl) solution for 2 minutes and rinsed with sterile distilled water. The sterilized fruit pieces were placed on sterile blotting paper and left to dry. Then, dried fruit pieces were placed on the PDA (Potato Dextrose Agar) medium in petri dishes with the help of forceps. The petri dishes were incubated at 25ºC for 5-7 days. Light yellow colonies with whitish

aerial mycelia and later black globose pycnidia were observed (Figure 1C). The classical diagnosis of fungal colonies developing on PDA-containing petri dishes was defined by using species diagnostic keys after examining the morphological and microscopic features (Sutton, 1980; Barnett & Hunter, 1998; Palou et al., 2010). The fungus was purified by the single spore isolation technique. The obtained single spore isolate was taken into PDA medium in an inclined tube and stored at +4ºC.

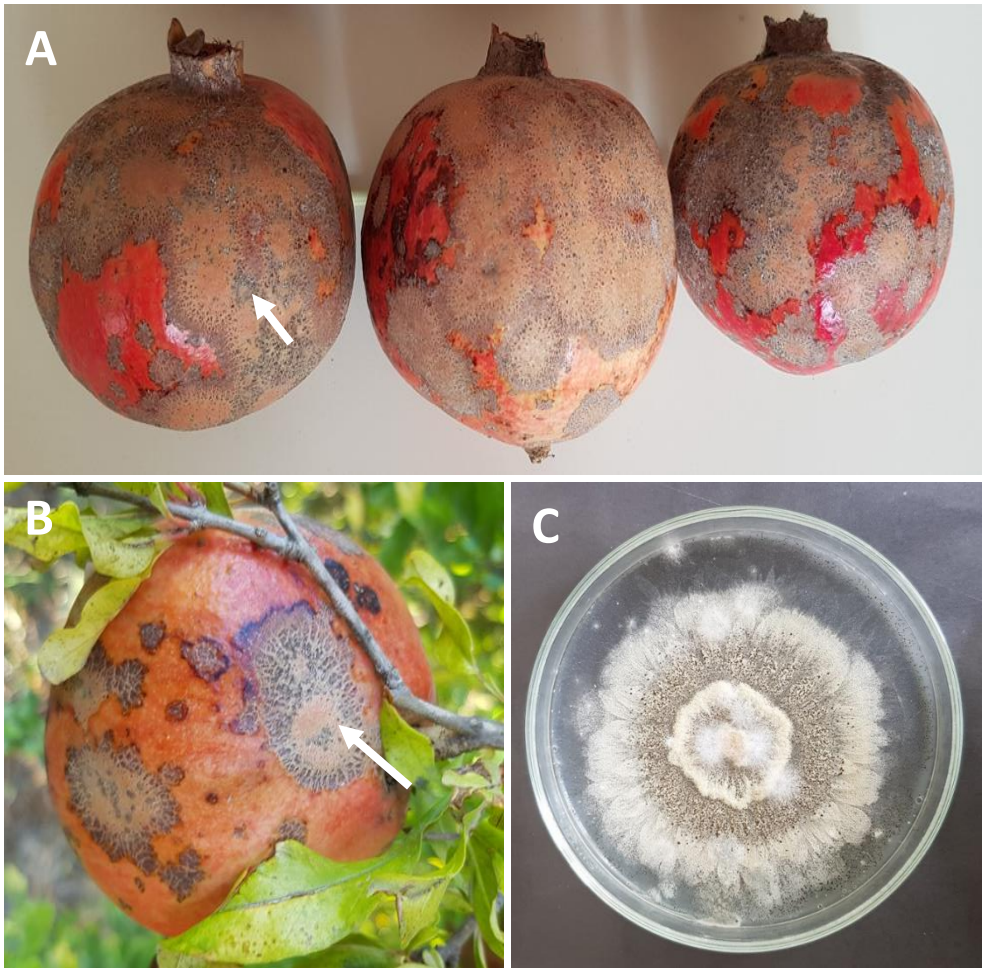

Figure 1. (A and B) Typical disease symtoms caused by *C. granati* on pomegranate fruit surface (arrows). (C) Tyical mycelial growth of *Coniella granati* on PDA nutrient medium.

Boron, Sodium and Potassium Salts

Boron salts used in the study; borax decahydrate ($Na_2B_4O_7.10H_2O$), boric acid (H_3BO_3) and etidot-67 ($Na_2B_8O_{13}4H_2O$) were obtained from the General Directorate of Eti Mining Operations (Turkey). Organic and inorganic sodium and potassium salts used in the study [sodium carbonate (Na_2CO_3), sodium nitrite ($NaNO_2$), sodium benzoate ($C_7H_5NaO_2$), and potassium sorbate ($C_6H_7KO_2$)] were purchased Merck (Darmstadt, Germany) and Sigma-Aldrich (Seelze, Germany) companies.

Determination of the Effects of Boron, Sodium and Potassium Salts on Mycelial Growth *in vitro* Conditions

Different concentrations of boron, sodium and potassium salts used in the study (0.0%, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06 and 0.07 g/l) were added to the PDA medium in 100 mL flasks sterilized in an autoclave and cooled to approximately 50ºC. It was ensured that the salts were mixed homogeneously into the whole nutrient medium. PDA medium containing different concentrations of salts was poured into sterile glass petri dishes with a diameter of 9 cm as 20 mL. 5 mm diameter mycelial discs taken from 7-10 days old *C. granati* culture, previously grown in PDA medium, were inoculated into these petri dishes. After the petri dishes were covered with parafilm, they were incubated at 24ºC for 7-10 days in the incubator. When the growth of fungi in the control petri dishes incubated with only PDA medium without salt added under the same conditions was close to coating the petri dish, the growth of the fungus in the petri dishes containing different concentrations of the control and salts was measured. The experiment was repeated 3 times for each salt concentration and was repeated 2 times.

Statistical Analysis

In vitro antifungal experiments were performed twice with at least three replications of each salt concentration. SPSS statistic program (version 17, USA) was performed for all calculations and statistical analysis. Analysis of variance was used to assess treatment effects. The significant differences between concentrations were determined by means of Duncan's Multiple Range Test ($P<0.05$). The efficient concentration (LD_{50} and LD_{90}) values for each salt were estimated by using Probit analysis.

RESULTS and DISCUSSION

The fungus was isolated from pomegranate fruits showing typical disease symptoms into PDA medium. The plates were incubated at 25°C for 5-7 days. The fungal colonies were yellowish cream-coloured and had abundant dark brown-black spherical pycnidia were (average 175–230 μm). The fungus was identified as *Coniella granati* (Saccardo) Petrak and Sydow (synonym *Pilidiella granati* Saccardo) based on morphological characteristics.

The inhibitory effects of different concentrations of boron, sodium and potassium salts (borax decahydrate, boric acid, etidote-67, sodium carbonate, sodium nitrite, sodium benzoate and potassium sorbate) used in the study on mycelial growth of *C. granati* differed (Figure 1). It was observed that the inhibitory effects on the pathogen of salts increased as the concentrations of the salts used in the study increased. Mycelial growth of *C. granati* was completely inhibited by borax at the concentration of 0.03%, etidote-67 at the concentration of 0.04%, boric acid, sodium nitrite, sodium carbonate and potassium sorbate at the concentration of 0.05%. The minimum inhibition concentrations at which inhibition occurred in all salts were statistically significantly different from all other concentrations

(P<0.05). It was determined that sodium benzoate inhibited mycelial growth at a relatively higher concentration (0.07%) compared to other applications (Table 1 and Table 2).

Table 1. Antifungal activities of different salts on the inhibition of mycelial growth (mm) of *Coniella granati*, the causal agent of pomegranate fruit and crown rot disease[a].

Doses	Borax	Boric acid	Etidote	Sodium benzoate	Sodium nitrite	Sodium carbonate	Potassium sorbate
0.0	90.00d	90.00f	90.00e	90.00h	90.00f	90.00f	90.00e
0.01	61.00cD	59.33eCD	68.00dE	80.00gF	40.33eB	56.67eC	30.33dA
0.02	20.67bA	51.33dC	24.67cA	68.33fD	31.00dB	51.33dC	23.00dA
0.03	0.00aA	44.33cE	7.33bB	48.33eF	22.00cD	43.33cE	15.00cC
0.04	0.00aA	21.33bC	0.00aA	42.33dD	11.00bB	19.67bC	7.67bB
0.05	0.00aA	0.00aA	0.00aA	31.67cB	0.00aA	0.00aA	0.00aA
0.06	0.00aA	0.00aA	0.00aA	13.33bB	0.00aA	0.00aA	0.00aA
0.07	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a
LD_{50}[b]	**0.013**	**0.019**	**0.014**	**0.031**	**0.011**	**0.018**	**0.008**
LD_{90}	**0.023**	**0.052**	**0.027**	**0.081**	**0.038**	**0.051**	**0.031**

[a] The values obtained are the average of fungus colony diameter (mm) values of 3 Petri dishes. Similar small letters next to average values in the same column or similar large letters next to average values in the row indicate that the difference between the applications is not statistically significant (Duncan's multiple range test, P<0.05).

[b] Effective concentrations of salts, inhibiting mycelial growth by 50% (LD_{50}) and 90% (LD_{90}), were determined by Probit analysis with the help of SPSS statistical program (version 17, SPSS Inc.) using the values of mycelium diameters obtained at different concentrations for each salt.

Table 2. Antifungal activities of different salts on the inhibition of mycelial growth (%) of *Coniella granati*, the causal agent of pomegranate fruit and crown rot disease.

Doses	Borax	Boric acid	Etidote	Sodium benzoate	Sodium nitrite	Sodium carbonate	Potassium sorbate
0.01	32.2	34.1	24.4	11.11	55.19	37.04	66.30
0.02	77.0	42.9	72.6	24.07	65.56	42.96	74.44
0.03	100.0	50.7	91.9	46.30	75.56	51.85	83.33
0.04	100.0	76.3	100.0	52.96	87.78	78.15	91.48
0.05	100.0	100.0	100.0	64.81	100.0	100.0	100.0
0.06	100.0	100.0	100.0	85.19	100.0	100.0	100.0
0.07	100.0	100.0	100.0	100.0	100.0	100.0	100.0

The results obtained from the study are in agreement with similar studies that revealed a positive relationship between increasing concentrations of different forms of boron, an essential microelement for plants, and their antifungal effects on mycelial growth of some plant pathogenic fungi (Qin et al.,

2010; Cao et al., 2012; Shi et al., 2012; Erper et al., 2019). Erper et al. (2019) investigated the efficacy of etidote-67 and borax decahydrate in apples against *P. expansum* both *in vitro* and *in vivo*. *In vitro* experiments, boron salts completely inhibited mycelial growth of *P. expansum* at 0.25% concentration, while these salts completely inhibited spore germination and grass tube elongation of the fungus at 0.125% concentration. They reported that the EC_{50} values of etidot-67 and borax decahydrate were 0.067 and 0.071, respectively.

The antifungal activity of organic and inorganic sodium salts against plant diseases has been investigated in previous studies (Hervieux et al., 2002; Mills et al., 2004; Latifa et al., 2011; Türkkan & Erper, 2014). Arslan et al. (2009) reported that concentrations of sodium carbonate that completely inhibited mycelial growth of different fungal disease agents were observed at 0.4% for *Macrophomina phaseolina* and *Rhizoctonia solani* AG-4 and 0.1% for *Sclerotium rolfsii*. Sodium carbonate completely inhibited mycelial growth for M. *phaseolina, R. solani* AG-4 HG I and *S. rolfsii* at 1.5%, 1.0 and 0.1, respectively. Türkkan & Erper (2015) investigated the effectiveness of 20 organic and inorganic sodium salts and two synthetic fungicides against bean root rot disease agents (*Fusarium equiseti, F. proliferatum, F. semitectum, F. solani* f. sp. *phaseoli, F. verticillioides, Rhizoctonia solani* AG4–HG I, *Macrophomina phaseolina* and *Sclerotium rolfsii*). According to *in vitro* tests, only captan, sodium benzoate and sodium metabisulfite (2%) completely inhibited mycelial growth of all disease-causing fungi. In this study, it was determined that the effectiveness of sodium benzoate on the inhibition of mycelial growth *of C. granati* was at relatively low doses.

Conclusion

As a result, some concentrations of boron, sodium and potassium salts used in this study could potentially be used in the control of pomegranate fruit and crown rot disease caused by *C. granati*. Boron, sodium and potassium salts appear to be an effective and alternative to synthetic fungicides against fungal disease agents, since they generally do not have a toxic effect on the environment and human health, and no resistance has been detected yet in disease-causing fungi against these salts.

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