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Research Article

Sensitivity of *Colletotrichum gloeosporioides* Isolates from Diseased Avocado Fruits to Selected Fungicides in Kenya

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Colletotrichum gloeosporioides is a serious postharvest pathogen of avocado fruits worldwide. Kenya lacks any registered fungicides for the management of the disease. Nevertheless, farmers commonly use commercially available fungicides such as Bayleton 25WP (Triadimefon 250 g/Kg), Milraz 76WP (Propineb 70% and Cymoxanil 6%), and Copper oxychloride 500WP for disease management. The efficacy of these fungicides against *C. gloeosporioides* is not known. The purpose of this study was therefore to test the inhibitory effect of these fungicides against 46 *C. gloeosporioides* isolates from avocado fruits collected from varieties grown at different agroecological zones in Murang'a County, a popular avocado-growing region in Kenya. Mycelial growth rate and sporulation for each isolate were measured *in vitro* on PDA plates amended with different concentrations of the fungicides. Plates were arranged in a completely randomized design with three replications per treatment. All fungicides were effective *in vitro* but there were significant differences in sensitivity among isolates. Bayleton had the highest mycelial inhibition followed by Milraz, while copper oxychloride had the lowest mycelial inhibition rates, ranging from 81% to 88%. However, copper oxychloride was more effective in inhibiting sporulation. The inhibitory effect of each fungicide was concentration-dependent, where twice the recommended concentration had the highest inhibitory effect, followed by the recommended concentration. Our results show that the fungicides used by farmers against *C. gloeosporioides*, the causal agent for anthracnose, are effective. We, however, recommend further field tests in different avocado-growing areas so as to validate their efficacy against various isolates and under different environments.

1. Introduction

Anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz & Sacc. has been reported on a wide variety of crops, such as avocado, almond, coffee, guava, apple, dragon fruit, cassava, mango, sorghum, and strawberry [1–3]. *Colletotrichum gloeosporioides* is a significant economic constraint to avocado production worldwide [4]. Anthracnose disease can occur on avocado fruits from setting until harvest. The disease results in fruit abortion and may also manifest as black spots on fruits which lower fruit quality [5, 6].

The disease inoculum is mainly conidia from diseased dead wood, leaves entangled in the tree canopy, branch terminals, mummified fruits, and flower bracts [7]. The conidia are disseminated through rainfall, irrigation, and heavy dew [8].

Disease management includes cultural, chemical, and biological control, along with the use of resistant cultivars [9–12]. Cultural control of anthracnose requires good orchard sanitation procedures. Furthermore, adequate postharvest handling practices must be applied to prevent cuts and bruises to fruit surfaces. Fruits showing symptoms of postharvest disease should not be packed into cartons containing healthy fruits. Biocontrol agents such as *Streptomyces* sp. A1022 [13], use of food preservatives such as carbonates, bicarbonates, and potassium sorbate [12], heat treatments [14], UV irradiation [15], microwaves [16], chitosan [17], and

Table 1: Fungicides concentrations used to determine sensitivity of *Colletotrichum gloeosporioides* isolates from diseased avocado fruits.

Trade name	Active ingredient (AI)	Concentration (g/l)	Concentration (g/l) for inhibition test
Bayleton 25WP	Triadimefon	0.25	0.500
			0.250
			0.125
Milraz 76WP	Propineb 70% and Cymoxanil 6%	0.50	1.00
			0.50
			0.25
Green Cop 500WP	Copper oxychloride	1.75	3.50
			1.75
			0.88

natural products such as essential oils [18, 19] have been used to control the postharvest disease.

Chemical control during fruit development is the main strategy to reduce the incidence of postharvest diseased fruits [20]. Application of fungicides such as Benomyl, Mancozeb, Metiram, Propineb, Thiabendazole, and Prochloraz has been used for disease management in South Africa, New Zealand, and Australia [21, 22]. Prochloraz, a nonsystemic fungicide, has been used in the packing line to control postharvest disease development. It affects the mycelial growth of the pathogens and acts as a sterol inhibitor impeding the synthesis of a fatty acid ergosterol, an important component in fungal cell wall development [9, 10].

In Kenya, management of anthracnose in avocado has been a challenge due to lack of registered fungicides. Fungicides registered for the management of fungal pathogens in other crops such as Bayleton 25WP (Triadimefon 250 g/Kg), Milraz 76WP (Propineb 70% and Cymoxanil 6%), and Green Cop 500WP (copper oxychloride 500 g/Kg) have been used for anthracnose management in avocado.

Inhibitory effect of Bayleton 25 WP (Triadimefon 250 g/Kg), Milraz 76 WP (Propineb 70% and Cymoxanil 6%), and Green Cop 500WP (copper oxychloride 500 g/Kg) used by farmers to control anthracnose in avocado in Kenya has not been determined. The aim of this study was to provide information on the efficacy of the fungicides against *C. gloeosporioides* isolates in Kenya with the aim of providing preliminary technical information required for further testing and possible registration of the effective fungicides against avocado anthracnose in Kenya.

2. Materials and Methods

2.1. Source of Isolates. Samples of infected fruits were randomly collected from avocado orchards in Murang'a County and taken to the laboratory. The fruits were washed using tap water and blotted to remove excess water. The fruits were surface-sterilized using 0.5% sodium hypochlorite for 30 seconds. Small sections from the edge of necrotic area were cut aseptically and plated on potato dextrose agar (PDA). Hyphal tips of the mycelia were subcultured to obtain pure cultures. *Colletotrichum gloeosporioides* was morphologically identified based on cultural and microscopic characteristics. Single-spore cultures were preserved on PDA slant universal bottle and stored at 4°C. A total of 46 isolates were used in this experiment.

2.2. Preparation of Fungicides Inhibition Treatments. In a previous survey, Bayleton 25 WP (Triadimefon 250 g/Kg), Milraz 76 WP (Propineb 70% and Cymoxanil 6%), and Green Cop 500WP (copper oxychloride 500 g/Kg) were identified as the fungicides used by farmers to control anthracnose. The concentrations used were determined based on the manufacturer's recommended doses on the fungicide label. Three concentrations of each fungicide were formulated for use as follows; twice the recommended rate, the recommended rate, and half the recommended rate (Table 1). Fungicide suspensions were prepared by dissolving specific quantities of each fungicide in warm PDA medium after autoclaving. About 15 ml of fungicide amended PDA medium was poured into sterile 9 cm diameter Petri dishes. Each fungicide concentration was replicated three times. Petri dishes with nonamended PDA media served as controls.

Disks of PDA, 5 mm in diameter, with actively growing mycelium of 7-day-old cultures, were cut out with sterile cork borer and placed in the centre of each plate. The amended and control Petri dishes inoculated with mycelia plugs were arranged in a completely randomized design in a sterile hood/chamber at room temperatures of 22–24°C in the Mycology Laboratory, KALRO, Kandara. The fungi were allowed to grow until mycelia growth covered the entire surface of the control plates.

2.3. Evaluation of Fungicides on Mycelia Growth Rate and Sporulation. After every two days, radial mycelial growth measurements were taken on each plate and recorded. Final measurements were taken after ten days when fungal growth in the control plates fully covered the plates and these measurements were used for data analysis. Inhibition of radial growth due to various fungicidal treatments was computed based on colony diameter on control plate using the following formula as described by [23]:

$$\% \text{ inhibition} = \frac{x - y}{x} \times 100, \quad (1)$$

where x is growth in control plate and y is growth in fungicide-treated plate.

Table 2: Effect of selected fungicides on mycelial growth of *Colletotrichum gloeosporioides* isolates.

Fungicide	Concentration (g/l)	Mean inhibition (%)
Milraz	0.25	82.1[*c]
	0.50 (recommended)	84.1[b]
	1.00	85.9[a]
	LSD	1.5184
	P value	<0.001
Copper oxychloride	0.88	80.9[c]
	1.75 (recommended)	83.3[b]
	3.50	85.1[a]
	LSD	1.035
	P value	<0.001
Bayleton	0.13	84.5[c]
	0.25 (recommended)	86.2[b]
	0.50	87.6[a]
	LSD	**0.6423**
	P value	<0.001

[*]Data represent means of three replications. Means followed by same letter are not significantly different at $P \geq 0.05$ according to Fisher's protected LSD test.

Further, the plates were flooded with 15 ml distilled water to bring the spores into suspension. Sporulation capacity was determined by counting the number of spores using a hemocytometer. Sporulation was expressed as the total number of spores as determined from 1 ml subsample.

2.4. Data Analysis. Analysis of variance (ANOVA) was used to compare fungicides inhibition percentages (radial mycelial growth and sporulation) using statistical Genstart version 6 software at 95% confidence level. Treatments were separated using Fisher's protected Least Significant Difference (LSD) test.

3. Results and Discussion

The mycelial growth of *C. gloeosporioides* isolates was inhibited significantly by the three fungicides' treatments with growth rates reduced by more than 80% (Table 2). Sanders et al. [24] observed similar results, where most of the *C. gloeosporioides* isolates obtained from diseased avocado fruits were highly sensitive to Prochloraz and Thiabendazole, with no growth being observed. The mycelial growth inhibition from the fungicide treatment significantly increased with an increase in the fungicide concentration (Table 2); however, none of the concentrations evaluated completely inhibited mycelial growth. Similar observations were made by Sanders et al., [24] when *C. gloeosporioides* isolates were subjected to various concentrations of Benomyl fungicide *in vitro*.

Among the fungicides, Bayleton had the highest mean inhibition percentages, with copper oxychloride showing the lowest inhibition percentages (Table 2). These fungicides probably acted as a sterol inhibitor impeding the ergosterol (fatty acid) synthesis, which is an important component of the fungal cell wall [12].

Inhibition of mycelial growth could further be explained by the multisite activity of the fungicide such as Milraz (Propineb and Cymoxanil), which inhibits synthesis of nucleic acids, amino acids, and other cellular processes [25].

Although farmers have been using these fungicides on avocado, they are not registered as preharvest chemical treatments for anthracnose on avocados in Kenya. Similarly, farmers have been using Benomyl for the management of anthracnose of avocado in South Africa although it is registered for the control of *Cercospora* spot of avocado [24]. Use of fungicides whose efficacy has not been tested might lead to incorrect application rate, which could favour the development of resistant strains of the causal agent [5].

The mean number of spores recorded from *C. gloeosporioides* isolates treated with different concentrations of fungicides differed significantly (Table 3). Isolates treated with different concentrations of Bayleton produced a higher number of spores as compared to Milraz and copper oxychloride treatments (Table 4). As the concentration of the fungicide was increased, spore production was significantly reduced but was not eliminated.

4. Conclusion

All the isolates were sensitive to Bayleton, Milraz, and copper oxychloride *in vitro*. Bayleton is the most effective fungicide in inhibiting mycelial growth and sporulation of *C. gloeosporioides in vitro*.

5. Recommendation

Further field tests should be done in different avocado-growing areas so as to validate these fungicides' efficacy against various isolates and under different environments.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Table 3: Mean number of spores/ml from 46 *Colletotrichum gloeosporioides* isolates treated with three fungicides ($\times 10^6$)/ml.

Copper oxychloride		Milraz		Bayleton	
Isolate	Mean	Isolate	Mean	Isolate	Mean
ko 2a	2.86^{*a}	G16b	3.25^{a}	ko20b	4.25^{a}
k19b	2.63^{ab}	ka14b	2.50^{b}	ka30b	3.75^{ab}
ka1c	2.63^{ab}	ka30b	2.50^{b}	ko 12c	3.63$^{a–c}$
ko24b	2.63^{ab}	ko20c	2.50^{b}	k20c	3.50$^{a–d}$
ko31c	2.63^{ab}	ko30a	2.50^{b}	ko14a	3.38$^{b–e}$
G16b	2.50$^{a–c}$	ko20b	2.38^{bc}	ko29a	3.25$^{b–f}$
k24c	2.50$^{a–c}$	k15a	2.25$^{b–d}$	k10b	3.13$^{b–g}$
ka12a	2.50$^{a–c}$	G22b	2.13$^{b–e}$	ko24b	3.13$^{b–g}$
ka30b	2.50$^{a–c}$	G8a	2.13$^{b–e}$	k20a	3.00$^{b–h}$
ko15c	2.38$^{a–d}$	ka22b	2.13$^{b–e}$	ka25 a	3.00$^{b–h}$
G20b	2.25$^{a–e}$	ko31c	2.13$^{b–e}$	ko 2a	3.00$^{b–h}$
k10c	2.25$^{a–e}$	G9a	2.00$^{b–f}$	ko13b	3.00$^{b–h}$
ka24a	2.25$^{a–e}$	k20a	2$^{b–f}$	ko20c	3.00$^{b–h}$
K20c	2.13$^{b–f}$	ko 2a	2.00$^{b–f}$	k15b	2.88$^{c–i}$
ka26 c	2.13$^{b–f}$	ko14a	2.00$^{b–f}$	k19b	2.88$^{c–i}$
ko11b	2.13$^{b–f}$	G19c	1.88$^{c–g}$	ka14b	2.88$^{c–i}$
ko17c	2.13$^{b–f}$	G20b	1.88$^{c–g}$	Ka 24a	2.88$^{c–i}$
G19c	2.00$^{b–g}$	k10b	1.88$^{c–g}$	ko13a	2.88$^{c–i}$
G8a	2.00$^{b–g}$	k10c	1.88$^{c–g}$	ko16a	2.88$^{c–i}$
G9a	2.00$^{b–g}$	K20c	1.88$^{c–g}$	ko7c	2.88$^{c–i}$
ka16 c	2.00$^{b–g}$	ka12a	1.88$^{c–g}$	k15a	2.75$^{d–j}$
ko13b	2.00$^{b–g}$	ko11b	1.88$^{c–g}$	ko17c	2.75$^{d–j}$
k15b	1.88$^{c–h}$	ko29b	1.88$^{c–g}$	G8a	2.63$^{e–k}$
ko29a	1.88$^{c–h}$	G12a	1.75$^{d–h}$	ka16 c	2.63$^{e–k}$
ko7c	1.88$^{c–h}$	Ka 31b	1.75$^{d–h}$	ka22b	2.63$^{e–k}$
G12a	1.75$^{d–i}$	ko16a	1.75$^{d–h}$	ko20a	2.63$^{e–k}$
k20a	1.75$^{d–i}$	ko17c	1.75$^{d–h}$	ko30a	2.50$^{f–l}$
ka9a	1.75$^{d–i}$	ko20a	1.75$^{d–h}$	G12a	2.38$^{g–m}$
ko13c	1.75$^{d–i}$	k19b	1.63$^{e–i}$	G16b	2.38$^{g–m}$
ko20c	1.75$^{d–i}$	ko13b	1.63$^{e–i}$	ka1c	2.38$^{g–m}$
k10b	1.63$^{e–j}$	ko13c	1.63$^{e–i}$	ka31b	2.25$^{h–n}$
ko13a	1.63$^{e–j}$	ko7c	1.63$^{e–i}$	ko31c	2.25$^{h–n}$
ko14a	1.63$^{e–j}$	ka16 c	1.50$^{f–j}$	G20b	2.13$^{i–o}$
ko27b	1.63$^{e–j}$	ka24a	1.50$^{f–j}$	G22b	2.13$^{i–o}$
ko30a	1.63$^{e–j}$	ka25 a	1.50$^{f–j}$	ka12a	2.13$^{i–o}$
ka25 a	1.50$^{f–j}$	ko13a	1.50$^{f–j}$	ko13c	2.13$^{i–o}$
ko16a	1.50$^{f–j}$	ko15c	1.50$^{f–j}$	G9a	2.00$^{j–o}$
ko20a	1.50$^{f–j}$	k15b	1.38$^{g–j}$	k10c	2.00$^{j–o}$
k15a	1.50$^{f–j}$	ka1c	1.38$^{g–j}$	ko29b	2.00$^{j–o}$
ka14b	1.50$^{f–j}$	ko27b	1.38$^{g–j}$	k24c	1.88$^{k–p}$
ka31b	1.38 $^{g–j}$	k24c	1.25$^{h–j}$	ka9a	1.88$^{k–p}$
ko29b	1.38$^{g–j}$	ka9a	1.25$^{h–j}$	ko15c	1.75$^{l–p}$
ko12c	1.25$^{h–j}$	ka26 c	1.13^{ij}	ka26 c	1.63$^{m–p}$
ka22b	1.13^{ij}	ko12c	1.13^{ij}	ko27b	1.50$^{n–p}$
ko20b	1.13^{ij}	ko29a	1.13^{ij}	ko11b	1.38^{op}
G22b	1.00^{j}	ko24b	1.00^{j}	G19c	1.13^{p}
LSD	**0.7251**	LSD	**0.6077**	LSD	**0.7833**
P value	<0.001	*P* value	<0.001	*P* value	<0.001

*Data represent means of three replications. Means within a column and followed by same letter(s) are not significantly different at $P \geq 0.05$ according to Fisher's protected LSD test.

Table 4: Effect of selected fungicides on *C. gloeosporioides* sporulation.

Fungicide	Concentration (g/l)	Mean number of spores ($\times 10^6$)/ml from 10-day-old cultures
Milraz	0.25	1.90*[b]
	0.50	1.33[c]
	1.00	0.35[d]
	Control	3.65[a]
	LSD	**0.1792**
	P value	**<0.001**
Copper oxychloride	0.88	1.95[b]
	1.75	1.22[c]
	3.50	0.59[d]
	Control	3.92[a]
	LSD	**0.2138**
	P value	**<0.001**
Bayleton	0.125	3.01[b]
	0.25	1.72[c]
	0.5	0.92[d]
	Control	4.69[a]
	LSD	**0.231**
	P value	**<0.001**

*Data represent means of three replications. Means followed by same letter are not significantly different at $P \geq 0.05$ according to Fisher's protected LSD test.

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