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Full Length Research Paper

Degradation of postharvest applied fludioxonil in pomegranate fruit cv. 'Wonderful' and efficacy on the gray mould

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Present study aimed to determine the effects of fludioxonil (FLU) against the gray mould disease development on pomegranate fruits; and the residue levels of FLU during 150 days of storage. Pomegranate (*Punica granatum*) fruits are very susceptible to postharvest decay (*Botrytis cinerea* Pers.) and very few chemicals are approved for the use in postharvest storage. Studies conducted with 'Wonderful' cultivar pomegranate fruits at 6±1°C and 90-95% relative humidity under cold storage conditions. Results suggested that FLU can protect fruits up to 60 days without facing any gray mould problem. Results also showed that, the FLU deposits on pomegranate fruits reduced far below to the maximum residue limit (MRL) of European Union: 3 ppm within 7 days after the treatment, but continue to protect the fruits by preventing conidial germination of fungus.

Key words: Storage, *Botrytis cinerea*, fungicide, maximum residue limit.

INTRODUCTION

Pomegranate (*Punica granatum* L.) is a non-climacteric fruit (do not ripen after harvest) which can grow in many tropical and subtropical regions of the earth (Roy and Waskar, 1997). Pomegranate tree has a long history of cultivation. The inside of the fruit is full of edible arils, which are a good source of juice (Al-Said et al., 2009). The fruit juice is high in essential minerals, vitamins, organic acids, polysaccharides and phenolic compounds. During last two decades, numerous researches have been conducted about pomegranates which come with a common conclusion by referring pomegranate fruit as "super fruit" due to its health benefits (Jurenka, 2008). Furthermore, the consumption of pomegranate fruit showed considerable increase throughout the world. The year-round demand for pomegranate fruit is being meet by postharvest storage (Kahramanoğlu and Usanmaz, 2016).

The limited postharvest life of pomegranate fruit is of the most important challenge limiting its consumption. Storage of fruits under ambient conditions is limited to only a few weeks (Fawole and Opara, 2013).

Pomegranate fruits are known to be sensitive to storage conditions with three most important problems i) weight loss, ii) chilling injury, and iii) gray mould (Elyatem and Kader, 1984). Most appropriate storage conditions for pomegranate fruits is reported to be temperature between 5 and 7°C with a relative humidity of 90 to 95%. Both temperature and relative humidity are crucial for

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preventing desiccation of the skin and weight loss (Arendse et al., 2014; Kahramanoğlu and Usanmaz, 2016). However, at the same time high humidity promotes the growth of microorganisms and enhances fruit decay (D'Aquino et al., 2009). *Botrytis cinerea* Pers. is the main causes of gray mould of pomegranate fruit, which reported to have an extensive host range including ornamentals, vegetables, and fruits with more than 200 plant species (Jarvis, 1977). Infection mainly occurs in orchards via surface defects and continues in storage (Zhao et al., 2010). Fludioxonil [4-(2,2-difluorobenzo {1,3}dioxol-4-yl)-1*H*-pyrrole-3-carbonitrile] is a specific fungicide belonging to the phenylpyrroles class and reported to prevent mould decay (Kahramanoğlu and Usanmaz, 2016).

Fludioxonil (FLU) prevents the conidial germination of fungus by inhibiting the osmosensing pathway (Li and Xiao, 2008) and inhibits spore germination, germ tube elongation, and mycelium growth of *B. cinerea*. It is considered a reduced risk pesticide by the US Environmental Protection Agency (EPA) (Rosslenbroich and Steubler, 2000). However, it was also reported that fludioxonil may have some negative effects on central nervous system and may damage human health (Coleman et al., 2012). FLU has 3 mg kg^{-1} maximum residue limit (MRL) in European Union allowed for pomegranate fruits (EU Pesticide Database, 2018). The aim of the present research was to study the effects of FLU on the gray mould development on pomegranate fruits in cold storage for five months; and to determine the residue levels of FLU during storage.

MATERIALS AND METHODS

'Wonderful' cultivar pomegranate fruits were used in the present study. Fruits were harvested by hand on 8 October 2017 from a 9-year old pomegranate orchard located in Güzelyurt in Northern Cyprus. The fruits were at commercial maturity (red color, around 17% TSS "total soluble solids" and nearly 1.80% TA "titratable acidity"). The harvested fruits were immediately transported to a packing house with a ventilated truck and promptly taken into packing line to be categorized according to EU standards (CBI, 2018) and selected for uniformity in size and color. Size 10 [10 fruits in a box of 30 cm × 40 cm dimensions] fruits from "extra" category was used in the present work. Studies continued for 150 days with 30-day intervals. Totally 10 boxes of fruits were selected for the studies where half of them were treated with i) dipping in pure water [control treatment]; and the other half with ii) dipping in fludioxonil (0.06%). Each fruit was dipped for 30 s into one of the prepared solutions (Palou et al., 2007). Fludioxonil is a non-systemic fungicide. The suggested application dose 0.06% for FLU (Celest Max 100 FS, 100 g a.i. L^{-1}, Syngenta) was used in the present work. Then the fruits were dried with a packing line dryer. The experiment was designed according to completely randomized design. All fruits were placed in corrugated cartons (40 cm × 30 cm) and stored at 6.5±1°C and 90-95% relative humidity for 30, 60, 90, 120 and 150 days. Quality measurements were conducted at 30-day intervals for both treatments: i) control and ii) fludioxonil (0.06%). After each storage period (30-day intervals), a box of fruit treated with control and a box of fruit treated with FLU were removed from the storage conditions and gray mould observation performed. Gray mould observation was done according to the 0 to 4 scale reported by Palou et al. (2007): 0 - no infection; 1 - mould at only the crown; 2 - mould covering less than 25% of the rind; 3 - mould covering 25 to 50% of the rind; and 4 - mould covering more than 50% of the rind. A parallel study conducted at the same time to determine the degradation of FLU on pomegranate fruits during time. Totally 60 fruits were treated with FLU, separated into 6 groups and groups were stored for 7, 30, 60, 90, 120 and 150 days. At the mentioned days, fruits were removed from the storage rooms and FLU concentration was determined according to AOAC (Wang and Wotherspoon, 2007). Comparison of the gray mould scores of FLU treated and untreated fruits were done with independent samples t-test by using SPSS 20.0. Degradation of the FLU on pomegranate fruits during time was then tried to be expressed with correlation and regressions analysis. Comparison of the gray mould scores among the DASs were done with one-way ANOVA and the mean separation was performed with LSD test at $P < 0.05$.

RESULTS AND DISCUSSION

Results showed that FLU has a significant influence on the gray mould arising from *B. cinerea* (Westerfield et al., 2011) (Figure 1). At the untreated control fruits, it was observed that the gray mould increased during storage time. The gray mould score of the control fruits was observed as 0.7 at 30 days after storage (DAS) and 3.3 at 150 DAS, while at the same time, the gray mould score of control fruits was measured as 0.0 and 0.5, respectively.

It is clear from the results that FLU is very effective in reducing the gray mould development. It is known that *B. cinerea* infects the pomegranate flowers or crowns in the field, and then spreads to the fruit and nearby fruits under favorable conditions (Caleb et al., 2012; Mulas, 2011). In present work, gray mould found to develop during time and FLU was found to be effective in preventing the development of gray mould similar to the findings of Palou et al. (2007). The first gray mould on the fruits treated with FLU was observed after 90 DAS with only 0.2 score and the highest score was measured at 150 DAS with 0.5 score. Results showed that FLU suppress *B. cinerea* and prevents its development, thus gray mould only found in crown even in 150 DAS. These results are not surprise, where it is reported that FLU inhibits fungal protein kinase which catalyzes phosphorylation of some enzymes in biosynthesis, thus prevents the development of fungus (Rosslenbroich and Steubler, 2000). Previously Schirra et al. (2008) reported that combination of FLU with heat improves the efficacy of fungicide and reduced the concentrations of active ingredient which is required to achieve a successful control of *B. cinerea*. Thus, it might be suggested that the storage temperature could also have influence on the degradation or the effectiveness of FLU.

According to the results obtained, FLU residue on fruits decreased to 0.193±0.016 mg kg^{-1} in 7 days after application and to 0.015±0.003 mg kg^{-1} in 120 days. The highest decrease observed from 7^{th} to 30^{th} days where it showed about 51% reduction. In a similar study, Szpyrka

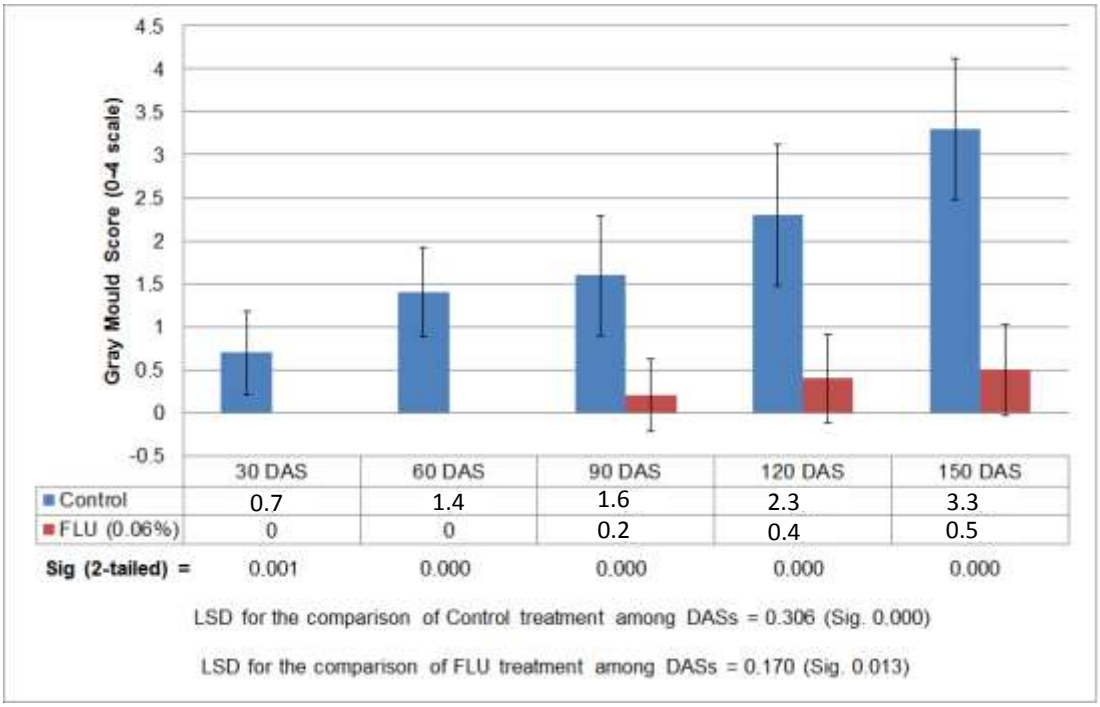

Figure 1. Effects of FLU on the development of gray mould.

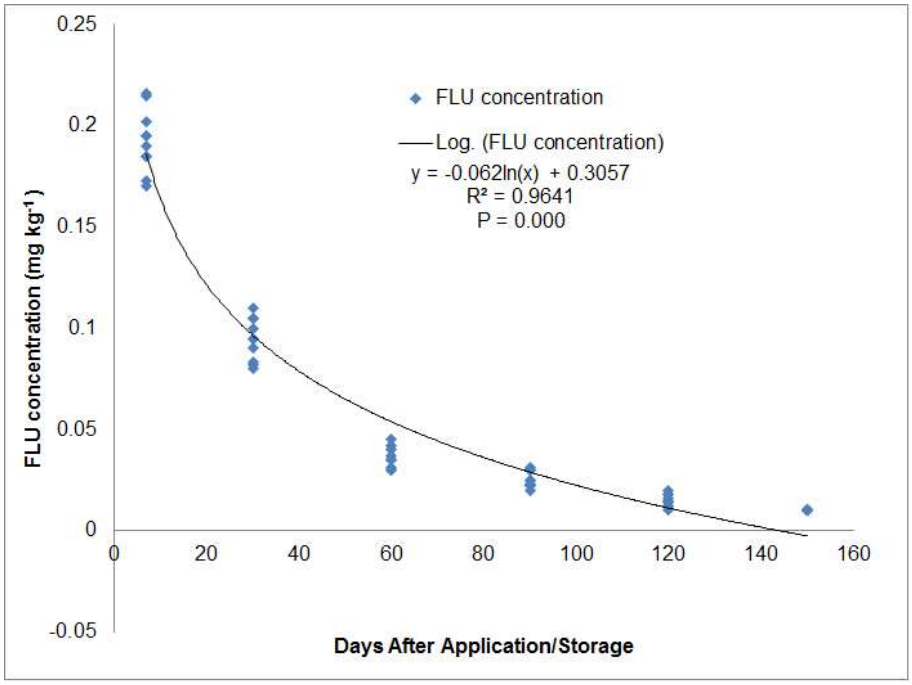

Figure 2. Correlation between the residual FLU concentration and storage duration.

and Sadło (2009) reported that FLU concentration decreases about 64% in 15 days when applied to tomatoes. After 150 DAS, the residue results were all below measurement limit (0.010 mg kg^{-1}). The FLU concentration shows a decrease according to equation y = -0.06ln(x) + 0.305 (R^2 = 0.964). Change of the residue levels according to the time is given in Figure 2. Residue analysis for FLU resulted with a high negative correlation

with time (-0.854, P > 0.01).

Conclusions

The present study showed that, within 7 days after the treatment, the FLU deposits on pomegranate fruits reduced under the MRL but continue to protect the fruits by preventing conidial germination of fungus. Degradation trends of FLU on pomegranate fruits and the gray mould development scores of present work suggest that FLU can protect pomegranate fruits within 60-days period of time. Results also suggest that, in case of high infestation by fungus, there would be a need for second treatment after 60-days period. However, further studies need to be performed to determine the degradation of FLU in case of second application. Studies about the fungicide behaviour on crops are crucial step for protection of fruits from diseases and to determine residue levels for ensuring human health.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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