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PREBIOTIC, *Bacillus subtilis* AND POTASSIUM PHOSPHITE ON ANTHRACNOSIS SEVERITY AND POST HARVEST QUALITY IN 'MÉNDEZ' AVOCADO

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ABSTRACT

Several factors affect the quality and safety of avocado (*Persea americana* Mill.) fruit during the production process, such as the agronomic management and climate. The objective of this research was to evaluate how foliar treatments affected postharvest control of anthracnose (*Colletotrichum* spp.) in two 'Méndez' avocado growing areas, Ziracuaretiro (ZI) and Salvador Escalante (SE), Michoacán, Mexico. Foliar sprays were used in the following treatments: 1) conventional management (CM) based on the technician's experience; 2) integrated management (IM) including *Bacillus subtilis*, a prebiotic (BioKakimu®), and potassium phosphite; and 3) absolute control without foliar sprays. The effects of the treatments were evaluated on fruit harvested at physiological maturity while taking into account the severity of post-harvest anthracnose and fruit quality up to consumption maturity. During the evaluation period, disease damage was less than 10 % of the fruit surface in all three treatments. However, IM treatment delayed maturation time by 12.4 days in ZI and 10.6 days in SE. Fruits of the IM treatment lost less weight in ZI (7.6 %) than in SE (8.48 %) at eating maturity. Fruit firmness in ZI was higher in the IM and control treatments, while there were no differences in SE. The fruits in ZI and SE showed a decreasing color index profile for the three treatments. It is concluded that the phytopathogenic fungus in 'Méndez' was not exposed to weather conditions favorable for its development for an extended period of time, given that the first anthracnose alerts were emitted when the fruits were harvested. The crop management method did not result in an increase in the nutrient concentration of the fruit mesocarp.

Keywords: *Colletotrichum*, *Persea americana* Mill., Phi.

INTRODUCTION

The *Colletotrichum* genus contains several species of phytopathogenic fungi of great economic and sanitary importance, causing considerable losses due to fruit lesions during storage and marketing (Udayanga *et al*., 2013). *Colletotrichum* infection occurs in avocado fruit (*Persea amaeicana* Mill.) on horticultural orchard trees prior to harvest when the conidium germinates and forms appressoria (Bruce da Silva and Michereff, 2013). However, due to quiescence of the pathogen, the disease becomes important during postharvest. Economic losses caused by to this fungus can amount up to 20 % of

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the total fruit production (Tapia-Rodríguez *et al*., 2020). Currently, chemical fungicides are used to control *Colletotrichum* species. However, their indiscriminate use represent a risk to human health (Gupta, 2017), environmental damage (Dong *et al*., 2013), and fungal resistance development (Fisher *et al*., 2018).

Due to the above, an adequate and effective management of this disease can be carried out through the use of antagonistic microorganisms that inhibit the growth of phytopathogens. Several antagonistic bacteria that secrete lytic enzymes are able to dissolve the fungal cell wall, resulting in fungal pathogen predatory activity (Xu *et al*., 2014). On the other hand, prebiotics are gaining popularity because they stimulate the antagonistic action of microbiota and may help control anthracnose while reducing the use of chemical pesticides (Guardado-Valdivia *et al*., 2018)Organisms found on avocado tree leaves have been associated to anthracnose control, such as *Wickerhamomyces anomalus* or *Candida intermedia* (Campos-Martínez *et al*., 2016). Oligosaccharides are a type of prebiotic substance, their main function is to serve as a source of energy to the antagonistic microorganisms group. There are preliminary studies on the use of prebiotics for the control of *Colletotrichum* in tree tomato (*Solanum betaceum* Cav.), using prebiotics and potassium fertilization to the soil, obtaining satisfactory results (Luengas-Gómez *et al*., 2012).

The use of potassium phosphite as an inducer of induced resistance (IR), which is a natural mechanism developed by plants to increase their resistance level to biotic or abiotic stresses, has become relevant (Machinandiarena *et al*., 2012). Many of the published works with phosphite are related to the control of oomycetes, and in some of them, there was an effective control of other fruit and vegetable crop pathogens, such as *Colletotrichum* spp. (Ogoshi *et al*., 2013; Costa *et al*., 2018). However, so far there has been no report on avocado.

Systemic fungicides are effective for disease control; however, active ingredients for disease management in avocado are limited. Therefore, the objective of this research was to determine the effect of a prebiotic, *Bacillus subtilis,* and potassium phosphite, as well as climatic conditions, on anthracnose control and postharvest quality of 'Méndez' avocado.

MATERIALS AND METHODS

The research was conducted out in two commercial 'Méndez' avocado orchards in the state of Michoacán, Mexico: orchard one, located in the municipality of Ziracuaretiro (ZI), at an average altitude of 1389 m; and orchard two, in the town of Jujucato, municipality of Salvador Escalante (SE), at an average altitude of 2070 m. The ZI orchard consisted of 18-year-old trees, and the SE orchard was composed of 10-year-old trees, both with the cv. 'Méndez' grafted on rootstocks originating from the Mexican race seed. They were established under a rectangular frame planting system with a row and tree spacing of 9 x 7 m, respectively. Agronomic management in both orchards included: chemical and organic fertilization, weeding, pest and disease control, and pruning. Micro-sprinkler irrigation was used from January to May.

Weather conditions

Climate data for precipitation (PP), temperature (T) and relative humidity (RH) for 2019 and 2020 were consulted in the iMetos 3.3 weather service (www.fieldclimate.com).

Treatments and experimental design

The pre-harvest treatments for *Colletotrichum* spp. Control were as follows: 1) conventional management (CM), according to the list of recommended pesticides for avocado cultivation (APEAM, 2014), which includes chemical control of pests and diseases by using foliar sprays; 2) integrated management (IM), by the application of foliar sprays of the following commercial products in the concentrations recommended by the manufacturer: 1 mL BioKakimu® L^{-1}, 1 g Fungifree® L^{-1}, 1 mL Nutri-Phite Magnum® L^{-1}, and the surfactant adherent Inex® (2 mL L^{-1}) were added at 15 to 20 day intervals from February to August in ZI and February to September in SE, for a total of 7 and 11 applications, respectively, pest control was carried out with chemical pesticides; and 3) absolute control. A completely randomized design was used with 4 homogeneous experimental units per treatment consisting of nine trees. From each treatment, avocado fruits were harvested at physiological maturity (26 and 29 % dry matter in ZI and SE, respectively). Subsequently, they were stored until consumption maturity under ambient conditions.

Post-harvest evaluation

For each treatment, 100 fruits were harvested from the four experimental units for evaluation of each variable. Anthracnose severity was evaluated in a sample of 20 fruits according to a hedonic scale: light damage (LD) when less than 10 % of the fruit surface had symptoms, such as dark lesions and rot; moderate damage (MD) when 10–20 % of the fruit surface was damaged; severe damage (SD), more than 20 % of the fruit surface had symptoms; and severe damage (SD) when more than 20 % of the fruit surface had symptoms.

Weight loss (differences in weight at consumption maturity with respect to the initial weight corresponding to physiological maturity) was measured daily with a digital scale on 10 fruits per treatment and expressed as a percentage. Mesocarp firmness was determined with a Chatillon texturometer (Wagner Force Five model FDV-30, Greenwich, USA) with a 7 mm conical probe at two opposite points of the equatorial zone of five fruits every two days and reported in Newtons (N).Exocarp color (EC) was measured in 10 fruits (the same used to evaluate weight loss) with a Hunter Lab reflection colorimeter, model D25 (Reston, VA, USA), by using the CIE Lab system to determine the values L*, a* and b* to calculate the color index ($IC=-10a^* b^*/L^*$) (Zarazúa-Escobar *et al*., 2005). The concentrations of P, K, Ca, and Mg were determined in four fruits as described by Alcántar-González and Sandoval-Villa (1999). The N concentration was determined by distillation of the sample and titration with sulfuric acid.

Statistical analysis

Analyses of variance were performed for the studied variables and treatment means were compared with Tukey's test ($p \leq 0.05$).

RESULTS AND DISCUSSION

In both municipalities, the rainy season began in May; there was more PP in SE (1666 mm) than in ZI (1263 mm), concentrated between July and October. Regarding T, in ZI the average maximum and minimum of the hottest (May and June) and coldest (January and February) months were 20, 21 and 15, 16 ˚C, respectively; in SE, the average maximum and minimum of the same months were 17.5, 17.4, and 12.7, 14 ˚C, respectively. September was the month with the highest RH in ZI and SE (91.35 and 93.16 % monthly average, respectively).

Regarding anthracnose damage on avocado fruit in both orchards, this was predominantly light, with less than 10 % of the fruit surface showing symptoms in the three treatments ($p \leq 0.05$), so the low incidence of the disease is ruled out as an effect of treatments. This study confirmed that both T and PP, as well as the monthly RH, are important factors in the incidence of anthracnose. The absence of MD and SD in 'Méndez' fruit could be attributed to the fruit not being exposed long enough to environmental conditions that favored the development of the fungus.

Tapia-Rodríguez e*t al.* (2020) mentioned that temperature, precipitation and monthly relative humidity are factors of great importance in the incidence of anthracnose. According to Bruce da Silva and Michereff (2013), the disease is severe with temperatures ranging from 24 to 28 ° C and high relative humidity, which could explain the LD found in the fruits from both locations. One explanation for the post-harvest LD of anthracnose in the three treatments is the orchard background, which included fertigation practices, pruning, weed control, chemical and biological pest and disease control, and chemical and organic fertilization; these are orchards typified as highly technical (Ramírez-Legarreta and Jacobo-Cuéllar, 2002).

Many of the causes of postharvest quality impairment and physiological disorders are often attributed to preharvest handling (Ferguson *et al*., 1999), which explains the quality and control of postharvest anthracnose in fruit from the orchard under study. Another reason for the predominantly light post-harvest anthracnose damage observed is that the fruits were not exposed for an extended period of time to weather conditions conducive to the development of the fungus, as the first anthracnose prediction alerts were issued in early June and the fruits were harvested in July and September in ZI and SE, respectively. Ávila-Quezada *et al.* (2002) mentioned that favorable environmental conditions are required during at least 4 hours for infection by *Colletotrichum gloesporioides* to occur.

At eating maturity, weight and firmness loss in SE were greater in the three treatments than in ZI (Tables 1 and 2); thus, the higher the dry matter (DM) percentage and the higher the altitude, the greater the weight and firmness loss, and the lower the durability. According to López-López and Cajuste-Bontemps (1999), physiological

Table 1. Weight loss of 'Méndez' avocado fruit from two commercial orchards (Ziracuaretiro (ZI) and Salvador Escalante (SE), Michoacán, Mexico), harvested and stored at room temperature (24±2 and 22±2 °C, respectively) until consumption maturity under conventional management, integrated management and no applications (control).

Treatment	Day									
	1	2	3	4	5	6	7	8	9	10
					ZI					
CM	0	0.8±0.1a	1.8±0.3a	2.6±0.5ab	3.7±0.7a	4.6±0.9a	5.4±1.1a	6.6±1.3a	7.4±1.6a	8.5±1.8a
IM	0	0.7±0.1b	1.5±0.2b	2.3±0.3b	3.2±0.4a	4.1±0.5a	4.8±0.7a	5.8±0.8a	6.6±0.9a	7.6±0.0a
Control	0	0.8±0.1a	1.9±0.3a	2.8±0.4a	3.8±0.5a	4.8±0.7a	5.7±0.7a	6.9±0.8a	7.8±0.9a	8.9±1.0a
					SE					
CM	0	1.2±0.2a	2.6±0.2a	4.1±0.3a	5.4±0.4a	8.4±0.8a	10.0±1.0a			
IM	0	1.0±0.2a	2.1±0.3b	3.2±0.4b	4.2±0.5b	6.4±1.0b	7.5±1.1b			
Control	0	1.3±0.3a	2.7±0.5a	4.3±0.7a	5.7±1.0a	8.4±1.4a	8.7±0.5ab			

a,b Means with different letters on each evaluation day and orchard indicate statistical difference (Tukey; $p \leq 0.05$); n = 4. CM: conventional management; IM: integrated management. Means ± standard deviation.

Table 2. Firmness of 'Méndez' avocado fruit from two commercial orchards (Ziracuaretiro (ZI) and Salvador Escalante (SE), Michoacán, Mexico), harvested and stored at room temperature (24±2 and 22±2 °C, respectively) until consumption maturity under conventional management, integrated management and no applications (control).

Treatment	Day				
	2	4	6	8	9
			ZI		
CM	17.5±0.2a	15.5±0.4b	12.2±0.7a	10.5±0.3b	8.3±0.3b
IM	17.4±0.2a	16.8±0.5a	12.4±0.7a	12.0±0.7a	9.6±0.7a
Control	16.3±0.4b	15.8±0.6b	11.5±0.7a	10.4±0.5b	9.4±0.3a
			SE		
CM	17.7±0.5a	15.3±0.1a	12.3±0.5a	0.3±0.2a	
IM	18.5±0.7a	15.4±0.4a	12.6±0.5a	0.7±0.7a	
Control	15.8±0.3b	14.8±0.3a	12.2±0.5a	0.1±0.2a	

a,b Means with different letters on each evaluation day and orchard indicate statistical difference (Tukey; $p \leq 0.05$); n = 4. CM: conventional management; IM: integrated management. Means ± standard deviation.

weight loss is associated with flowering and altitude, with medium and high altitudes (1700 and 2100 m) and advanced flowering reporting higher values. These results differ from those reported by Fischer *et al.* (2019), who concluded that fruits produced at higher altitudes (2580 m) have greater weight, showing less weight loss and firmness in postharvest. Weight loss and dehydration during storage and ripening determine the shelf life and quality of ripe avocados (Escobar *et al*., 2019).

Avocado firmness correlates well with maturity, decreasing gradually from harvest to a smooth flesh texture (Uarrota *et al*., 2020). Even though the weight loss in SE was greater than in ZI, the fruits of each treatment at maturity for consumption presented an external and internal appearance that was free of damage, and there were no fruits with dehydration symptoms. The data obtained for the external color variable (CI) show that at the time of harvest, fruits from SE had higher values than those from ZI. Fruits with a higher chromatic value also had a higher oil content (Table 3). These ZI results match those reported by Rosas-Flores *et al*. (2016) who found a decrease in IC in the exocarp of avocado fruit cv. 'Mendez' during ripening, from 37.06 to 2.17. There was no significant effect of treatments on the 'Méndez' avocado exocarp color ($p \leq 0.05$).

Table 3. Color index of 'Méndez' avocado fruit from two commercial orchards (Ziracuaretiro (ZI) and Salvador Escalante (SE), Michoacán, Mexico), harvested and stored at room temperature (24±2 and 22±2 °C, respectively) until consumption maturity under conventional management, integrated management and no applications (control).

Treatment	Day						
	1	2	3	4	5	6	7
				ZI			
CM	32.2±3.7a	30.3±5.9a	28.2±4.3a	22.7±2.4a	18.4±1.6a	15.2±2.9a	13.3±3.3a
IM	34.4±2.6a	29.5±4.9a	23.9±4.6a	17.6±3.1b	14.2±2.1b	13.9±2.8a	10.5±2.3a
Control	35.4±1.9a	31.8±3.5a	24.5±2.1a	21.4±2.9ab	17.3±4.4ab	12.4±2.5a	11.4±4.1a
				SE			
CM	46.7±6.7a	43.7±5.1a	43.4±3.5a	40.6±6.2a	38.3±2.4a	34.9±3.1a	31.2±2.6a
IM	42.6±5.3a	41.0±5.7a	39.2±4.6a	38.1±4.5a	34.1±5.5ab	31.4±5.4a	30.0±2.6a
Control	41.0±0.9a	40.9±2.6a	38.7±0.6a	34.6±6.7a	30.6±6.9b	9.1±4.5b	6.4±4.5b

a,b Means with different letters on each evaluation day and orchard indicate statistical difference (Tukey; $p \leq 0.05$); n = 4. CM: conventional management; IM: integrated management. Means ± standard deviation.

The nutritional analysis of the mesocarp of ZI fruits (Table 4) shows that the IM treatment had a higher concentration of P (0.24 %) compared to the CM and control treatments (0.19 and 0.17 %, respectively). This difference may be attributed to the application of potassium phosphite; however, in the SE location, this response was not present, although it presented higher concentrations of Ca and Mg. Salazar-García *et al*. (2019) indicate that, in addition to the type of management and avocado cultivar, there are many factors that could be responsible for the nutrient concentration per treatment and per orchard. On the other hand, K concentrations were low and medium for N, P, Ca, and Mg at both locations. With the exception of K, the concentrations of N, P, Ca, and Mg in the fruit are similar to those reported for the cv. 'Méndez' by Salazar-García *et al.* (2021).

Table 4. Macronutrient concentration (dry matter) in the mesocarp of 'Méndez' avocado fruit from two commercial orchards (Ziracuaretiro (ZI) and Salvador Escalante (SE), Michoacán, Mexico), under conventional management (CM), integrated management (IM) and without applications (control).

Location	Treatment	(%)				
		N	P	K	Ca	Mg
ZI	CM	1.13±0.08a	0.19±0.00b	0.70±0.40a	0.09±0.00b	0.08±0.00b
	IM	1.27±0.18a	0.24±0.01a	0.68±0.08a	0.08±0.00b	0.09±0.00a
	Control	1.12±0.1a	0.17±0.00b	0.58±0.03a	0.10±0.00a	0.07±0.00b
SE	CM	0.84±0.08a	0.16±0.00ab	0.58±0.04a	0.09±0.00c	0.07±0.00b
	IM	0.71±0.18b	0.12±0.01b	0.50±0.08b	0.11±0.00a	0.08±0.00a
	Control	0.70±0.1b	0.18±0.00a	0.55±0.03a	0.10±0.00b	0.07±0.00b

a,b Means with different letters in each nutrient and orchard indicate statistical difference (Tukey; $p \leq 0.05$); n = 4. CM: conventional management; IM: integrated management. Means ± standard deviation.

CONCLUSIONS

The 'Méndez' avocado fruit did not show anthracnose damage because fruit development coincided with the dry season and was harvested before being exposed to anthracnose damage. It is convenient to include other locations, agricultural cycles and times of the year where climatic conditions are more conducive to disease development.

Fruits with a high dry matter content and harvested at higher altitude showed greater weight loss and decreased firmness. Fruits from both locations showed a decreasing color index profile. The type of crop management had no effect on the nutrient concentration of the fruit mesocarp.

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