African Journal of Rural Development, Vol. 7 (4): October-December, 2022: pp.480-491. ISSN 2415-2838

In vitro activity of *Bacillus* and *Trichoderma* species in the management of crucial bacterial plant diseases

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ABSTRACT
This study aimed to screen isolates of *Bacillus* and *Trichoderma* spp. for antagonistic activity against *Ralstonia solanacearum, Xanthomonas campestris* pv *campestris,* and *Pseudomonas* sp. in vitro. Twenty-eight Trichoderma and nineteen Bacillus isolates were used as antagonists. Molten nutrient agar was seeded with 2 108 CFU/ml of each pathogen, and the paper disc method was used to test the efficacy of the antagonists. Filter paper discs (5mm diameter) soaked for 10 minutes in Bacillus broth and 5mm discs of actively growing Trichoderma on potato dextrose agar were placed on the seeded media. Antagonistic activity was assessed by measuring the radius of the pathogen's zone of inhibition (ZOI) by the antagonist. The 10 Bacillus and 11 Trichoderma isolates that were tested had varied antagonistic activity against the pathogens. Trichoderma isolate T1 was the most potent in inhibiting the growth of *R. solanacearum*, with a mean ZOI measuring 13.5mm. In contrast, Bacillus isolate CB64 was the best antagonist, with a mean ZOI measuring 4.3mm. Trichoderma isolate T28 and Bacillus isolate CA5 showed the highest ZOI of 15.2mm and 6.6mm, respectively, against *X. campestris*. The antagonists screened gave lower activity against *Pseudomonas* sp. compared to other pathogens. Trichoderma isolate T28 showed the highest ZOI of 9.3mm, and Bacillus isolate CB14 and CB22 gave similar ZOI of 5.3mm. Trichoderma isolates showed better activity by more than 56.67% than Bacillus isolates. The results showed that twelve Trichoderma isolates (with ZOI of 10mm) and nine Bacillus isolates (with ZOI of ≥ 4.3mm) have high potential in managing various bacterial diseases. This implies that Trichoderma and Bacillus have antagonistic activity against bacterial pathogens and can be incorporated in an integrated disease management package.

Keywords: Biocontrol, *Ralstonia solanacearum, Xanthomonas campestris, Pseudomonas* sp.

RÉSUMÉ
Cette étude visait à cribler des isolats de Bacillus et Trichoderma spp. pour leur activité antagoniste contre *Ralstonia solanacearum, Xanthomonas campestris* pv *campestris* et *Pseudomonas* sp. in vitro. Vingt-huit isolats de Trichoderma et dix-neuf isolats de Bacillus ont été utilisés comme antagonistes. L'agar nutritif fondu a été ensemencé avec 2 108 CFU/ml de chaque pathogène, et la méthode du disque de papier a été utilisée pour tester l'efficacité des antagonistes. Des disques de papier filtre (diamètre de 5 mm) trempés pendant 10 minutes dans du bouillon de Bacillus et des disques de 5 mm de Trichoderma en croissance

Cite as: Kariuki, C. K., Mutitu, E. W. and Muiru, W. M. 2022. In vitro activity of Bacillus and Trichoderma species in the management of crucial bacterial plant diseases. *African Journal of Rural Development* 7 (4): 480-491.

active sur de l'agar de pomme de terre dextrose ont été placés sur le milieu ensemencé. L'activité antagoniste a été évaluée en mesurant le rayon de la zone d'inhibition (ZOI) du pathogène par l'antagoniste. Les 10 isolats de Bacillus et les 11 isolats de Trichoderma testés ont présenté une activité antagoniste variable contre les pathogènes. L'isolat de Trichoderma T1 était le plus puissant pour inhiber la croissance de *R. solanacearum*, avec une ZOI moyenne de 13,5 mm. En revanche, l'isolat de Bacillus CB64 était le meilleur antagoniste, avec une ZOI moyenne de 4,3 mm. L'isolat de Trichoderma T28 et l'isolat de Bacillus CA5 ont montré la ZOI la plus élevée de 15,2 mm et de 6,6 mm respectivement contre *X. campestris*. Les antagonistes testés ont montré une activité plus faible contre Pseudomonas sp. par rapport aux autres pathogènes. L'isolat de Trichoderma T28 a montré la ZOI la plus élevée de 9,3 mm, et les isolats de Bacillus CB14 et CB22 ont donné une ZOI similaire de 5,3 mm. Les isolats de Trichoderma ont montré une activité supérieure de plus de 56,67 % par rapport aux isolats de Bacillus. Les résultats ont montré que douze isolats de Trichoderma (avec une ZOI de 10 mm) et neuf isolats de Bacillus (avec une ZOI de 4,3 mm) ont un potentiel élevé pour la gestion de diverses maladies bactériennes. Cela implique que Trichoderma et Bacillus ont une activité antagoniste contre les pathogènes bactériens et peuvent être incorporés dans un package de gestion intégrée des maladies.

Mots-clés : Biocontrôle, *Ralstonia solanacearum, Xanthomonas campestris, Pseudomonas* sp.

INTRODUCTION

Management of bacterial plant diseases to increase the productivity of various important crops like tomato has been a great challenge. Management of bacterial diseases has been achieved through cultural practices, resistant plant varieties, and chemicals (Narasimhamurthy *et al*., 2018). Most bacterial pathogens such as *R. solanacearum*, *X. campestris* pv *campestris* and *Pseudomonas* sp. cause devastating diseases to important crops (Sundin *et al*., 2016). *R. solanacearum*, the causal agent of bacterial wilt, is the most devastating pathogen in tomato production (Sinha, 2016). In Kenya, bacterial wilt disease causes 64-100% yield loss (Mbaka *et al*., 2013). *X. campestris* pv *campestris* causes black rot, a major disease of vegetables in brassica family (Massomo *et al*., 2003). *Pseudomonas* sp. that causes bulb rot disease of Ornithogalum has led to 40% losses in cut-flower production (Mwangi, 1993).

Available strategies for controlling bacterial pathogens are limited in effectiveness; hence, managing the diseases is difficult (Mbaka *et al*., 2013). New strains of the pathogens keep on arising and are capable of infecting resistant crop varieties (Onduso, 2014). Furthermore, there are hardly known chemicals to manage bacterial diseases except for antibiotics that are used for human and animal diseases, which are highly regulated to prevent the development of resistance to human pathogens (Yendyo *et al*., 2017). Challenges in management of bacterial wilt has led to decline in tomato productivity and high yield losses by farmers.

Therefore, alternative strategies for managing bacteria diseases are necessary (Kago *et al*., 2019). Fungi and bacteria that are antagonistic against pathogens and promote plant growth, have been used to manage diseases (Brotman *et al*., 2008: Singh *et al*., 2012: Nikolic *et al*., 2013). Biological control of bacterial wilt disease of solanaceous crops through antagonistic agents has been reported (Narasimha and Srinivas, 2012: Kumar, 2017). Plant growth-promoting rhizobacteria have antagonism against *Xanthomonas campestris* pv *campestris* (Compant *et al*., 2005: Liu *et al*., 2016).

The action of certain plant growth-promoting agents (PGPA), including Bacillus strains and *Trichoderma* spp, is effective in managing Pseudomonas spp (Kloepper *et al*., 2004: Sundin *et al*., 2016). Studies have reported that microbial antagonists such as *Rhizobium, Bradyrhizobium*, and *Trichoderma* spp have shown greater residual effects after application in the soil in one season (Yaqub and Shahzad, 2011). The ability of these Biocontrol agents to persist in the soil protects the next crop. Application of the antagonist after every season increases their population in the soil, thus increases their competitiveness and effectiveness (Yaqub and Shahzad, 2011). Therefore, this study aimed to identify biological control agents that are antagonistic to these important bacterial pathogens in vitro. Selected Biocontrol Agents (BCAs) from this study will be researched further in the field and developed into commercial products that will overcome bacterial wilt disease challenge and toxicity caused by chemicals.

MATERIALS AND METHODS

Experimental Site and Design. The experiment was conducted from January to December 2019 in the Plant Pathology laboratory at the University of Nairobi, College of Agriculture and Veterinary Sciences, Kenya. A complete randomized design with three replicates was used.

Isolation of bacterial pathogens. Stem samples of bacterial wilt diseased tomato plants were collected from heavily infected fields in Mwea region, Kirinyaga County. The stems were cut into small pieces (2cm long) and were surface sterilized with 1% NaOCl solution for two minutes followed by three rinses in sterile distilled water. Then, the stem pieces were macerated using a sterile glass rod in sterile distilled water contained in a sterile universal bottle. A sterile wire loop was dipped in the upper layer of the suspension and was streaked on Petri plates containing 2, 3, 5 Triphenyl Tetrazolium Chloride (Kelman's TZC agar) medium for isolation of *R. solanacearum* (Kelman, 1954). *Pseudomonas* sp. was isolated on sucrose nutrient agar (SNA) medium (EPPO, 2014) from bulb rot diseased Ornithogalum samples collected from infected field at Kabete Field Station. *X. campestris* pv *campestris* was isolated on yeast peptone sucrose agar (YPSA) medium (Schaad, 1988) from black rot diseased cabbage leaves collected from heavily infected fields at Kabete Field Station using the same procedure outlined above.

The Petri plates were incubated upside down at 28^{o}C for 48 hours. Isolated pathogens were purified on nutrient agar (NA), transferred to NA on agar slants, and incubated for 48 hours. Sterile distilled water was added in each slant for preservation and stored at room temperature (23 ±2^{o}C) for later experimental use (Kelman and Person, 1961: Khasabuli *et al*., 2017). The pathogens' identity was confirmed through cultural and morphological characteristics of each pathogen.

Retrieval of Bacillus strains and isolation of Trichoderma. A total of 19 coded *Bacillus* spp. preserved in sterile modified loam soil were obtained from the Culture Collection Center of the Department of Plant Science and Crop Protection, University of Nairobi. Bacillus strains were retrieved by sprinkling particles of the modified loam soil on nutrient agar as described by Wagacha *et al*. (2007). The Petri plates were incubated for 48 hours at room temperature. Pure cultures of the isolates were obtained by sub-culturing each isolate on fresh NA and incubating for 24h at 23±2^{o}C.

Soil samples for Trichoderma isolation were acquired randomly from cultivated coffee fields, pasture land, kitchen waste disposal site, tomato field, and the non-cultivated area at Kabete Field Station. The samples from the six areas were composited into one sample, transported in paper bags, and stored at 4^{o}C in the laboratory. Trichoderma strains from the soil were isolated using a serial dilution technique. One millilitre aliquots of the suspension were plated on Trichoderma selective medium (TSM) as described by Maina *et al*. (2015). Pure cultures of Trichoderma isolates were maintained on potato

dextrose agar (PDA) at 28°C (Watts *et al*., 1988). The isolates were identified using morphological and microscopic characteristics as described by Samuel *et al*. (2004), and coded T1 up to T28.

Preparation of metabolites from Bacillus isolates. One hundred millilitre of TSCHEN's medium was prepared in a 500ml conical flask for each Bacillus isolate (Tschen and Kou, 1984). The medium was sterilized at 121°C for 15 minutes and then cooled to about 40°C. Ten millilitre of Bacillus suspension for each isolate, previously prepared by flooding a 10 days old pure culture with sterile water, was transferred to the conical flask containing the TSCHEN's medium. The conical flask was corked using cotton wool and properly sealed with aluminium foil to avoid contamination. The inoculated liquid media were incubated at room temperature for seven days on a rotary shaker at 120rpm (Wagacha *et al*., 2003).

In vitro screening of Bacillus and Trichoderma antagonists against *Ralstonia solanacearum, Xanthomonas campestris* pv *campestris* and *Pseudomonas* sp. Paper disc method was used to screen the antagonists against the bacterial pathogens. A virulent *R. solanacearum* isolate was multiplied on nutrient broth and cultured for 48 hours at 28°C. Ten millilitres of 48-hour old bacterial suspension containing 2×108 CFU/ml was mixed with 1 litre of 50°C molten sterilized NA medium for Bacillus and PDA medium for Trichoderma isolates so as to get a good spread of bacteria on agar medium (Singh and Jagtap, 2017). The seeded medium was poured into sterile Petri plates and was allowed to solidify. Previously sterilized filter paper discs measuring 5mm in diameter were soaked in different antagonist broths of Bacillus isolates for 10 minutes. Three paper discs were placed at equidistant points, one centimetre from the edge of each plate. The plates were laid out in a complete randomized design (CRD) with three replicates and incubated at 28±2°C for 72 hours. Filter paper disc dipped in sterile distilled water for 10 minutes served as control. Observations on formation of zones of inhibition (ZOI) between the antagonists and the pathogen around the filter paper discs were made, and their radius in mm were recorded at 24, 48, and 72 hours of incubation (Singh and Jagtap, 2017).

Fungal discs of Trichoderma isolates measuring 5mm in diameter from the margin of actively growing four days old cultures were used in place of filter paper discs. The fungal discs were removed with a cork borer and placed in the centre of the plates containing pathogen-seeded PDA. The plates were arranged in a CRD with three replicates on sterile laboratory bench and incubated at 28±2°C for six days. Observations and measurements of ZOI in mm around the mycelial discs against *R. solanacearum* were recorded after 2, 4 and 6 days. Discs of solidified sterile PDA medium that had not been cultured with the fungus served as control (Singh and Jagtap, 2017).

The same procedure was used for evaluating the antagonistic activity of Bacillus and Trichoderma isolates against *X. campestris* pv *campestris* and *Pseudomonas* sp. The medium was seeded with these pathogens in place of *R. solanacearum*.

Data analysis. Analysis of variance for data recorded on measurements of ZOI was carried using GenStat 15th edition to evaluate the antagonistic ability of Bacillus and Trichoderma isolates. The model for ANOVA was mean equals treatment plus residual. Mean comparisons was bulk, all the treatment including the control were compared using Fisher's Protected Least Significant Difference (LSD) test ($P \leq 0.05$).

RESULTS

Isolation of bacterial pathogens

Colony characteristics of the pathogens. White, fluidal, pinkish red-centred colonies of *R. solanacearum* with round, irregular margins (8.0mm) were observed on TZC medium. In addition, some colonies appeared round, deep red with narrow bluish borders. *Xanthomonas* campestris pv campestris appeared as mucoid, circular, convex, shiny yellow colonies when

grown on YPSA medium. *Pseudomonas* sp. colonies on SNA were smooth, elevated with entire margins, and pearly whitish-yellow in colour.

Isolation and identification of Trichoderma. The pure colonies of Trichoderma grown on PDA medium showed different growth patterns and colony characteristics. The colour of the colonies varied from light green to dark green. Twenty-eight isolates of Trichoderma were identified from these characteristics.

In vitro screening of Bacillus and Trichoderma antagonists against *Ralstonia solanacearum, Xanthomonas campestris* pv *campestris* and *Pseudomonas* spp. There were clear zones of inhibition (ZOI) around the filter paper discs by some of the Bacillus isolates against the test pathogens. Similarly, clear ZOI were formed around the mycelia discs of Trichoderma isolates that had activity against the three test pathogens. Four isolates of Bacillus (CB64, CA7, CA5 and CA10) showed activity against *Ralstonia solanacearum*. Isolate CB64 gave the highest ZOI (Mean = 4.3mm) at 24 hours of incubation and remained constant up to 48 hours of incubation. This was followed by CA7 (ZOI = 3.4mm); while ZOI by the other isolates was 2.2mm. However, the ZOI of CB64 decreased to 3.0mm at 72 hours. Similarly, the other three Bacillus isolates recorded reduced ZOI after 48 hours of incubation as shown in Table 1. For *Xanthomonas campestris*, 10 Bacillus isolates showed activity against the pathogen (Table 1). Isolate CA5 had the largest ZOI of 6.6mm, followed by CA51 and CB8 at 5.7 and 4.1mm, respectively. The other seven isolates had significantly lower ZOI (2.5mm) compared to the first three isolates. There was no significant difference among the antagonists at 24, 48 and 72 hours of incubation. Ten isolates of Bacillus had activity against *Pseudomonas* sp., and seven of these showed activity with a ZOI of more than 3.7mm. Bacillus isolates with the highest ZOI against *Pseudomonas* sp. were CB14 and CB22, which had similar measurements of 5.3mm while the control had no clear ZOI as indicated in Table 1.

Out of the 28 Trichoderma isolates screened, 21 isolates showed activity (Table 2). The maximum ZOI of Trichoderma isolates against the pathogens were formed at 4 days of incubation and generally remained stable and constant even at 6 days. Control plates had no visible ZOI. Data were not recorded for isolates with no ZOI against the test pathogens. The ZOI of Bacillus and Trichoderma isolates against the three pathogens were significantly different at P 0.05 compared to control. At four days of incubation, nine Trichoderma isolates had the highest activity against *R. solanacearum* with ZOI of 9.2mm (Table 2). From the screened Trichoderma isolates against *X. campestris* pv c*ampestris*, 11 isolates showed activity against the pathogen with ZOI of 7.7mm and above. For *Pseudomonas* sp, 11 Trichoderma isolates showed activity against the pathogen. The isolate with the largest ZOI was T28 with 9.3mm, followed by T12 at 7.7mm. The other nine isolates had ZOI of 3.4mm, which was significantly lower than the ZOI recorded for T28 and T12. Isolate T1, which had the highest activity against *R. solanacearum* with a ZOI of 13.5mm, also showed high activity against *Xanthomonas campestris* with a ZOI of 10.3mm but recorded significantly lower activity for *Pseudomonas* sp with a ZOI of 3.4mm. Isolates T28 and T12, which had the highest ZOI of 15.2 and 11.4mm against *Xanthomonas campestris* pv *campestris*, respectively, also had the highest activity against *Pseudomonas* sp. with ZOIs of 9.3 and 7.7mm, respectively. Further, the activity of these isolates (T28 and T12) against *Xanthomonas campestris* pv *campestris* was significantly higher than against *Pseudomonas* sp. The mean ZOI against the three test pathogens was significantly different at P 0.05. *R. solanacearum* had the highest mean (10.3mm), followed by *Xanthomonas campestris* pv *campestris* (9.3mm) and *Pseudomonas* sp. (3.5mm).

Table 1. Radius of the zone of inhibition (mm) due to Bacillus against three bacteria pathogens at 48 hours of incubation

	Measurements of radius in mm		
Bacillus isolates	*Ralstonia solanacearum*	*Xanthomonas campestris*	*Pseudomonas* sp.
CB64	4.3a	_	_
CA7	3.4b	1.5e	1.6f
CA5	2.2c	6.6a	3.7cde
CA10	1.5c	1.8de	4.0bcd
CA51	_	5.7b	3.0e
CB8	_	4.1c	3.8bcd
CA48	_	2.5d	4.4b
CB4	_	2.2de	_
CB24	_	2.2de	3.3de
CB12	_	2.0de	4.2bc
CA9	_	1.8de	_
CB14	_	_	5.3a
CB22	_	_	5.0a
Control	0d	0f	0g
Mean	2.3	2.8	3.5
CV (%)	19	20	11
LSD (P≤0.05)	0.79	0.91	0.67

Means followed by the same letter(s) along a column are not significantly different at P 0.05. CV: Coefficient of Variation, LSD: Least significant difference of means, and dashes (_) imply that the isolate had no activity against the test pathogen.

DISCUSSION

In vitro assessment of the ability of various antagonistic agents that act against different plant pathogens is the first step in selecting potential biological control agents (Ramesh *et al*., 2009). These antagonists use different mechanisms such as competition, lysis, antibiosis, siderophore production, and hyper parasitism (Revathi *et al*., 2017). Virulent *Ralstonia solanacearum* colonies were white with pinkish centre while avirulent colonies appeared round, deep red with narrow bluish border. These observations were similar to those of Champoiseau and Momol (2008) who reported similar characteristics about *R. solanacearum* colonies. EPPO, (2014) reported that *X. campestris* pv *campestris* appears as mucoid, circular, convex, shiny yellow colonies when grown on YPSA medium, which supports the current study's findings. In addition, they also reported that colonies of *Pseudomonas* spp are smooth, elevated with entire margins, and pearly whitish-yellow on SNA medium, similar to observations made in the current study. Schaad (1988) also reported similar findings for *Pseudomonas* sp. colony characteristics. Observations of the Trichoderma isolates colony colour that varied from light green to dark green were in agreement to the Trichoderma colony characteristics reported by Kannangara *et al*. (2017).

Table 2. Radius of the zone of inhibition (mm) induced by Trichoderma against three bacteria pathogens after four days of incubation

	Measurements of radius in mm		
Trichoderma isolates	*Ralstonia solanacearum*	*Xanthomonas campestris*	*Pseudomonas* sp.
T1	13.5a	10.3cd	3.4c
T4	13.1ab	_	2.2ef
T2	12.5b	_	_
T8	11.6c	_	_
T3	11.2cd	_	3.3c
T7	11.0cd	_	_
T6	10.8de	9.4de	_
T14	10.2e	10.7bc	3.2cd
T16	9.2f	_	3.3c
T28	_	15.2a	9.3a
T12	_	11.4b	7.7b
T18	_	10.6bc	_
T5	_	10.0cd	_
T19	_	9.4de	_
T13	_	8.5ef	_
T10	_	8.4ef	_
T17	_	7.7f	_
T11	_	_	2.8de
T26	_	_	2.4f
T25	_	_	2.2ef
T9	_	_	2.2ef
Control	0g	0g	0g
Mean	10.3	9.3	3.5
CV (%)	3.70	6.70	9.70
LSD (P≤0.05)	0.64	1.04	0.57

Means followed by the same letter(s) along a column are not significantly different at P 0.05. CV: Coefficient of Variation, LSD: Least significant difference of means, and dashes (_) imply that the isolate had no activity against the test pathogen.

In this study, strains of *Bacillus* and *Trichoderma* spp. effectively suppressed the growth of *Ralstonia solanacearum, Xanthomonas campestris* pv *campestris* and *Pseudomonas* sp. in vitro, as reported earlier (Lwin and Ranamurkhaarachchi, 2006: Liza and Bora, 2009). The radius of zones of inhibition caused by Bacillus isolates against *Ralstonia solanacearum* ranged between 1.5mm and 10mm which was comparable to the findings reported by Ramesh *et al*. (2009) and Singh *et al*. (2012). Bacillus isolate CB64 had the highest activity (ZOI = 4.3mm). The results observed in the current study showed that Bacillus and Trichoderma isolates had ability to suppress the pathogens. This could have been because these isolates secrete antibiotics and secondary metabolites that suppress bacterial pathogen through antibiosis. The antagonistic ability of Bacillus isolates against *Ralstonia solanacearum* observed in the present study was similar to the results observed by Seleim *et al*. (2011) who indicated that the mechanism used by the antagonist was antibiosis.

The activity of Bacillus isolates was more effective against *Xanthomonas campestris* pv *campestris* and *Pseudomonas* sp than against *R. solanacearum*. Inhibition zones of Bacillus isolates against *Xanthomonas campestris* pv *campestris* ranged between 1.5mm to 6.6mm in radius, and isolate CA5 showed the highest ZOI (6.6mm), which was in agreement with Monteiro *et al*. (2005). Compant *et al*. (2005) and Liu *et al*. (2016) also reported that certain endophytic bacteria can reduce the in vitro growth of *Xanthomonas* spp by producing siderophore and antibiotics compounds. *Bacillus* isolates had higher activity against *Pseudomonas* sp. than the other test pathogen and its control. Zones of inhibition ranged from 3.0mm to 5.3mm in radius, and isolates CB14 and CB22 recorded similar ZOI (5.3mm), in line with the results reported by Bais *et al*. (2004). *Bacillus* strains secrete different antibiotics such as polymyxin, circulin, and colistin that are active against gram-positive and gram-negative bacteria and many pathogenic fungi (Maksimov *et al*., 2011; Tapwal *et al*., 2011; Mukherjee *et al*., 2013). A larger ZOI was observed after 48 hours of incubation than after 24 hours, which could be because of the additive secretion of antibiotics by the antagonists over time (Singh *et al*., 2012; Chen *et al*., 2013).

Trichoderma isolates tested against the pathogens did better than Bacillus isolates, possibly because the latter are fungi that produce volatile compounds such as β-1, 3-glucanase, chitinases, protease, gliotoxis and dermadine, among others, with higher inhibitory properties than for Bacillus isolates. Hernandez-Castillo *et al*. (2020) also reported higher in vitro inhibition of *Fusarium* spp by *Trichoderma* spp (62.4-54.8%) than *Bacillus* spp. (44.5-36.9%). Zones of inhibition of Trichoderma isolates against *R. solanacearum* ranged from 7.4mm to 14mm, similar to the findings of Yendyo *et al*. (2017). Trichoderma isolate T1 (13.5mm) was the most potent in inhibiting the growth of *R. solanacearum*. Zones of inhibition of Trichoderma isolates against *Xanthomonas* sp. ranged between 7.7 mm to 15.2mm in radius, which was comparable to earlier studies (Nikolic *et al*., 2013: Sharma, 2018). Trichoderma isolate T28 (15.2mm) showed the highest ZOI against *Xanthomonas campestris*. The activity of Trichoderma isolates against the test pathogen was most likely due to the production of metabolites and secondary compounds that can suppress the growth of the pathogen. The present study showed that Trichoderma isolates had significantly lower activity against *Pseudomonas* sp. but isolate T28 showed the highest ZOI (9.3mm). This could be because Pseudomonas species are able to produce resistance endospores and compounds which are metabolically active even under harsh conditions. *Pseudomonas* spp also produces its own metabolites that have antagonistic properties against some pathogens, which could have caused the lower activity observed in Trichoderma isolates (Brotman *et al*., 2008). Bacillus isolates CA10, CA7, CA5, and Trichoderma isolates T1 and T14 had antagonistic activity against all the pathogens,

possibly because the secondary metabolites and chemical factors like antibiotics synthesized by the antagonists have a broad-spectrum activity (Gross and Vidaver, 1990). However, since the activity of Bacillus and Trichoderma isolates under field conditions may be different from the in vitro activity, there is a need for greenhouse and field trials to confirm these findings. It appears that Biocontrol agents are highly specific against target pathogen. This implies that BCA with antagonistic activity against one pathogen may not have any antagonistic activity against another pathogen. Therefore, management of certain diseases may require multiple BCAs to be used. Further BCAs remain active for a relatively short-time and therefore unable to provide long lasting control unless continuous application is done especially for use in the field. Results of this study can be used further, in production and formulation of selected BCA isolates by industries to increase their shelf-life and retain Biocontrol activity similar to that of fresh cells. Therefore, proper methods of industrial scale-up and fermentation have to be developed.

CONCLUSION

This study aimed to identify biological control agents that are antagonistic to important bacterial pathogens *in vitro*. Screening of Bacillus had challenges due to its endophytic nature that easily caused contamination. In addition, Trichoderma and Bacillus isolates remained active for a relatively short time, six days and 48 hours, respectively. This implies that they are unable to provide long lasting control. Ten Bacillus and 11 Trichoderma isolates had varied antagonistic activity against the pathogens tested. *Trichoderma* isolates T1, T28 and T28 were the most potent in inhibiting the growth of *R. solanacearum, X. campestris* and *Pseudomonas* sp, respectively. Bacillus isolates CB64 and CA5 had the highest activity against *R. solanacearum* and *X. campestris*, respectively while CB14 and CB22 had the highest activity against *Pseudomonas* sp. Isolates of Trichoderma showed better activity by more than 56.67% than isolates of Bacillus. The high difference in means of ZOI implied that Bacillus isolates had the lowest activity against *R. solanacearum* followed by *X. campestris* but showed high activity against *Pseudomonas* sp. The results showed that 12 Trichoderma isolates (with ZOI of 10mm) and nine Bacillus isolates (with ZOI of 4.3mm) have high potential in managing various bacterial diseases.

Production and formulation of selected isolates to increase their shelf-life and retain biocontrol activity similar to fresh cells is recommended. However, since the activity of Bacillus and Trichoderma isolates under field conditions may be different from the in vitro activity, greenhouse and field trials are recommended.

ACKNOWLEDGEMENT

The authors are grateful to the Kenya National Research Fund for providing financial support to conduct this research and the University of Nairobi for providing facilities to carry out the experiments.

STATEMENT OF NO-CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in this paper.

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