ISSN: 2347-4688, Vol. 9, No.(2) 2021, pg. 83-90

Current Agriculture Research Journal

www.agriculturejournal.org

Efficacy of Two New Fungicides Against *Colletotrichum kahawae* Infecting Coffee in Kenya

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Abstract

Coffee Berry Disease(CBD) caused by *Colletotrichum kahawae* is a destructive fungal disease of coffee in Kenya, leading to a loss of about 75%. This study aimed to assess the *In Vitro* efficacy of two fungicides:- CRI 1 (Pyraclostrobin 150 g/L + Fluxapyroxad 75 g/L) and CRI 2 (Pyraclostrobin 128 g/Kg + Boscalid 252 g/Kg) against *C. kahawae* using poisoned food technique on Potato Dextrose Agar. A total of 170 coffee berries with *C. kahawae* symptoms were purposively collected from both sprayed and unsprayed plots. Ten rates of each of the two fungicides were assessed ranging from 0.01% to 0.1% at an interval of 0.01%. Two commercial standard fungicides Pyraclostrobin 250 g/L at 0.04% and Tebuconazole 200 g/L + Trifloxystrobin 100 g/L at 0.1% were used as positive controls. Fungal inoculum in PDA media devoid of the fungicide acted as the negative control. Data on colony diameter was collected after every 24 hours for 13 days. Analysis of Variance (ANOVA) of the data on colony diameter was done using CoStat software version 6.400. The results revealed that all the rates of CRI 1 and CRI 2 fungicides controlled the colony diameter of *C. kahawae* compared to the control treatment. CRI 1 fungicide suppressed the growth of *C. kahawae* even at the lowest concentration of 0.01% with a percentage control of 64.74 %. The highest concentration of 0.1% had a percentage control of 66.15%. CRI 1 is more effective in controlling *C. kahawae* since it controls the fungus at a rate even lower than Pyraclostrobin 250 g/L which had a percentage control of 66.10 at a recommended rate of 0.04% and Tebuconazole 200 g/L + Trifloxystrobin 100 g/L fungicides which had a percentage control of 65.76 at a recommended rate of 0.1%. CRI 1 also hade a better percentage control as compared to CRI 2. CRI 2 had a percentage control of 54.63% at the highest rate of 0.1% and a percentage control of 35.60% at the lowest rate of 0.01%. Further studies on CRI 1 and CRI 2 fungicides should be carried out for yield assessment in the field.

Article History
Received: 21 June 2021
Accepted: 26 August 2021

Keywords
Boscalid;
Coffee;
Colletotrichum Kahawae;
Efficacy,
Fluxapyroxad;
Fungicide;
Poisoned Food Technique;
Pyraclostrobin.

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Doi: dx.doi.org/10.12944/CARJ.9.2.03

Introduction

Coffee is the most important raw material traded throughout the world after crude oil.[1] Coffee sustains over 100 million people globally and is rated among the largest export commodities in the world.[2] Coffee is the fifth largest foreign exchange earner in Kenya after tourism, tea, horticulture and diaspora remittances[3] and has largely contributed to the Kenyan economy through foreign exchange earnings, as well as creating employment opportunities.[4]

Despite its importance, coffee production in Kenya faces a major challenge from Coffee Berry Disease (CBD). The disease is caused by *Colletotrichum kahawae*, an anthracnose of green and ripe berries. The pathogen is an ascomycete that reproduces both sexually and asexually.[5] The fungi can infect the plant during any stage from the flower to the berry. Mummified berries, twigs, bark and dead branches are the sources of primary inoculum. *Colletotrichum* conidium forms within 24 hours under favourite conditions, after which elongation of the germ tube follows.[6] The apical section of the germ tube later differentiates to form an appressorium. The coffee fruit is then colonized by infection hyphae that arise from the appressoria. The symptoms then start to appear that includes; dark, necrotic sunken spots and brown, superficial lesions.[7] The disease causes premature fruit fall and mummified or damaged fruits which leads to a loss of about 75% of coffee.[8]

Coffee Berry Diease was first detected in Sotik in Western Kenya in 1922.[9] The disease has spread since then to several countries, including Uganda, Tanzania, Angola, Cameroon, Malawi, DRC Congo, and Rwanda among other countries. Rain is almost 100% responsible for transmission of the spores of *C. kahawae*, from the surface of infected berries to other berries by raindrops. The disease is mainly reported in coffee farms located at an altitude of between 1400 and 2200 m above sea level.[9]

Colletotrichum kahawae in Kenya can be managed using resistant coffee cultivars such as Ruiru 11 and Batian.[3, 8,10,11] However, most farmers have retained traditional varieties like SL 28, SL 34, and K7 which are highly susceptible to CBD due to their high cup quality.[10] Other management strategies, include cultural practices like use of shade trees to reduce the impact of raindrops, and timely and proper pruning of coffee plants.[9,12] Cultural practices are not 100% reliable for management of CBD, and therefore incorporation of chemical control into integrated disease management programmes is critical.[6, 13] There are over 200 commercial fungicides tested and registered in Kenya for management of CBD.[14] The registered fungicides can be broadly classified into Strobulins, Coppers, Triazoles and biopesticides. Despite having a number of fungicides to use, most coffee farmers in Kenya use fungicides from the Family of strobulins because of their high efficacy. However, the number of registered fungicides in this family is very narrow, an aspect that increases the risk of resistance development 15. It is therefore recommended by the Fungicide Resistance Action Committee (FRAC) for an interchangeable use of fungicides with different modes of action to avoid fungicide resistance.[15]

Pyraclostrobin active ingredient has been used for the control of anthracnose disease of sorghum, *Septoria tritici*, *Puccinia* spp, *Pyrenophora teres* and even *Colletotrichum kahawae*.[26] A premix of Fluxapyroxad +Pyraclostrobin active ingredients have been used for the control of several plant pathogens including anthragnose and powdery mildew which has resulted to an increase in mango production.[13] The same combination has controlled several other fungal pathogens including *Fusarium virguliforme, Macrophomina phaseolina, Rhizoctonia solani,* and *Sclerotinia sclerotiorum.*[25]

This study, aims to evaluate (Pyraclostrobin 150 g/L + Fluxapyroxad 75 g/L) and (Pyraclostrobin 128 g/Kg + Boscalid 252 g/Kg) as alternative fungicides for management of Coffee Berry Disease to be used interchangeably with the available fungicides as recommended by FRAC.

Material and Methods

Study Area

The study was conducted in the Plant Pathology laboratory, at the KALRO- Coffee Research Institute, Ruiru, Kenya. The centre lies at an altitude of 1620 m above sea level, on latitude 0° 41' 0 S and longitude 34° 46' 0 E , in Kiambu County. It receives a annual bimodal rainfall of 1063 mm with an average temperature of 16ºC (Min 12.8ºC, max 25.2ºC).

Sample Collection

Purposive sampling technique was used[16] whereby, only coffee berries with CBD symptoms were collected to be used for isolation of *C. kahawae*. A total of 170 premature green berries and partially ripe beans with slightly sunken and dark brown lesions were collected randomly from sprayed and unsprayed plots of SL 28, Geisha and K7 coffee varieties in Rukera farm at KALRO-CRI, for isolation of C.kahawae.

Fungal Isolation

The diseased berries were washed once in sterile distilled water with Teepol® (Teepol GD 18, Shell Chemical Industries Limited, Nairobi, Kenya). Five drops of Teepol® in 200 ml sterile distilled water using a teat pipette was used to remove initial dust.[7] The berries were rinsed with sterile distilled water until no foam was formed. Clean coffee berries were spread on a cellulose wadding on a sterile bench and left for 30 minutes to air-dry. After air-drying, the berries were incubated at 24ºC for 24 hours in sterile lunch boxes containing cellulose wadding to promote sporulation. After sporulation, the lesions were cut carefully and placed in sterile distilled water and serially diluted to 10^{-3} concentration. Using the spread plate technique, 10^{-2} and 10^{-3} dilutions were spread in a previously prepared Potato Dextrose Agar(PDA) (HiMedia Laboratories Pvt. Ltd. India) on 90mm × 15mm Petri plates. The plates were transferred in a sterile room for growth for seven days at a temperature of 24 ± 1ºC. A mixed culture of several isolates was obtained after seven days. Pure cultures were obtained by subculturing from regions where culture characteristics corresponded with those documented for *C. kahawae* 7. The C.kahawae obtained was used in subsequent experiments for Fungicide efficacy assessment.

Anti-Fungal Activity Assay using Poisoned Food Technique

Two new proprietary fungicides, CRI 1 and CRI 2 were subjected to anti-fungal activity assay using poisoned food technique.[17] CRI 1 has a combination of Pyraclostrobin and Fluxapyroxad while CRI 2 has a combination of Pyraclostrobin and Boscalid. Two commercial fungicides Pyraclostrobin 250 g/L and Tebuconazole 200 g/L + Trifloxystrobin 100 g/L were used as standard controls. The discs on the media devoid of the fungicide acted as negative controls. Potato Dextrose Agar was prepared and sterilized at 121ºC, and 15 atmospheres pressure, for 15 minutes in an autoclave (Priorclave Ltd).

The PDA was supplemented with test fungicides at ten concentrations of (0.01 %, 0.02 %, 0.03 %, 0.04 %, 0.05 %, 0.06 %, 0.07 %, 0.08 %, 0.09 %, 0.1 %) for CRI 1 and CRI 2 and two commercial standards Pyraclostrobin 250 g/L (0.04%) and Tebuconazole 200 g/L + Trifloxystrobin 100 g/L (0.1%). The fungicides containing media were poured into the Petri plates under aseptic conditions and allowed to cool and solidify. After complete solidification of the medium, 5mm disc of seven days old culture of *C. kahawae* was cut together with the PDA media using a sterile cork borer and placed aseptically upside down at the centre of the Petri plates. The treatments were replicated three times. The plates were incubated at 24 ± 1ºC and the growth of fungus colony was measured after every 24 hours for 13 days. The colony diameter was measured in millimetres using a digital vernier calliper.

Data Analysis

Data on fungal colony diameter was converted into angles corresponding to percentage angles,[18] and Analysis of Variance (ANOVA) was done using CoStat software version 6.400 (Cohort software Limited, United Kingdom).

Fig.1: Dark gray C. kahawae on PDA media

Results

Isolation of *Colletotrichum Kahawae* from Infected Coffee Berries

Isolated *Colletotrichum kahawae* fungal pathogen on PDA media is presented in figure 1. The fungal isolates were identified as *C. kahawae* based on the morphological appearance when grown on PDA media. Isolate 1 of *C. kahawae* fungal pathogen was obtained from Rukera farm at CRI where fungicides are applied for control of CBD while

isolate 2 of *C. kahawae* was obtained from Coffee Research Institute coffee farm, where fungicides are not applied.

Efficacy of CRI 1 and CRI 2 Fungicides against *C. Kahawae*

The various candidate fungicide rates recorded significantly (P<0.05) lower colony diameters for both isolate 1 and isolate 2 compared with the control treatment (Table 1). Although there was a reduction in colony diameter with the increasing concentration of CRI 1 fungicide, there was no significant difference that was established among the treatment rates and the standard controls (Pyraclostrobin 250 g/L (0.04%) and Tebuconazole 200 g/L+Trifloxystrobin 100 g/L (0.1%) as indicated in Table 1. CRI 1 Fungicide percentage control increased with the increasing concentration from 0.01% to 0.1% (Table 2). The highest colony diameter that differed significantly (P<0.05) from the candidate fungicides was recorded in the untreated control (40.13 mm and 36.85 mm) for isolate 1 and isolate 2 respectively (Table 1 and figure 2).

Table 1: Mean colony diameter of *C. kahawae* Isolate 1 and Isolate 2

	CRI 1		CRI 2	
	Colony diameter(mm)		Colony diameter(mm)	
Fungicide % rates	**Isolate 1**	**Isolate 2**	**Isolate 1**	**Isolate 2**
Control	40.13 a	36.85 a	35.96 a	38.74 a
0.01	13.62 b	13.52 b	22.63 b	25.48 b
0.02	13.31 b	13.31 b	21.51 bc	23.93 bc
0.03	13.22 b	13.22 b	21.21 bcd	23.37 bcd
0.04	13.09 b	13.22 b	20.52 cde	19.59 cde
0.05	13.09 b	13.05 b	20.26 cde	19.17 de
0.06	13.05 b	13.05 b	19.85 cdef	18.64 de
0.07	13.05 b	13.05 b	19.43 def	18.21 e
0.08	13.05 b	13.05 b	18.72 efg	17.19 ef
0.09	13.05 b	13.05 b	18.3 fg	16.81 ef
0.1	13.01 b	13.05 b	17.29 g	16.60 ef
Pyraclostrobin 250 g/L (0.04%)	13.05 b	13.05 b	13.05 h	13.05 f
Tebuconazole 200 g/L+Trifloxystrobin 100 g/L (0.1%)	13.31 b	13.31 b	13.31 h	13.31 f
LSD (P=0.05)	1.06	1.75	0.87	2.28
CV (%)	8.6	14.47	5.32	13.88

Means followed by the same letter in the same column are not significantly different. (LSD test P < 0.05)

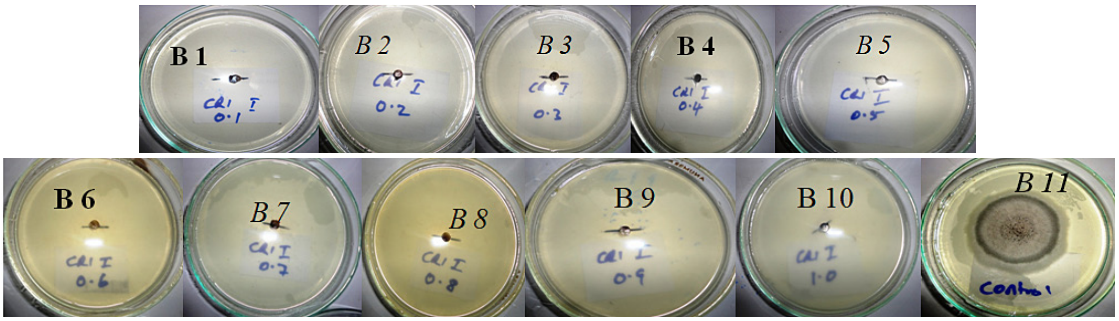

Fig. 2: B1-B10 shows highly suppressed colony diameter on the ten rates of CRI 1 fungicide. B 11 shows the highest colony diameter on the untreated control

Table 2: CRI 1 and CRI 2 Fungicide percentage rates and percentage control

	% Control	
Fungicide % rates	**CRI 1**	**CRI 2**
0.01	64.74	35.6
0.02	65.42	39.17
0.03	65.65	40.32
0.04	65.82	46.31
0.05	66.04	47.22
0.06	66.1	48.47
0.07	66.1	49.61
0.08	66.1	51.93
0.09	66.1	53
0.1	66.15	54.63
Pyraclostrobin 250 g/L (0.04%)	66.1	66.1
Tebuconazole 200 g/L+ Trifloxystrobin 100 g/L (0.1%)	65.76	65.76

The colony diameter decreased with the increasing concentration of CRI 2 fungicide with the lowest colony diameter recorded in the plates with rate 0.1% for both isolate 1 and isolate 2 (17.29 mm and 16.60 mm respectively). The fungi colony diameter was significantly (P<0.05) reduced by CRI 2 at 0.01% ,0.02%, 0.03%,0.04%, 0.05%,0.06%,0.07%,0.08%,0.09% and 0.1% in relation to the control treatment although the reduction for rate 0.08%,0.09% and 0.1% did not differ significantly (P<0.05) from that of the standard control treatments (Table 1). The fungicide percentage controls increased from the lowest concentration of 0.01% to the highest concentration of 0.1% (Table 2 and figure 3).

Table 3: Fungicides, isolates and their interaction on the control of C.kahawae

Source	
Main Effects	**P-value**
Treatment	0.0000 ***
Fungicide	0.0000 ***
Plot	0.8268 ns
Interaction	
Treatment x Fungicide	0.0000 ***
Treatment x Plot	0.9353 ns
Fungicide x Plot	0.5241 ns
Treatment x Fungicide x Plot	0.4175 ns
LSD (P=0.05)	1.59
CV (%)	11.12%

The interaction between the treatments and the fungicides was significant (P<0.05) in controlling the CBD colony diameter (Table 3). There was no significant (P>0.05) difference between the interaction of the treatment and the plot, the fungicide and the plot and the interaction of the treatments, the fungicides and the plot in controlling the CBD colony diameter (Table 3).

The fungal colony diameter decreased with the increasing fungicide concentration. The mean colony diameter of CRI 1 was smaller than that of CRI 2. The mean colony diameter of CRI 1 decreased with the increasing fungicide concentration from 0.01 % upto 0.05 % and then the colony diameter remained constant upto rate 0.1% (Figure 4).

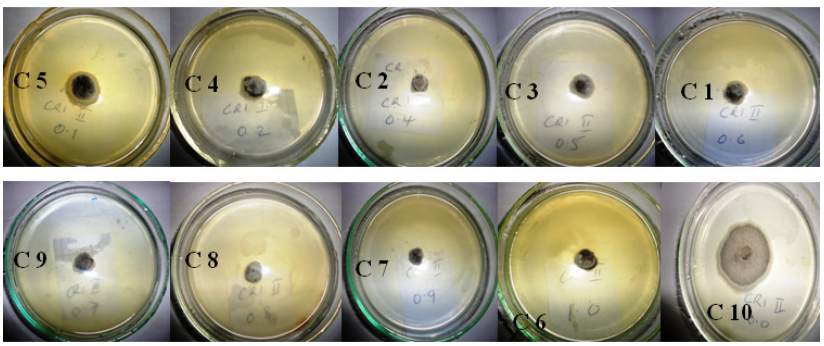

Fig. 3: C1-C9 shows colony growth on the ten rates of CRI 2 fungicide. C10 shows the highest colony diameter on the untreated control

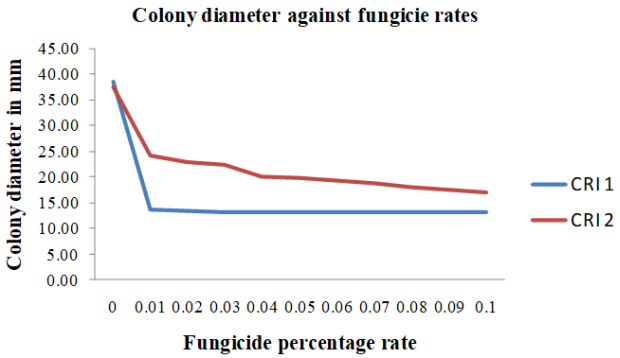

Fig. 4: Mean colony diameter of *C. kahawae* against fungicide rates

Discussion

Colletotrichum kahawae fungus was isolated and identified based on its morphological appearance on PDA media.[19,20 ,21] The results of this study have revealed that both CRI 1 and CRI 2 fungicides significantly controlled the growth rate of *Colletotrichum kahawae in vitro*.

The suppressive active ingredient Pyraclostrobin in the two chemicals hinders spore germination, germ tube elongation, mycelia growth and sporulation.[22]

In addition, the study revealed that CRI 1 was more suppressive on the colony diameter compared to CRI 2. This could be attributed to the differences in the chemical ingredients in the two products (CRI 1 and CRI 2). Fluxapyroxad is more effective in suppressing the fungal growth alone.[23] Pyraclostrobin has also been found controlling the fungal pathogens independently as in the case of Pyraclostrobin 250 g/L.[24]

The mode of actions in the two active ingredients, Fluxapyroxad and Pyraclostrobin in CRI 1 could be the reason for its high efficiency in controlling *C. kahawae*. The results of this study are in agreement with.[13, 25, 26] In a study on the *in vitro* evaluation of commercial fungicides against some of the major soil borne pathogens of soybean, Navi concluded that a combination of Pyraclostrobin and Fluxapyroxad controlled several fungal pathogens that included; Fusarium virguliforme, Macrophomina phaseolina, Rhizoctonia solani, and Sclerotinia sclerotiorum.[25]

In a study on the evaluation of pre-mix fungicide, fluxapyroxad and pyraclostrobin 500 sc against powdery mildew disease of mango (Oidium mangiferae), Ravikumar confirmed that the fungicide significantly reduced the disease severity and increased the mango productivity.[13]

In a study on effectiveness of fungicides and their application timing for the management of sorghum foliar anthracnose in the mid-Atlantic United States, Acharya concluded that Pyraclostrobin and Pyraclostrobin plus Fluxapyroxad controlled anthracnose disease of sorghum.[26] These studies reveal that a combination of Pyraclostrobin and Fluxapyroxad is effective in the control of Anthracnose and other fungal pathogens.

Conclusions and Recommendations

All the CRI 1 fungicide rates controlled the growth rate of *C. kahawae*. All the CRI 2 fungicide rates also controlled the growth rate of *C. kahawae*. CRI 1 demonstrated to be the best fungicide on the control of *C. kahawae* even at the lowest rate of 0.01% as compared to Pyraclostrobin 250 g/L and Tebuconazole 200 g/L + Trifloxystrobin 100 g/L at 0.04 % and 0.1 % respectively. CRI 1 is also more suppressive on the control of *C. kahawae* as compared to CRI 2. CRI 1 is more effective in controlling *C. kahawae* since it controls the fungus at a rate even lower than Pyraclostrobin 250 g/L and Tebuconazole 200 g/L + Trifloxystrobin 100 g/L. The best rates of CRI 2 for control of *C. kahawae* are 0.08 %, 0.09%, and 0.1%. This *In vitro* study has revealed that CRI 1 and CRI 2 can be used interchangeably as alternative fungicides for management of Coffee Berry Disease with the available fungicides like Pyraclostrobin 250 g/L (0.04%) and Tebuconazole 200 g/L+Trifloxystrobin 100 g/L (0.1%). Further studies on CRI 1 and CRI 2 fungicides should be carried out in the field on commercial farms to assess their effect on CBD and coffee yield.

Acknowledgements

The authors acknowledge Kenya Agricultural and Livestock Research Organization -Coffee Research Institute (KALRO-CRI) and Kenyatta University scientists for reading the paper and suggesting improvements. We also thank the KALRO-CRI plant pathology staff for participating in the study. We also acknowledge Kenya Agricultural and Livestock Research Organization -Coffee Research Institute (KALRO-CRI) for funding the project.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest

The authors do no have the conflict of interest regarding the work and publication of results for this article.

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