

Control of spot on pomegranate fruit in field and phytosanitary quality during postharvest

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ABSTRACT: The pomegranate is a fruit with high productive potential; however, the hot and humid climate favors the occurrence of diseases that cause spots on the fruit peel, reducing their commercial value. Therefore, this study aimed to evaluate different controlling methods of spots on pomegranate fruit under field conditions and their relation with the diseases incidence during the postharvest. The experiment consisted of the following treatments: Test: control group; VM: Viçosa mixture; MZ: Mancozeb; MZ+PC: Plastic cover + Mancozeb; CZ: Carbendazim; KP: Potassium phosphite; P+C: Picoxystrobin + Ciproconazole. Evaluations were performed at 29, 51, 57, 65 and 72 days after application. The treatments MZ, MZ+PC and CZ were more efficient in reducing the severity and incidence on field, with AUDPC-Sev of 67.50; 89.72 and 198.33, respectively. At the post-harvest treatments MZ+PC, MZ, CZ and P+C, the rates of fungal incidence were lower, with 29.2, 37.5, 45.8 and 62.5%, in that order. A linear relationship was found between severity and spots incidence on field with the fungi incidence during postharvest. The Mancozeb treatment was efficient in controlling fruit spots, in addition to reducing the occurrence of diseases during the postharvest of pomegranate.

Key words: *Colletotrichum* sp.; *Punica granatum* L.; *Thyrostroma carpophilum*

Controle de mancha em frutos de romã a campo e qualidade fitossanitária em pós-colheita

RESUMO: A romã é um fruto de alto potencial produtivo, todavia, o clima quente e úmido favorece a ocorrência de doenças que causam manchas nas cascas dos frutos, reduzindo o valor comercial. Assim, o objetivo do trabalho foi avaliar diferentes métodos de controle de manchas em frutos de romã sob condições de campo, e sua relação com a incidência de doenças em pós-colheita. O experimento foi composto pelos seguintes tratamentos: Test: Testemunha; CV: Calda Viçosa; MZ: Mancozeb; MZ+CP: Cobertura plástica + Mancozeb; CZ: Carbendazim; FK: Fosfito de potássio; P+C: Picoxistrobina + Ciproconazole. As avaliações foram realizadas aos 29, 51, 57, 65 e 72 dias após a aplicação. Os tratamentos MZ, MZ+CP e CZ foram mais eficientes para redução da severidade e incidência a campo, com AACPD-Sev de 67,50; 89,72 e 198,33, respectivamente. Em pós-colheita os tratamentos MZ+CP, MZ, CZ e P+C apresentaram menor incidência de fungos, com 29,2, 37,5, 45,8 e 62,5%, nesta ordem. Foi observado relação linear entre a severidade e a incidência de manchas no campo com a incidência de fungos em pós-colheita. O tratamento com Mancozeb foi eficiente no controle das manchas nos frutos, além de reduzir a ocorrência de doenças em pós-colheita de romã.

Palavras-chave: *Colletotrichum* sp.; *Punica granatum* L.; *Thyrostroma carpophilum*

Introduction

Pomegranate (*Punica granatum* L.) is a fruit tree from temperate climates, whose ancestor has its origin in the region that includes both Transcaucasia and Central Asia, from Iran and Turkmenistan to the northern India (Holland et al., 2009; Chandra et al., 2014). It is one of the oldest edible fruits known to man and is associated with the remote Middle-Eastern civilizations, and is famous for its medicinal properties, which affect the antioxidant capacity of different parts from the plant, especially the fruit (Pal et al., 2014).

Pomegranate production in 2017 was estimated in 3.8 million tons, with India, Iran, Turkey, China and the United States of America as the main producers. However, Spain, Egypt and Israel are the most developed countries in terms of exporting, research, productive performance and the market itself (Kahramanoglu, 2019).

The pomegranate tree can be used in gardens, as a decorative plant; in the chemical industry, for producing paints; and, mainly, *in natura*, since its aril, peel and seed all have nutraceutical characteristics, promoting health when consumed (Venkitasamy et al., 2019).

Despite the few cultivated areas in Brazil, pomegranate is an option as an income source for small and medium producers, whose expansion of areas cultivated with it has been increasing over the last years (Suzuki, 2016). However, in regions with a subtropical climate, phytosanitary problems related to the occurrence of shot hole, having *Thyrostroma carpophilum* (Lév.) B. Sutton as the causal agent, and anthracnose, caused by *Colletotrichum* sp., are becoming more frequent (Bellé et al., 2018; Silva-Cabral et al., 2019) and thus compromising the fruit quality (Honorato, 2019), commercial value (Gowdar & Hugar, 2017) and the competitiveness of the Brazilian pomegranate when faced with the international market.

Studies about controlling the diseases that cause spots on pomegranate fruit are still scarce. Nargund et al. (2012), when working with fungicides and bioagents (*Trichoderma viride*) in controlling anthracnose, observed that the active ingredient Propiconazole, from the triazoles chemical group, demonstrated an efficient anthracnose control after seven applications. Whereas Cardoso et al. (2010), evaluating different fungicides and resistance inducers applied in a pomegranate orchard contaminated with anthracnose, verified that the inducers were not effective in controlling the disease, yet the fungicides, whose active ingredients were Carbendazim and Tebuconazole, were efficient in controlling it. Kumari et al. (2015) found similar results, in which Propiconazole and Carbendazim had the highest controlling percentage of spots on fruit and leaf in pomegranate. As an alternative, especially for the small rural producer, using potassium phosphite, due to its resistance-inducing action; or physical controlling by using protectors (Abd El-Rhman, 2010; Samra & Shalan, 2013), such as plastic cover, can be adopted on the disease management.

In light of the foregoing, the present study was conducted with the objective of evaluating the efficiency of different controlling methods on the management of the spots on the peels of pomegranate fruit, under field conditions, as well as its relation with the incidence of the crop post-harvest diseases.

Materials and Methods

Controlling the spots on pomegranate fruit under field conditions

The experiment was conducted in the municipality of Assaí/PR, in a commercial area with the 'Valenciana' cultivar, planted in 2011, in a 4.5 m x 2.0 m spacing, located at 23°22'26.8" S 50°52'36.8" W, with altitude of 570 m and the cfa climate (subtropical with hot summer), according to the Köppen classification. The accumulated precipitation was of 980.3 mm and the mean temperature was 23.13 °C, varying between the maximum mean of 28.66 °C and the minimum mean of 19.8 °C, as according to the historical series of climatic data collected during the experiment period, from September 25 to December 6, 2015.

The experimental design was the completely randomized having seven treatments and three replicates, with one plant per replicate with six fruits each. The treatments were composed by Test: control group, VM: Viçosa mixture (1 kg ha⁻¹ of Ubyfol Viçocalda + 1 kg ha⁻¹ of quicklime), MZ: Mancozeb 800 g kg⁻¹ (Dithane NT[®]), MZ+CP: Mancozeb 800 g kg⁻¹ (Dithane NT[®]) + plastic cover, CZ: Carbendazim 800 g L⁻¹, FK: Potassium phosphite (Reforce[®]), P+C: Picoxystrobin 200 g ha⁻¹ + Ciproconazole 80 g ha⁻¹ (Aproach prima[®]).

The treatments MZ and MZ+CP were applied seven days apart, in the 250 mL dose of commercial product and 100 L of mixture volume, using the fruit plastic cover, model "Chinese hat", for MZ+CP; the VM in the 1 kg ha⁻¹ dose of Ubyfol Viçocalda[®] (400 L ha⁻¹) and 1 kg ha⁻¹ of quicklime, spaced seven days apart; the FK and CZ treatments at an interval of 14 days, in doses of 2 and 1 L ha⁻¹ of the commercial product, respectively; and the P+C treatment at a commercial product dose of 400 mL ha⁻¹, in three applications (early sprouting, full flowering and 28 days after flowering).

All products were applied using an electric backpack sprayer (brand: Yamaho[®], model FT16), with capacity for 16 L and two nozzles (Magno fan no. 03), with pressure of 40 lb, during the morning.

The analyzed variables were incidence and severity of spots on pomegranate fruit under field conditions, with the severity evaluated based on an eight-point scale (0; 5; 15; 25; 40; 60; 80; and 100%) (Figure 1) adapted from the proposed by Gowdar & Hugar (2017) and the incidence by the number of fruit with symptoms.

The disease control started when 80% of the orchard was at stage C:09 (red tip), with the fruit harvesting occurring from stage L:81 to 85 (fruit ripening), according to the phenological scale described by Melgarejo et al. (1997). The evaluation of the incidence and severity of spots was performed from

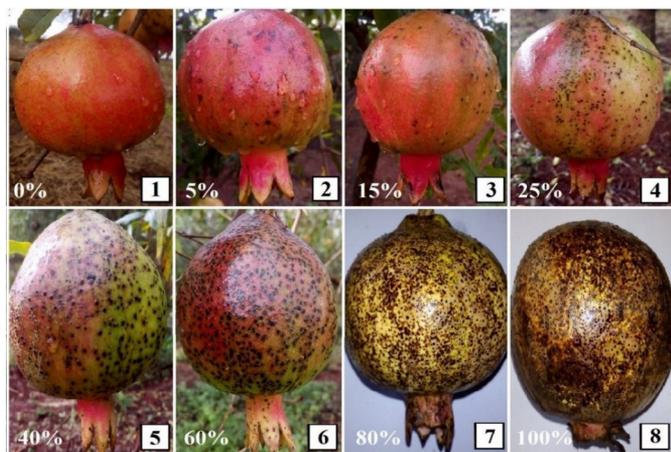


Figure 1. Scale adapted from the one prepared by Gowdar & Hugar (2017) to evaluate the severity of pomegranate fruit spots.

the early stage J:73 (fruit growth) onwards, totaling five evaluations, at 29, 51, 57, 65 and 72 days after application.

With the data from the incidence and severity, the area under the disease progress curve was calculated considering both severity (AUDPC-Sev) and incidence (AUDPC-Inc), using the formula of Campbell & Madden (1990), Equation 1.

$$\text{AUDPC} = \sum [(y_i + y_{i+1}) \times 0.5] \times (t_{i+1} - t_i)$$

in which: y = severity or incidence of spots in the i -th evaluation and t = time at the time of the i -th evaluation.

Fungi incidence during postharvest

The test was conducted at the Phytopathology Laboratory from the Department of Agronomy, part of the State University of Londrina (UEL). The experimental design used was the completely randomized one, composed of the same treatments as the previous test with three replicates, having two fruit as the experimental unit.

Fruits under field conditions were collected with plastic boxes, then packed in polypropylene plastic and immediately transported to the laboratory. These fruits were wounded with a perforator at four points equidistant from their respective equatorial zone and placed in a humid chamber,

with mean temperature of 20 ± 2 °C under white fluorescent light bulbs (40 cm distance) and a 12-hour photoperiod, for five weeks. After incubation, they were evaluated with the aid of a magnifying glass and a stereoscopic microscope (Brand: Olympus) in order to determine the occurrence of fungi fructification.

Statistical analysis

The data obtained from the analysis of severity and spots incidence on the peel from pomegranate fruit under field and post-harvest conditions were submitted to analysis of variance and the means compared by the Scott-Knott test at the 5% significance level. Assumptions of error normality and variance homogeneity were tested by means of the Shapiro-Wilk and Bartlett tests at the 5% significance level.

Data on severity (X_1) and incidence (X_2) of the fruit spots were analyzed with multiple linear regression, in order to explain the disease incidence during the postharvest (Y) of pomegranates. Thus, the model was defined based on the Akaike information criterion (AIC) and the Bayesian inference (BIC) through the Stepwise algorithm. The assumptions of error normality (Shapiro-Wilk) and multicollinearity were tested in order to validate the model. All analyzes were processed by using the R software (R Core Team, 2019).

Results and Discussion

Controlling spots on pomegranate under field conditions

There was a significant effect of treatments for the variable severity and AUDPC-Sev. All treatments demonstrated a lower AUDPC-Sev when compared to the control group. The treatments with application of Mancozeb (T4), Mancozeb + plastic cover (T6), Carbendazim (T6) and Viçosa Mixture (T2) had the lowest severity in all evaluations held. The same treatments, except for T2, had the lowest AUDPC-Sev with 89.72, 67.50 and 198.33, respectively (Table 1).

For the variables fruit spot incidence and AUDPC-Inc, treatments T3 and T4 had the lowest disease incidence, similar to what was found regarding the severity variable (Table 2). Treatments based on potassium phosphite and Picoxystrobin + Ciproconazole did not demonstrate any statistical difference when compared to the control group.

Table 1. Severity, area under the disease progress curve of severity (AUDPC-Sev.) from fruit spots (*Colletotrichum* sp. and *T. carpophilum*) in function of applying fungicides and leaf fertilizer in a commercial cultivation of Pomegranate (Londrina-PR, 2015).

Treatment	Severity of fruit spots (%)					AUDPC-Sev.
	24/Oct	15/Nov	21/Nov	29/Nov	06/Dec	
Control group	24.17 C	63.61 D	72.22 D	88.89 C	92.22 C	2651.39 E
Viçosa mixture	3.61 A	6.67 A	10.83 A	11.94 A	13.61 A	346.11 B
Mancozeb	1.67 A	1.95 A	1.95 A	2.78 A	2.78 A	89.72 A
Mancozeb + Plastic Cover	1.11 A	1.67 A	1.67 A	1.67 A	2.22 A	67.50 A
Carbendazim	3.61 A	4.17 A	4.17 A	6.11 A	7.22 A	198.33 A
Potassium Phosphite	13.17 B	40.61 C	55.28 C	64.72 B	77.78 B	1857.97 D
Picoxystrobin + Ciproconazole	9.72 A	31.67 B	45.28 B	64.72 B	67.78 B	1589.86 C
CV (%)	60.99	18.39	20.26	15.64	17.35	8.88

Means followed by the same letter do not differ from each other by the Scott-Knott test ($p \geq 0.05$).

Table 2. Incidence and area under the disease progress curve of incidence (AUDPC-Inc) from fruit spots (*Colletotrichum* sp. and *T. carpophilum*) in function of applying fungicide and foliar fertilizer in a commercial cultivation of Pomegranate (Londrina-PR, 2015).

Treatment	Incidence of fruit spots (%)					AUDPC-Inc.
	24/Oct	15/Nov	21/Nov	29/Nov	06/Dec	
Control group	100.0 B	100.0 B	100.0 B	100.0 B	100.0 B	56.6 C
Viçosa mixture	72.2 B	83.3 B	88.9 B	100.0 B	100.0 B	43.9 B
Mancozeb	33.3 A	38.9 A	38.89 A	55.6 B	55.6 A	21.0 A
Mancozeb + Plastic Cover	22.2 A	33.3 A	33.3 A	33.3 A	44.4 A	18.5 A
Carbendazim	72.2 B	83.3 B	83.3 B	100.0 B	100.0 B	45.4 B
Potassium Phosphite	100.0 B	100.0 B	100.0 B	100.0 B	100.0 B	55.4 C
Picoxystrobin + Ciproconazole	88.9 B	100.0 B	100.0 B	100.0 B	100.0 B	53.0 C
CV (%)	18.77	12.5	13.23	4.32	6.00	7.1

Means followed by the same letter do not differ from each other by the Scott-Knott test ($p \geq 0.05$).

The results do not corroborate with those obtained by Nargund et al. (2012), in which the treatment with Propiconazole (Triazole) at a 0.1% concentration was significantly higher than other fungicides in controlling anthracnose, with the Ciproconazole, an active ingredient from the same chemical group, not resulting in efficient controlling of diseases in the present study.

As for the treatments with Carbendazim and Mancozeb, similarity was found with other experiments available in the literature (Gaikwad, 2000; Patel, 2009), in which the Carbendazim molecule, together with Mancozeb, demonstrates a positive response in the controlling percentage of the fruit spots on pomegranate, emphasizing that the ingredients can be used by themselves or in a mixture. Cardoso et al. (2010) found a similar result, obtaining the best responses for treatments based on Carbendazim and Tebuconazole.

The results of the treatment with potassium phosphite did not differ from the control group, which corroborates with Cardoso et al. (2010), in which the authors observed no statistical difference from the control group with the application of potassium phosphite. Using phosphites for controlling phytopathogenic species depends on several environmental factors and, above all, on the host susceptibility, and may have no effect; or have direct effects, by inhibiting the mycelial growth and the spore production, or then indirect, by stimulating the defense responses of the host (Lobato et al., 2010; Eshraghi et al., 2011).

The treatment with Mancozeb associated with the plastic cover did not significantly differ from the treatment with only Mancozeb, indicating that its adoption did not contribute effectively in controlling the spots on pomegranate peels under field conditions. Shlomo (2015) found contradictory results in terms of reducing the incidence of pests and diseases, especially in the initial stages after the setting the cover up, hence concluding that such a technique should be better studied, since the high demand for labor and its using complexity can preclude the technique.

Hollomon (2015) mentions Mancozeb as a multi-site fungicide, which contributes in managing the disease resistance. Corroborating with the present study, which, even though it was conducted in a rainy year and therefore suffered

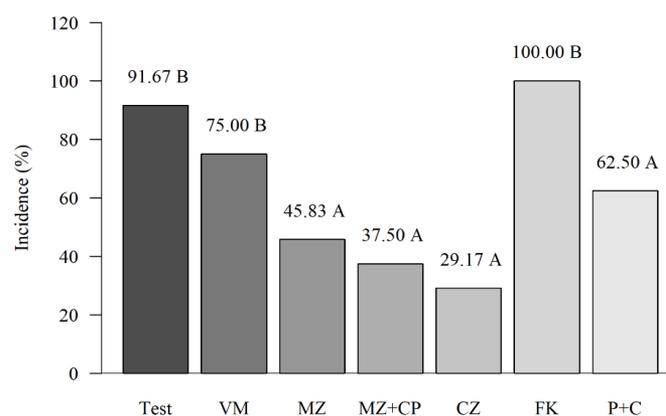
possible losses from the product protective action, Mancozeb stood out in controlling spots on pomegranate peel.

The results in controlling anthracnose, on pomegranate fruits, through the biweekly application of Carbendazim consolidated with those obtained by Cardoso et al. (2010), showing to be efficient in controlling the disease.

In a study of the *Thyrostroma carpophilum* control, in species from the genus *Prunus*, Alves et al. (2011) found an efficient control of shot hole (*T. carpophilum*) in peaches when using Mancozeb and methyl thiophanate, similarly to the results found in the present study. However, to date, no study has been published regarding the controlling of pomegranate peel spots caused by *T. carpophilum*, according to the consulted literature.

Fungi incidence during the postharvest

The treatments Carbendazim, Mancozeb + plastic cover, Mancozeb and Picoxystrobin + Ciproconazole had a fungi incidence during postharvest of 29.17%; 37.5%; 45.83% and 62.5%, respectively, statistically superior to the other evaluated treatments (Figure 2). This is related to the proper



Means followed by the same letter do not differ from each other by the Scott-Knott test ($p \geq 0.05$). CV = 22.46%.

Figure 2 . Fungus incidence in pomegranate postharvest submitted to different chemical treatments (Test: Control group; VM: Viçosa mixture; MZ: Mancozeb; MZ+CP: Mancozeb + Plastic Cover; CZ: Carbendazim; FK: Potassium Phosphite; P+C: Picoxystrobin + Ciproconazole) during the crop development (Londrina-PR, 2015).

management throughout the crop development, the fruit inoculum reduction and the reduction of fungal diseases incidence during postharvest, especially in the treatments with systemic fungicides.

Multiple linear regression analysis

Multiple linear regression analysis, considering the complete model and using the severity (X1) and incidence (X2) parameters, indicated a significant effect for the model coefficients (Figure 3). Thereby, the severity and incidence of fruit spots on the pomegranate pre-harvest are directly related to the post-harvest disease incidence ($Y = 0.3995X1 + 0.53574X2$, $R^2 = 0.91$).

Ritenour et al. (2004) found that applying systemic fungicides such as methyl thiophanate and Benomil during the pre-harvest reduced by half the rot incidence in the postharvest of citrus fruits. Hence, based on the obtained results, it is suggested that the management of post-harvest diseases of pomegranate before and after the harvesting should be performed, as it reduces the inoculum potential on them, reinforcing the preventive and eradicating techniques of phytopathogens proposed by Senhor et al. (2009) and Montecalvo et al. (2019).

In conclusion, it is noteworthy mentioning that fungicide applications may not reduce the diseases incidence on pomegranate peel, such as those caused by the fungi *Colletotrichum* sp. or *Thyrostroma carpophilum*, yet if they reduce the number of lesions on their surface, they can be used in the integrated disease management of this crop, thus associating with other controlling measures in the fruit post-harvest treatment (Tatagiba et al., 2002).

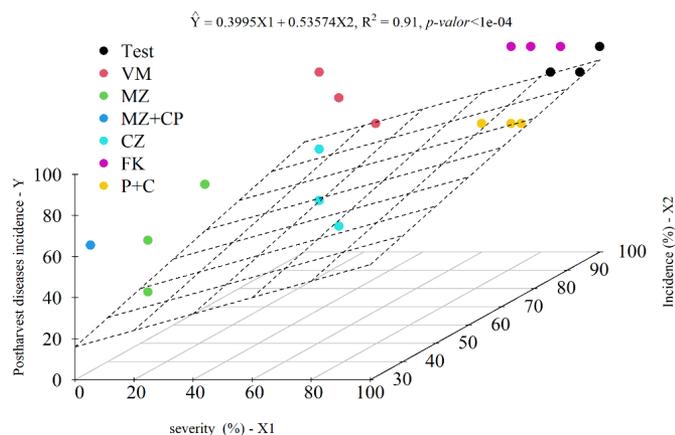


Figure 3. Multiple regression analysis between the independent variables fruit severity (X1) and field incidence (X2) with the dependent variable (Y) post-harvest diseases incidence.

Conclusions

The application of Mancozeb is efficient in controlling the spots on pomegranate fruit.

The treatments Mancozeb, Mancozeb + plastic cover, Carbendazim and Picoxystrobin + Ciproconazole resulted in a lower fungi incidence in post-harvest fruits.

The severity and incidence of spots on pomegranate fruits under field conditions have a direct relation with the fungi incidence during postharvest.

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