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Antimicrobial Activity of Foliar Fertilizer Formulations and their Effect on Ice Nucleation Activity of *Pseudomonas syringae* Pv. *garcae* Van Hall; the Causal Agent of Bacterial Blight of Coffee

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ABSTRACT

This study was carried out with the broad objective of assessing the potential for control of Bacterial Blight of Coffee (BBC) using foliar fertilizer applications to Inhibit Ice Nucleation Activity (INA) of the causative agent (*Pseudomonas syringae pv. garcae*). Bacteria isolates from four coffee growing areas in Kenya were characterized based on visual, biochemical, physiological and pathogenicity characteristics to distinguish the pathogenic isolates of *P. syringae* from other phylloplane epiphytic bacteria. Isolates of bacteria from diseased coffee plants were categorised in three groups. Isolates that were pathogenic to coffee and produced typical symptoms of BBC fell under group 1 and were assumed to be of *P. syringae* pv. *garcae*. Other isolates that fell under groups 2 and 3 were considered to be saprophytic epiphytes. Four commercially available fertilizer formulations; Bayfolan™, Mboleasafi™, Farmphoska™ and Farmfoliar™ were tested on four pathogenic bacteria isolates collected from the four regions. All formulations significantly ($p<0.05$) reduced the number of bacterial colony forming units. Bayfolan™ treatment had the highest bacterial growth inhibition potential while Farmfoliar™ was least inhibitive. All the isolates were Ice Nucleation Active (INA+) and all the fertilizer formulations were potentially capable of suppressing the bacterial ice nucleation activity with Farmphoska™ being the most suppressive. Findings presented in this report indicate a potential for the management of BBC using foliar fertilizers to suppress the bacterial INA.

Key words: Bacterial blight of coffee, *Pseudomonas syringae* pv. *garcae*, foliar fertilizers, ice nucleation activity, bacteria

INTRODUCTION

Coffee is second most traded commodity after petroleum and the most widely consumed beverage worldwide (Berecha *et al*., 2011). The three top producers of coffee in the world are Brazil, Vietnam and Colombia and these countries contribute around 55% of global production (Sureshkumar *et al*., 2010). Its production provides a livelihood for over 120 million people worldwide (Gichuru *et al*., 2008). Coffee cultivation in Kenya began around 1900 and it was introduced by the French missionaries (Kathurima *et al*., 2010). Kenya produces mainly Arabica

coffee of reputable quality that is classified within the Colombian milds known for balanced acidity and body with pleasant distinctive aroma (Omondi, 2008). However, coffee production in Kenya is seriously constrained by diseases across the coffee growing areas especially the Coffee Berry Disease (CBD) caused by *Colletotrichum kahawae,* Coffee Leaf Rust (CLR) caused by *Hemileia vastatrix* Berk and Br. and Bacterial Blight of Coffee (BBC) caused by *Pseudomonas syringae* pv. *garcae* Van Hall (Gichimu and Omondi, 2010; Agwanda *et al*., 1997).

BBC is characterized by necrotic, water-soaked lesions on leaves, shoot tips and young fruits culminating in die-back (Ito *et al*., 2008). It has been described in Brazil, Kenya, Uganda and China where it has been causing concern among coffee growers and researchers due to its high incidences and severity (Chen, 2002). The bacteria persist on all healthy surfaces of the tree and during cool and wet weather, the pathogen multiplies and initiate epidemics (Silva *et al*., 2006). The disease can be a serious problem in high altitudes, where plants are injured from heavy winds and/or hailstorms (Jansen, 2005). Dash and Gummadi (2007) also reported that *Pseudomonas* sp. is also capable of degrading the quantity of caffeine in coffee. Although copper based fungicides are recommended for BBC control, these sprays become less effective as bacterial blight infection pressure increases (Kairu *et al*., 1991). Other challenges associated with chemical control approaches include high costs, phytotoxicity and residual effects of the fungicides (Abera *et al*., 2011). Hofte and De-Vos (2006) reported that there is no single control strategy that is effective against the plant pathogenic *Pseudomonas* and control should therefore be based on a combination of chemical, biological and cultural strategies.

Ice Nucleation Activity (INA) is the induction of ice formation at super cooled temperatures of -2 to -10°C in the presence of suitable ice nuclei (Sarhan *et al*., 2005). The amount of supercooling, however, depends on the presence and activity of ice nucleators which can be extrinsic, such as the ice nucleating bacterium *Pseudomonas syringae* or intrinsic compounds of plant origin (Fuller *et al*., 2003). The role of bacterial ice nucleation in frost injury and the subsequent development of plant diseases have been extensively reviewed (Hirano and Upper, 2000; Morris *et al*., 2004; Kennelly *et al*., 2007). The strains of *P. syringae* and *Erwinia herbicola* have been shown to contain ice nucleation protein encoding genes and are the most studied active naturally occurring ice nuclei (Sarhan *et al*., 2005). Strains of the bacterium *Pseudomonas syringae*, can express ice nucleation activity at -1 or -2°C (Mohler *et al*., 2008). The coffee plant is sensitive to frost which easily damages and eventually completely kills the coffee tree (DaMatta and Ramalho, 2006). Lethal temperature for coffee leaves in field conditions is very close to freezing and the damage becomes more severe with the increase of exposition to critical temperatures (Petek *et al*., 2005). Such frost damage provides the pathogen with infection entry avenues and in one case in Kenya, frost injury was followed by severe BBC leaf and shoot infection (Kairu, 1994). In view of this observation, it is important to find out at what temperature is *P. s. garcae* ice nucleation active.

The application of field applied compounds for frost protection has been an elusive dream of many researchers and agri-chemical companies (Fuller *et al*., 2003). Frost injury to coffee plants was, however, prevented by spraying the trees with organophosphorous insecticide disulfoton in Brazil (Bach *et al*., 1994). Wowk and Fahy (2002) demonstrated the effect of polyglycerol polymers in inhibiting ice nucleation activity of *P. syringae*. Wisniewski *et al*. (2002) reported the use of a hydrophobic kaolin as a protectant of freezing in tomato. In Kenya the connection between ice nucleation activity of *P. s. garcae* and frost damage on one hand and development of bacterial blight of coffee on the other has not been fully focused in research probably on the assumption that

frost damage is insignificant in Kenya's warm tropical climate. However, with currently experienced global climate change, it's apparent that frost occurrence in tropical countries can not be overruled. This research work was carried out with the objectives of: (1) Isolating and characterizing the bacterial species occurring on coffee phylloplane in Kenya (2) assessing the antimicrobial activity of selected foliar fertilizer formulations on selected *P. s. garcae* isolates and (3) evaluating the potential of the foliar fertilizer formulations to suppress BBC by inhibiting bacterial INA.

MATERIALS AND METHODS

The study was carried out under laboratory conditions at Egerton University, Njooro Campus in Kenya between 2008 and 2009. Leaves and secondary branches infected with BBC were collected from coffee trees growing in Mt. Elgon region (Elg) in Western Kenya, Ruiru (Rru) near Nairobi, Mweiga (Mwg) in Central Kenya and Solai (Sli) in the Kenyan Rift Valley.

Experiment 1: Isolation and characterization of bacteria from coffee phylloplane: Bacteria from the diseased material were isolated using the method of Goszcznska *et al.* (2000). The isolates were subjected to biochemical, physiological and pathogenicity tests for characterization as described by Kairu (1997) and Lelliott and Stead (1987). The isolates were evaluated for colony colour, colony growth rate, colony margin characteristics, fluorescent pigment production, Arginine dihydrolase reaction and pathogenicity on coffee. Characterization variables were organized into a matrix and subjected to hierarchical cluster analysis based on Euclidean distance using XLSTAT Version 2010 statistical software.

Experiment 2: Antimicrobial activity of the foliar fertilizers against *P.s. garcae* isolates: The commercial foliar fertilizers Bayfolan™, Mboleasafi™, Farmphoska™ and Farmfoliar™ were used for this test. Their choice was based on their widespread utilization in Kenya, their availability and differences in composition of Chloride ion (Cl^-), Sulphur (S), Copper ions (Cu^{2+}), Vitamin B_1 and Cobalt (Co) (Table 1).

Table 1: Chemical formulations of four commercial foliar fertilizers

	Chemical composition																
Fertilizer	N	P	K	C	Fe	Mg	Mn	B	Cu	Zn	Co	Mo	Cl	Na	S	VB_1	GH
Bayfolan™	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+
Mboleasafi™	+	+	+	-	+	+	+	-	-	+	-	+	-	-	+	-	-
Farmphoska™	-	-	-	+	+	-	+	+	+	+	+	+	-	-	-	-	-
Farmfoliar™	+	+	+	-	-	-	Unspecified trace elements										

+: Present, -: Absent, VB1: Vitamin B1, GH: Growth hormone

These foliar fertilizers were assessed for antimicrobial activity using two methods.

Method I: Effects of the foliar fertilizers on colony growth of *P. s. garcae* isolates: Four isolates Rru005, Sli008, Mwg010 and Elg006 from Group 1 (Annex 1) were selected for use in the subsequent experiments. The isolates were selected on the basis of their pathogenicity on coffee seedlings and to represent the different geographical regions where coffee is grown in Kenya. Each isolate was suspended in sterile distilled water in vials and the cell concentration adjusted to

10^6 cfu mL^{-1}, using Spectronic 20 spectrophotometer. Thereafter serial dilution was carried out so as to attain a final bacterial cell concentration of 10^8 cfu mL^{-1}. Using a pipette, 1 mL of the bacterial suspension was added into 9 mL of each fertilizer formulation in vials. The vials were left to stand for 24 h. Controls were prepared by serially diluting the bacterial suspension with distilled sterile water to attain 10^8 cfu mL^{-1}. Aliquots of 0.1 mL were withdrawn from each vial after 24 h and plated on nutrient agar. Each treatment had six replicates and the plates were completely randomized. The plates were incubated at 27°C for 48 h. The number of colonies formed for each fertilizer formulation/isolate combination and the control were enumerated under the magnifying lens of a bacteria cell colony counter. The data was analysed using SAS general Linear Model Procedure (SAS Institute, 2004).

Method II: Growth inhibitory effects of the foliar fertilizers against isolates of *P.s. garcae*: The four isolates were cultured on liquid nutrient broth by suspending the bacteria in vials containing the broth and incubating at 27°C for 24 h with regular shaking. An aliquot of 2.5 mL of the broth containing 10^6 cfu mL^{-1} was added to 247.5 mL of autoclaved Nutrient Sucrose Agar at 40°C. After swirling to distribute the cells of the bacteria within the agar media, the media was poured into Petri dishes and left to solidify. Sterile filter paper discs measuring 15 mm in diameter were soaked in the various fertilizer solutions for 15 min and then placed aseptically at the centre of Petri dishes on the surface of the media. Two controls; one positive and the other negative were set up. For the positive control, the filter paper discs were soaked in a solution of streptomycin sulphate antibiotic. For the negative control, filter paper discs were soaked in sterile distilled water and placed on the surface of the media at the centre of the Petri-dishes. The plates were then incubated at 27°C for 48 h. The experiment had six replicates and the replicates completely randomized. The sizes of inhibition zones were measured with a transparent ruler under the magnifying lens of a bacterial cell colony counter. The length from the edge of the filter paper disc to the colony margin was measured. This was then recorded as the length from the filter paper edge to the colony margin on both sides.

Experiment 3: Bacterial INA suppression by the fertilizers: Bacterial INA suppression by the foliar fertilizer solutions was assessed using two methods:

Method I: Effects of foliar fertilizer formulations on ice nucleation temperatures: The method developed by Vali (1971) was used with some modifications to measure the number of active bacterial ice nuclei at various sub-zero temperatures. Cultures of the four isolates grown on Nutrient Sucrose Agar for 48 h were suspended in sterile distilled water in culture tubes and the number of cfu mL^{-1} adjusted to 10^6 using Spectronic 20 spectrophotometer. Two millilitres of the bacterial suspensions were measured out into four culture tubes and an equal volume of each fertilizer formulation was added to the culture tubes. An equal volume of sterile distilled water was added to the fifth culture tube to serve as a control. Aluminium foil sheets measuring 5 cm×5 cm were sprayed with 1% solution of paraffin in xylene and then dried at 55°C in a hood for 10 min. The foils were then folded using empty Petri dish covers as moulds to form flat bottomed troughs. These were floated on a 1:1 mixture of methanol and water at a depth of about 5 cm in an aluminium tray. Using a micropipette, 10 drops each measuring 30 μL of the bacterial/fertilizer suspension, were placed on the flat bottomed aluminium foil troughs and placed in an incubator set at: 0, -2, -4, -6, -8, -10, -12 and -14°C respectively for 2 h. The number of bacterial/fertilizer

suspension droplets that froze at each of the set temperatures were counted and recorded. The experiment had six replicates set up in a completely randomized design.

Method II: Number of ice nuclei active at a given sub-zero temperature: Due to unavailability of an ice nucleus spectrometer, a modification of the method of Makino (1982) and Vali and Stansbury (1966) as described by Kairu (1994) was used. Only one isolate Mwg010 was used in this experiment because its ice nucleation activity was the most suppressed by most of the fertilizer formulations in method 1 above. Bacterial/fertilizer suspensions and aluminium foil sheet troughs were prepared as outlined in Method I. The fertilizer/bacterial isolate combinations were prepared in culture tubes. The troughs were then floated on a 1:1 mixture of methanol and water in an aluminium tray. The solution was cooled to sub-zero temperature in salt/ice freezing mixture in a perspex container placed inside the freezer compartment of a refrigerator. Using a micropipette 200 μL of each suspension was withdrawn from the culture tubes and placed in the floating aluminium trough. A temperature probe was immersed into the drop. The temperature at which the drop froze was recorded. The experiment had six replicates in a completely randomized design.

RESULTS

Characterization of bacteria from coffee phylloplane: The biochemical, physiological and pathogenicity tests on bacteria isolated from diseased coffee material resulted in three groups of isolates (Fig. 1; Annex 1). Group I contained translucent, levan forming, fast growing isolates with a tenacious colony margin and a negative arginine dihydrolase reaction. These isolates produced fluorescent pigment on King's B (King *et al*., 1954) and were pathogenic on coffee seedlings. Isolates in this group were considered to be the pathogenic types of *P. syringae* due to their pathogenicity on coffee seedlings. Group II isolates were opaque ash-white in colour, positive levan formation, slow growing, had a non-tenacious margin and positive arginine dihydrolase reaction. These isolates did not produce fluorescent pigment on nutrient sucrose agar and were not pathogenic on coffee. Group III isolates had inconsistent results on most of the tests (Annex 1). However, all of them were yellow in colour. They were also non-pathogenic on coffee seedlings. Four isolates, Rru005, Sli008, Mwg010 and Elg006, from the pathogenic Group 1 were selected for use in the subsequent experiments.

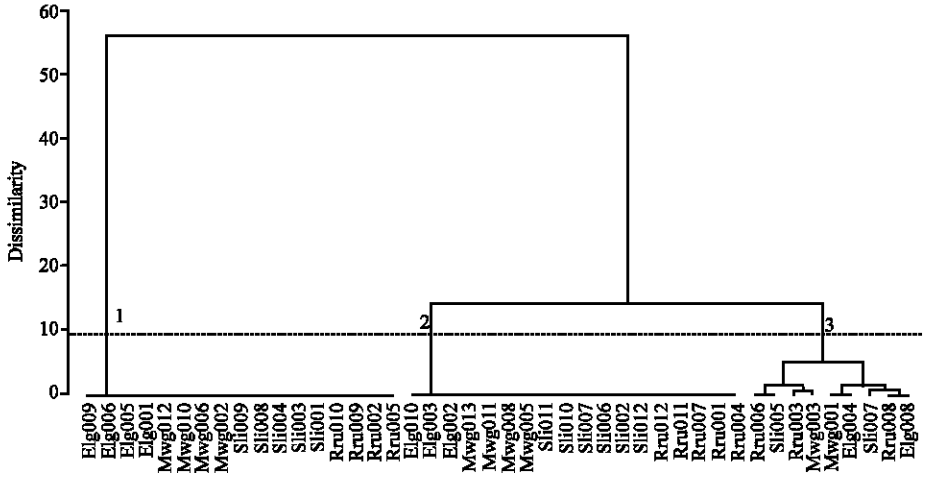

Fig. 1: Dendrogram depicting the three distinct groups of isolates

Antimicrobial activity of the foliar fertilizers against selected *P.s. garcae* isolates

Effects of the foliar fertilizers on colony growth of selected *P. s. garcae* isolates: All the four foliar fertilizer formulations tested had antimicrobial effect on the bacterial isolates. This was expressed in form of reduced number of colony forming units as compared to the control treatment (Table 2). In the control treatment between 99.2 and 100.0 colonies grew on the agar plates. The mean number of colonies in the control treatment was 99.6. The fertilizer formulation treatment with the lowest colony count represented the greatest bactericidal effect. Farmphoska™ had the highest antimicrobial effect with a mean of 60.9 colonies growing while Farmfoliar™ had the lowest bactericidal effect with a mean of 65.6 colonies (Table 2). Thus the foliar fertilizers significantly ($p<0.05$) reduced the number of bacteria colonies. The isolate Elg006 was most sensitive with a mean of 63.4 colonies growing while the Mweiga isolate (Mwg010) was least sensitive with a mean of 64.6 colonies.

Table 2: Colony counts of four *P. s. garcae* isolates treated with four foliar fertilizer formulations

	Isolates				
	Mwg010	Rru005	Sli008	Elg006	Means
Farmphoska	64.2a	59.2a	61.2a	60.7a	60.9
Bayfolan	62.8a	65.2b	62.5a	60.8a	62.8
Mboleasafi	64.2a	67.0b	62.7a	63.0b	64.2
Farmfoliar	67.2b	66.3b	66.5b	62.3a	65.6
Control	100	99.2	99.7	100	99.6
Means*	64.6	64.4	63.2	63.4	

Means followed by the same letter in a column are not significantly different ($p \geq 0.05$). *Excluding control

Growth inhibitory effects of the foliar fertilizers against isolates of *P.s. garcae*: The results of bacterial growth inhibition by the fertilizer formulations are presented in Table 3. Distinct bacterial growth inhibition zones were observed around filter paper discs soaked in the various fertilizer formulations as compared to the negative control. They were however, not as extensive as the zones around the positive control. The mean inhibition zone by streptomycin, the positive control on the four isolates was 10.2 mm whilst the mean inhibition zone for the negative control was 0.00 mm. In the negative control experiment, bacteria colonies grew up to the margin of the filter paper discs. Bayfolan treatment had the highest bacterial growth inhibition potential while Farmfoliar was least inhibitive. The growth inhibition zone exhibited by Farmfoliar was not significantly different ($p>0.05$) from the control treatment except for the isolate Mw010 (Table 3).

Table 3: Effects of foliar fertilizer formulations on growth inhibition (mm) of *P.s. garcae*

	Isolates				
Fertilizer	Mwg010	Elg006	Sli008	Rru005	Means
Bayfolan	7.7a	8.3a	8.2a	6.4a	7.7
Mboleasafi	6.3b	6.4b	6.2b	5.5b	6.1
Farmphoska	4.2c	3.4c	3.0c	3.9c	3.7
Farmfoliar	0.8d	0.2d	0.5d	0.1d	0.4
Control (-ve)	0	0	0	0	0.0
Control (+ve)	10.3	10.2	10.2	10.2	10.2
Means*	4.8	4.5	4.5	4	

Means followed by the same letter in a column are not significantly different ($p \geq 0.05$). *Excluding control

Bayfolan showed the largest mean inhibition zone of 7.7 mm. The second best performing fertilizer was Mboleasafi with mean inhibition of 6.1 mm. The isolate Rru005 was least sensitive to the fertilizer formulations with a mean of 4.0 mm, while Mwg010 was most sensitive with a mean of 4.8 mm.

Suppression of bacterial INA by the fertilizers

Effects of foliar fertilizer formulations on ice nucleation temperatures: Results of experiment to determine bacterial ice nucleation temperatures as affected by the fertilizer formulations demonstrated significant ($p<0.05$) different suppressive effects of bacterial ice nucleation activity by the four foliar fertilizer formulations. Ice nucleation active bacterial nuclei were observed as "frosty" spots where the droplets of water had frozen. All the fertilizer formulations suppressed the freezing temperatures of the suspensions to temperature below zero. In the control experiment, the suspension froze even at 1.0°C (Table 4). Farmphoska was the most suppressive of the ice nucleation activity of all the isolates, with a mean freezing point of -11.3°C. This represents the greatest suppression of bacterial Ice Nucleation Activity (INA). Farmfoliar was least suppressive of bacterial INA with a mean freezing point of -8.7°C. The ice nucleation activity of the Mwg010 isolate was the most suppressed by most of the fertilizer formulations. It recorded an average ice nucleation temperature of -10.8°C compared to Rru005, Elg006 and Sli008 isolates which recorded average ice nucleation temperatures of -10.6, -9.0 and -7.8°C, respectively. Mboleasafi was, however, more suppressive of bacterial INA on Ruiru isolate (Rru005) at -10.0°C than on the Mweiga isolate (Mwg010) at -9.3°C.

Table 4: Effects of foliar fertilizer formulations on ice nucleation temperatures of *P.s. garcae*

	Isolates				
Fertilizer	Mwg010	Rru005	Elg006	Sli008	Means
Farmphoska	-13.0a	-12.7a	-10.8a	-8.5a	-11.3
Bayfolan	-11.0b	-10.0b	-9.2b	-7.5b	-9.4
Mboleasafi	-9.3c	-10.0b	-8.2c	-7.5b	-8.8
Farmfoliar	-9.5c	-9.5b	-7.8c	-7.8a	-8.7
Control	+1.0d	+1.0c	+1.0d	+1.0c	1
Means*	-10.8	-10.6	-9	-7.8	

Means followed by the same letter in a column are not significantly different ($p>0.05$). *Excluding control

Mean number of ice nuclei active at a given sub-zero temperature: Table 5 shows the mean number of ice nuclei active at various sub-zero temperatures on the Mweiga isolate (Mwg010) compared to the control. The Mwg010 isolate was used because its ice nucleation activity was the most suppressed by most of the fertilizer formulations in the experiment 3 (i) above. In the control treatment 2, 4 and 8 droplets out of 10 were active ice nuclei at 0, -2 and -4°C, respectively and all the 10 droplets were ice nucleation active at -6°C. There were no active ice nuclei at temperatures as low as -6°C in all the four fertilizer treatments. Ice nucleation active nuclei were first observed at -8°C in the Bayfolan, Mboleasafi and Farmfoliar treatments which recorded mean droplets of 1, 2 and 3, respectively. There were no active ice nuclei in the Farmphoska treatment at -8°C. This represented the highest bacterial ice nucleation suspension. Even at -14°C when all the 10 droplets in the Bayfolan, Mboleasafi and Farmfoliar treatments were ice nucleation active, 1 out of the 10 droplets in the Farmphoska treatment was still ice nucleation inactive (Table 5).

Table 5: Average active ice nuclei at various sub-zero temperatures in the Mwg010 isolate

	Temperature (°C)							
Fertilizer	0	-2	-4	-6	-8	-10	-12	-14
Farmphoska	0	0	0	0	0	4	6	9
Bayfolan	0	0	0	0	1	6	9	10
Mboleasafi	0	0	0	0	2	4	8	10
Farmfoliar	0	0	0	0	3	7	9	10
Control	2	4	8	10	10	10	10	10
Means*	0	0	0	0	1.5	5.25	8	9.75

*Excluding control

DISCUSSION

Characterization of bacteria from coffee phylloplane: The biochemical, physiological and pathogenicity tests on bacteria isolated from diseased coffee material resulted in three groups of isolates but only one group was pathogenic to coffee and produced typical BBC symptoms. Although it was not possible to characterise the bacteria isolated up to pathovar level, the pathogenic isolate was assumed to be *P. s.* pv *garcae* as its characteristics were similar to those reported by Kairu (1997). Two other groups of bacteria, one that had yellow colonies and another with opaque-white colonies were consistently isolated from diseased coffee material. These two groups were non-pathogenic and probably consisted of saprophytic epiphytes and contaminants like the ones earlier isolated by Otieno (1988). Some of the epiphytic phylloplane bacteria isolates have been previously shown to be antagonistic to the pathogenic isolate (Otieno and Gathuru, 1995). There is need to examine further the interaction between these non-pathogenic and the pathogenic phylloplane bacteria.

Antimicrobial activity of the foliar fertilizers against selected *P.s. garcae* isolates: Antimicrobial activity shown by foliar fertilizers in both bactericidal effect and growth inhibition can be explained on the basis of the chemical composition of these fertilizers. The fertilizer ingredients thought to play a role in the observed antimicrobial activity are copper and sulphur. These components have been shown, in other formulations, to be bactericidal and bacterial INA inhibitors (Mittelstadt, 1996). Some isolates were more sensitive to fertilizer formulations than others, a factor which was attributed to differences in pathotypes of *P.s. garcae* in different locations or different history of fungicide use in these areas. Routine integration of copper based fungicides in sprays to control CBD/CLR/BBC in some areas might have induced some Cu^{2+} tolerance/resistance to some *P. s. garcae* isolates as reported by Kairu (1994). However, it would be expected that Rru005 which was isolated from a plot at Ruiru that had never been sprayed with fungicides would be most sensitive but this was not the case. The interaction observed between the isolates and fertilizer formulations in bactericidal and growth inhibition could have arose from the interaction between different chemical composition of fertilizers and isolate biochemical characteristics. In the overall however, when compared to the bacterial growth inhibition by streptomycin sulphate (positive control), these inhibitions can only be described as mild and therefore, may have minimum or negligible contribution to the observed suppression of BBC by the fertilizer formulations.

Suppression of bacterial INA by the fertilizers: The experiment to determine bacterial ice nucleation temperatures as affected by the fertilizer formulations demonstrated potential ability

of the fertilizers to suppress the ice nucleation activity of *P. s. garcae* below 0°C. All the fertilizer formulations suppressed the freezing temperatures of the suspensions to temperature below zero. This concurred to Savvides *et al.* (2000), who reported that Ice nucleation active bacteria (INA) was able to catalyse ice formation at temperatures below -1.5°C. Mohler *et al.* (2008) demonstrated that some *Pseudomonas spp* and *Erwinia herbicola* bacteria are mainly ice active in the temperature range between -7 and -11°C. Farmphoska was the most suppressive of the ice nucleation activity of all the isolates. This can be attributed to the fact that only Farmphoska contained Copper (Cu) ions and no Phosphorus (P) in its chemical formulations (Table 1). Copper salts are reportedly capable of inhibiting bacterial ice nucleation (Wowk and Fahy, 2002). Non-ice nucleation active (INA^-) bacteria have been shown to convert to ice nucleation active (INA^+) under conditions of phosphate deficiency (Blondeaux and Cochet, 1994; Fall and Fall, 1998). Bach *et al.* (1994), managed to prevent frost injury to coffee plants in Brazil by spraying the trees with organophosphorous insecticide, disulfoton. Wowk and Fahy (2002) demonstrated the effect of polyglycerol polymers in inhibiting ice nucleation activity of *P. syringae*. Fuller *et al.* (2003) reported that some acrylic compounds provide frost protection by covering the leaf surfaces with an inert layer.

CONCLUSION

Findings presented in this report indicate a potential for using foliar fertilizers on the coffee phylloplane to suppress BBC infection by inhibiting bacterial INA. Being salts in nature, these fertilizers act in the same way as other inorganic salts used in cloud seeding to avoid hail-storms or to initiate frost thawing in temperate regions during winter. It is not possible to recommend a particular fertilizer type since the effects of interaction between the fertilizer types and isolate source were found to be significant. However, it could be ideal to study the effects of some selected combinations of fertilizer formulations.

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ANNEX

Annex 1: Biochemical, physiological and pathogenicity characteristics of the bacteria isolates

	Isolate	Col	Lev	Rate	Marg	Arg	Flo	Path
Group I	Rru005	T	+	F	Te	-	+	+
	Rru009	T	+	F	Te	-	+	+
	Rru010	T	+	F	Te	-	+	+
	Sli001	T	+	F	Te	-	+	+
	Sli003	T	+	F	Te	-	+	+
	Sli004	T	+	F	Te	-	+	+
	Sli008	T	+	F	Te	-	+	+
	Sli009	T	+	F	Te	-	+	+
	Mwg002	T	+	F	Te	-	+	+

Annex 1: Continued

	Mwg006	T	+	F	Te	-	+	+
	Mwg010	T	+	F	Te	-	+	+
	Mwg012	T	+	F	Te	-	+	+
	Elg001	T	+	F	Te	-	+	+
	Elg005	T	+	F	Te	-	+	+
	Elg006	T	+	F	Te	-	+	+
	Elg009	T	+	F	Te	-	+	+
Group II	Rru004	O	+	S	Nt	+	-	-
	Rru007	O	+	S	Nt	+	-	-
	Rru011	O	+	S	Nt	+	-	-
	Rru012	O	+	S	Nt	+	-	-
	Sli012	O	+	S	Nt	+	-	-
	Sli002	O	+	S	Nt	+	-	-
	Sli006	O	+	S	Nt	+	-	-
	Sli007	O	+	S	Nt	+	-	-
	Sli010	O	+	S	Nt	+	-	-
	Sli011	O	+	S	Nt	+	-	-
	Mwg005	O	+	S	Nt	+	-	-
	Mwg008	O	+	S	Nt	+	-	-
	Mwg011	O	+	S	Nt	+	-	-
	Mwg013	O	+	S	Nt	+	-	-
	Elg002	O	+	S	Nt	+	-	-
	Elg003	O	+	S	Nt	+	-	-
	Elg010	O	+	S	Nt	+	-	-
GroupIII	Rru006	Y	+	S	Nt	-	-	-
	Rru008	Y	+	F	Te	+	+	-
	Sli005	Y	+	S	Nt	-	-	-
	Sli007	Y	+	F	Te	-	+	-
	Mwg001	Y	+	S	Te	+	+	-
	Mwg003	Y	+	F	Te	-	-	-
	Elg004	Y	+	S	Te	+	+	-

Col: Colony colour, Lev: Levan formation, Rate: Growth rate, Marg: Margin characteristics, Arg: Arginine dihydrolase reaction, Flo: Fluorescent pigment production, Path: Pathogenicity on coffee, T: Translucent, O: Opaque-ash white, Y: Yellow, F: Fast growing, S: Slow growing, Te: Tenacious, Nt: Non-tenacious, Elg: Mt. Elgon region, Mwg: Mweiga, Sli: Solai, Rru: Ruiru

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